Study Title

The Impact of GAUCHO® and TI-435 Seed-Treated Canola on Honey Bees, *Apis mellifera* L.

Data Requirement

None

Authors

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Completion Date

April 11, 2001

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Study / Project Number

Not Applicable

Report Number

110403

Statement of Confidentiality

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d)(1)(A),(B) or (C).

Company: Bayer Corporation
Agriculture Division
Research and Development Department
Environmental Research Section

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Certification of Good Laboratory Practice / Quality Assurance Statement

The overall study described in this document was not conducted under Good Laboratory Practice requirements. However, the study was conducted in the spirit of GLP (see page 1a) and the analytical portion was conducted in compliance with the Good Laboratory Practice (GLP) requirements of 40 CFR Part 160, dated October 16, 1989. For the analytical report, a Certificate of Compliance and a Quality Assurance Statement are presented on pages 24 and 25, respectively, of this report.

Submitter: Bayer Corporation
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Certification of Authenticity

With the exception of the following items, subsequent pages of this report are presented as received from the performing laboratory:

1) Addition of a Bayer report number;
2) Addition of page numbers to pages 1a, 18a, 18b, 19a, 19b, 19c, 19d, 21a, 21b, 21c, and 23;
3) Repagination of pages 24 -117; and
4) Clarified shaded text on page 59.

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FINAL REPORT

The impact of GAUCHO® and TI-435 Seed-Treated Canola on Honey Bees, *Apis mellifera* L.

Principal Investigators:

[redacted]

STUDY TEAM APPROVAL PAGE

The study team takes this opportunity to assure the reader that the honeybee behaviour portion of this project was done "in the spirit of GLP". The analytical phase of this study and the report submitted by Enviro-Test Laboratories (Appendix 4) was done in compliance with Good Laboratory Practises according to EPA-FIFRA section 40 CFR part 160 (Oct. 16, 1989).

[redacted]

April 5, 2021
Date

April 9, 2021
Date

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INTRODUCTION

GAUCHO® (Bayer Corp.) is a seed treatment containing the chloronicotinyl insecticide known as imidacloprid. Imidacloprid is the first compound in the chloronicotinyl family to act on an insect's nicotinic acetylcholine receptors (Leicht, 1993). Since its initial registration in France in 1991, imidacloprid has become widely used receiving acknowledgment for its biological activity on a broad range of homopteran insect pest including aphids, leafhoppers, planthoppers, thrips and whiteflies (Elbert *et al.*, 1991; Schmuck, 1999). In addition, this compound has been found to be active against some species in the orders Coleoptera, Diptera and Lepidoptera (Elbert *et al.*, 1991). Today, imidacloprid is registered for use in many countries, having considerable agricultural importance as a broad spectrum multi-formulation insecticide that can be used on a wide variety of crops.

Imidacloprid is highly water-soluble with considerable molecule mobility in the xylem of treated plants (Elbert *et al.*, 1998). These systemic characteristics make imidacloprid particularly suited for seed treatment and soil application. Imidacloprid's systemicity is enhanced by its residual activity, which in seed treatments, has been established at up to 60 days after planting of the treated seed (Tröltzsch, 1995; Schmuck, 1999). Therefore, imidicloprid as a seed treatment can be used with confidence on crops, such as canola, that bloom >60days after planting and are pollinated by insects such as the honey bee (*Apis mellifera* L.). Further more, the systemic nature and residual activity of imidicloprid make it a valuable tool in integrated pest management programs for many agricultural insect pests.

Despite worldwide recognition of imidacloprid as an efficacious agricultural chemical, claims were made by French beekeepers in 1997 that GAUCHO® treated sunflowers were responsible for the decline and subsequent failure of French honey bee colonies. French beekeepers maintain that honey bees from hives placed in GAUCHO® treated sunflower fields display high rates of mortality, disorientation and low honey production all leading to a severe decrease in colony strength and in some instances colony death. Recent studies European studies examined the effects of GAUCHO® seed treated sunflowers and contaminated sugar syrup on honey bees, and found no evidence to support the claims made by French beekeepers (Schmidt and Schmuck, 2000)

With the registration of GAUCHO® in Canada in 1998 and the U.S. in 1997 it was important to determine whether honey bee colonies used to pollinate the massive expanses of canola grown in these two countries would be negatively impacted by this new seed treatment. The objectives of this study were to: 1) determine whether GAUCHO® and TI-435, a new unregistered second generation chloronicotinyl, seed treated canola grown in Ontario, Canada and Minnesota, USA had any effect on the honey producing ability, and foraging and hive behavior of honey bees; and, 2) determine whether pollen and nectar collected by honey bees from seed treated canola blossoms contained residues of imidicloprid plus two metabolites, olefin-imidicloprid

and hydroxy-imidicloprid, or TI-435 above the "no observable adverse effect concentration" (NOAEC) of 20ppb (0.02ppm) (Schmidt and Schmuck, 2000).

MATERIALS AND METHODS

Ontario, Canada Study

Agronomic Information. The experiment was conducted at three locations in southern Ontario:

- **Site 1** -Windy Acre Farms, Grand Valley, Ontario (GPS = 43^{0}55'N, 80^{0}15'W)
- **Site 2** - Windy Acre Farms, Grand Valley, Ontario (GPS = 43^{0}47'N, 80^{0}13'W)
- **Site 3** - located 2km south of Elora Research Station (GPS = 43^{0}39'N, 80^0 25'W).

Sites 1 and 2 were approximately 3.0 km from each other. Site 3 was located 47.0 and 44.0 km southwest of Sites 1 and 2, respectively.

The soil at the three sites was identified as "loam-textured" by the University of Guelph's Laboratory Services Department.

At each site a 1 ha planting of spring canola, *Brassica napus* var. #46865, was established. Seed, for both the Ontario and Minnesota studies, was provided by Pioneer Hi-Bred Production Ltd.-Plant Breeding Division (Georgetown, Ontario) and treated by the Gustafson Partnership (Guelph, Ontario). Weather constraints and differences in regional microclimates resulted in the fields being seeded on different dates. On 3 May, Site 3 (the most southerly experimental plot) was seeded, using a Hege Model 80 - small plot planter, with AMS 13945 (TI-435 (Bayer Corp.), 600Al/ 100 kg seed) plus RS Vitavax Fungicide (3.3% carbathiin + 6.6% thiram (Uniroyal Chemical Co.)), treated seed at 6 lbs./acre. On 16 May, Site 1 was seeded, using a Case IH 500 Minimum till drill seeder, with Vitavax RS Flowable (3.3% carbathiin, 6.6% thiram and 50% lindane (Uniroyal Chemical Co.), 2250ml of formulated product/100kg seed) treated seed at 6-7 lbs./acre. On the same day, Site 2 was seeded using a Case IH Minimum till drill seeder, with GAUCHO® (1000g AI/100 kg seed (Bayer Corp.)) plus RS Vitavax Fungicide (see above) canola seed at 6-7 lbs./acre.[1] All test sites were treated with the herbicide TREFLAN EC® (2.3 L/ha (Dow AgroSciences Canada Inc.)) prior to planting. Sites 1 and 2 were fertilized prior to planting with composted chicken manure (100 lbs N/acre). Site 3 was fertilized on 2 May with a mixed 20-10-10 fertilizer (300kg/ha).

Approximately 18 and 30 days after planting emergence rates were determined by counting the number of canola seedlings per 1metre of row in 10 random locations throughout each test field. An average emergence rate was computed for each of the

[1] Seed treated with the 3 products were sent to Bayer AG-Monheim for analysis. Two separate analyses of 2 separate sub-samples indicated: Lindane content = 1492 g a.i./ 100 kg (99.5%); Imidicloprid content = 976 g a.i./ 100 kg (97.6%); TI-435 content = 606 g a.i./100 kg (100.9%).

three sites (Table 1). Unseasonally cool temperatures and heavy rainfall following planting was likely responsible for the low emergence rates at Site 1 (Vitavax) and Site 2 (Gaucho) compared to Site 3.

Table 1. Emergence rates for seed treated canola at three test sites in southern Ontario *during the 2000 field season.*

Treatments	Seeding Date	Collection Date	Percent Emergence	Collection Date	Percent Emergence
Vitavax RS (Site 1)	16-05*	08-06	17.1	20-06	24.8
Gaucho (Site 2)	16-05*	08-06	29.2	20-06	28.2
TI-435 (Site 3)	3-05	22-05	65.3	08-06	62.6

* Planting delayed to 16-05 due to unseasonal weather.

During the 2000 field season, flea beetle pressure in southern Ontario was excessive on canola. Flea beetle damage estimates were undertaken at all sites. This was not part of the original protocol. Flea beetle damage estimates were recorded by determining the percent damage to new leaves and the entire plant, on 15 randomly selected plants in a marked 4-meter row from 5 random locations throughout each test field.

Meteorological and soil moisture data was recorded from planting to the end of the bloom period for canola using the Elora-Fergus Monthly Climatological Summaries (**Appendix 1**). To determine soil moisture 0 to 15 cm cores were collected twice over the course of the experiment, from six locations (in an E-shaped pattern) throughout each test field. Soil moisture measurements were calculated for each of the two sampling dates and then averaged for each of the three test sites (**Appendix 2**).

Honey Bee Information. When 20% of the canola blossoms were opened, 4 two-super colonies of honey bees containing sister queens of approximately the same age were placed at the easterly edge of each of the three sites. With hive entrances facing east, colonies were placed 1 m apart, on a 2 m x 5 m sheet of white cloth that was weighed down at each corner with a brick (Figure 1).

Prior to placement at each site, colonies were equalized for strength, food stores, sealed brood and adult bees (covering at least 10 frames). To determine if changes in colony strength occurred over the course of the experiment, the total amount of sealed brood and frames of adult bees were again estimated prior to colony removal at the end of the canola bloom period.

Data collection was consistent for each of the test sites, however, due to the differences in seeding dates, data collection dates varied from Grand Valley (Site 1 and 2) to Elora (Site 3) (Table 2). Over the course of the experiment all hives were weighed

weekly using a tripod scale (Figure 2). The scale was "tared" prior to weighing each hive. Increases or decreases in honey super and/or brood super weights were assumed to be related to either nectar collection or consumption respectively. Therefore, changes in colony weights were used to determine the total honey production per colony.

Table 2. Time line for data collection at each of the test sites in southern Ontario during the 2000 field season.

Operation/ Data Collected	Vitavax® **Grand Valley – Site 1**	GAUCHO® **Grand Valley – Site 2**	TI-435 **Elora – Site 3**
Colonies in	07-04**	07-04	06-26
Hive weight	07-04, 07-11, 07-17, 07-25, 08-02	07-04, 07-11, 07-17, 07-25, 08-01	06-26, 07-06, 07-11, 07-20
Mortality	07-05, 07-07, 07-09, 07-11, 07-13, 07-15, 07-17, 07-19, 07-21, 07-23, 07-25, 07-27	07-05, 07-07, 07-09, 07-11, 07-13, 07-15, 07-17, 07-19, 07-21, 07-23, 07-25, 07-27	06-27, 06-29, 07-01, 07-03, 07-05, 07-07, 07-09, 07-11, 07-13, 07-15, 07-17, 07-20
Foraging activity	07-05, 07-07, 07-10, 07-11, 07-13, 07-15, 07-17, 07-19, 07-21, 07-23, 07-25, 07-27	07-05, 07-07, 07-10, 07-11, 07-13, 07-15, 07-17, 07-19, 07-21, 07-23, 07-25, 07-27	06-27, 06-29, 07-01, 07-03, 07-05, 07-07, 07-09, 07-10, 07-13, 07-15, 07-17
Hive behavior	07-05, 07-07, 07-09, 07-11, 07-13, 07-15, 07-17, 07-19, 07-21, 07-23, 07-25, 07-27	07-05, 07-07, 07-09, 07-11, 07-13, 07-15, 07-17, 07-19, 07-21, 07-23, 07-25, 07-27	06-27, 06-29, 07-01, 07-03, 07-05, 07-07, 07-09, 07-11, 07-13, 07-15, 07-17, 07-20
Sealed brood	07-04, 08-02	07-04, 08-01	06-26, 07-20
Frames of adult bees	07-04, 08-02	07-04, 08-01	06-26, 07-20
Nectar	07-10, 07-19	07-10, 07-19	07-03, 07-10
Pollen	07-11, 07-21	07-11, 07-17	07-05, 07-11
Colonies out	08-02	08-01	07-20

** month/day

The number of dead bees found on the white sheet surrounding each colony was recorded and removed every other day (Figure 3).

To determine honey bee foraging activity, 6 x 1 m² collection plots were placed in each of the test fields. At each site, a collection plot was placed at each corner and two were placed 4 m into the field directly behind the colonies. The amount/type of foraging activity was determined by recording the number of bees entering each collection site (per 1 min interval), while noting the type of foraging activity which they performed (landing on flowers no nectar or pollen collection; landing with pollen and/or nectar collection). To better the chance of observing foraging activity data collection alternated between morning and afternoon at each site.

Honey bees were monitored for abnormal behavior (aggressiveness, convulsiveness, or other erratic behavior) while foraging in the field and as they returned to the hive. For each hive, behavior of foragers (returning to the hive) was monitored and recorded every other day for 2 minutes intervals.

Statistical Analysis. All honey bee information data were subjected to ANOVA and Fisher's protected LSD test using Statistix® software.

Pollen and Nectar Collection Information. Pollen and nectar samples were collected twice (7 and 14 days after placement in the field) from each hive at all of the three test sites. Prior to nectar collection (usually the day before) one empty hive frame was place in each of the colonies to be sampled. At collection time, liquid nectar (5g/hive) samples in these new frames were collected using a pipette with a disposable tip (Figure 4). Nectar from each of the 4 colonies at a site was pooled. Comparably, pollen collection devices were placed on colonies one day prior to collection. Pollen samples (3g/hive) were simply scooped out of pollen tray in each of the pollen collection devices. Pollen from each of the 4 colonies at a site was pooled. All nectar and pollen samples were placed into marked 60ml pre-cleaned amber jars and maintained in a freezer at -24^{0}C until they were shipped to Enviro-Test Laboratories (Edmonton, Alberta) for residue analysis conducted in compliance with GLP guidelines according to EPA-FIFRA section 40 CFR part 160 (Oct. 16, 1989).

Minnesota, U.S.A., Study

Agronomical Information. The experiment was conducted at the Rosemount Research and Outreach Center, Dakota County, Minnesota, 44°N., 93°W. Specifically, the locations of each site were as follows, and can be seen on the map in the **Appendix 3**:

- **Site 1 -control**: Section 28, range 19W, Township 115N.
- **Site 2 Gaucho**: Section 35, range 19W, Township 115N.
- **Site 3 TI-435**: Section 3, range 19W, Township 114N.

Sites 1 and 2 were 3 km from each other. Site 3 was located 4.5 km from site 2 and 7.5 from site1. There were no flowering plants in the area, which was predominantly planted with corn, soybeans, and peas.

The soil at the three sites was identified as Waukegan Silt Loam by the University of Minnesota Soils Department.

At each plot, a 1 ha planting of *Brassica napus* var #46865 was planted, as described in the Methods section for Ontario, with the following modifications: All sites were seeded on 16 May, 2000 with a Tye No-Till Drill at 4.5 lbs/acre (5 kg/ha), spaced 7 inches (17.5 cm) apart. The land was chisel-plowed in the fall, and a field cultivator was used in the spring. All sites were treated with TREFLAN® (PrePlant, Inc.) at 24 oz/acre prior to planting.

Bloom initiated between 22-24 June at all sites. Emergence rates were determined on 26 June, 2000 (two days before the bees were brought into the sites) by counting the number of canola plants per 1 meter of row in 10 random locations throughout each site (Table 3). In Site 2, the Gaucho treated site, an additional 10 locations (20 total) were counted because of the anomalous growth of canola at this site (see paragraph below.

Table 3. Emergence rates for seed treated canola at three test sites in Minnesota during the 2000 field season.

	Seeding Date	Collection Date	Percent Emergence
SITE 1	16 May	26 June	15.6
SITE 2	16 May	26 June	20.0 *
SITE 3	16 May	26 June	32.7

* Average of 20 x 1-m rows

It is important to note that although the emergence rate of the plants at Site 2 was similar to the rate at Site 1, the majority of the plants on Site 2 did not develop normally, particularly in the center of the field. Many of the plants were stunted in the center of the field, relative to the far edges. During the course of the study, many of the plants in the center of the field died, while others bloomed although the total plant height was stunted. When these plants in the center did bloom, approximately 7-10 days after the edge rows, the bees did visit the blossoms normally. After inquiry to the Rosemount station planting supervisor as to why the plants emerged but were stunted in the field, we learned that the site had been treated with HORNET® (herbicide) in 1999 and the carryover in the soil negatively affected the growth of the canola. Unfortunately, the records of this herbicide treatment were not consulted prior to planting the canola in 2000.

Meteorological data were monitored by the Rosemount Research and Outreach Center staff, and are included in **Appendix 3** Additional weather data (cloud cover, wind speed) for the St. Paul (closest weather station) area can be obtained from weather records at http://www.wxusa.com/MN/

Honey Bee Information. On 28 June, when approximately 20% of the canola blossoms were open at each site, four honey bee colonies (each in two "deep" standard boxes) were placed at the edge of each site, all facing south. The colonies contained sister queens that were reared in MN in mid-May, and were introduced into the experimental colonies in early June. On 26 June (prior to placing the colonies at the sites) the colonies were equalized for adult bee population, sealed brood, and food (nectar and pollen stores). To determine if colony strength changed over the course of the experiment, sealed brood areas and adult populations were measured again at the end of the bloom. Sealed brood area was measured using a wire grid divided into 3cm

squares and adult bee populations were estimated by counting the number of frames covered by adult bees.

To approximate honey yield, the colonies were weighed weekly using a battery operated digital scale (type****) that weighed up to 200 lb (90 kg). The change in colony weight (difference between the final and initial colony weights) was calculated, and averaged for the four colonies in each site. This difference in weight was assumed to be due to increase in honey stored in the colonies.

To measure adult bee mortality, a 2m x 5m white sheet was secured on the ground in front of the colonies and the number of dead bees was counted every other day during the bloom (**Table 4**).

Foraging activity was determined in 6 x 1m plots as described in the Ontario methods section, with one exception. Because few bees were noted landing on flowers without collecting pollen, nectar, or both, this measure was not taken. As in Ontario, foraging activity data collection alternated between morning and afternoon at each site.

Honey bees were monitored for abnormal behavior (any convulsions, aggressiveness, or other erratic behavior) in the field at the hive entrances for 2 minute intervals every other day during the bloom.

Table 4. Time line for data collection at each site in Minnesota, US. All data was collected on same day for each site.

Data Collected	**All sites**
Colonies in	June 28
Hive weight	June 28; July 5, 12, 20
Mortality, Foraging activity, hive behavior	June 28, 30; July 3, 5, 7, 10, 12, 14, 17, 20
Measurements of sealed brood, frames adult bees	June 28; July 25
Nectar and pollen samples	July 6, 12 and 20 *
Colonies out	July 28

* see section on Pollen and nectar collection, below

Statistical Analysis. All honey bee information data were subject to ANOVA and Tukey's studentized range test (HSD) using SAS statistical software.

Pollen and Nectar Collection Information. Pollen and nectar samples were collected from all sites on 6 and 12 July (8 and 14 days after placement in the field). An extra sample of nectar and pollen was collected on 20 July at the control and GAUCHO sites (Sites 1 and 2, respectively) because the plants in the center portion of the GAUCHO site, which were stunted, bloomed about 1 week later than the surrounding edge plants. A corresponding extra sample was collected from the VITAVAX RS FLOWABLE site, but the TI-435 site was done blooming at that time.

The method of collection of nectar samples from the colonies was the same as described in the Ontario Methods section. However, the method of collecting pollen samples differed in the following regard: Pollen traps were placed on each colony one day prior to collecting samples, and samples were scooped out of the pollen trays in each of the pollen traps, and frozen at –20°C. However, because the colonies collected a variety of pollens from different plants, the pollens were later sorted in the lab by color (while still frozen), and were compared to representative color samples of pollen collected on bees specifically visiting canola. Samples of the same color were analyzed using standard pollen analysis techniques in our lab to identify the pollen to genus. Only pollen from the genus *Brassica*, presumed to be solely from canola blossoms was sent for analysis. All pollen and nectar samples were shipped to Enviro-Test Laboratories (Edmonton, Alberta) for residue analysis conducted in compliance with GLP guidelines according to EPA-FIFRA section 40 CFR part 160 (Oct. 16, 1989).

Residue Analysis of Samples from Ontario and Minnesota

The objectives of the residue analysis section of this study were to determine the limit of quantification (LOQ) for the analytes, the limit of detection (LOD) for imidicloprid, validate the methods and analyze the pollen and nectar samples using the following Bayer Methods: 1) "Residue Analytical Method 00554 for the Determination of Residues of TI-435 in Nectar, Honey, Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS-detection" - Bayer Report Number MR-812/98; and, 2) "Residue Analytical Method for the Determination of Residues of Imidicloprid, Hydroxy-Metabolite and Olefin-Metabolite in Nectar, Honey,Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS Detection" - Bayer Report Number MR-551/98.

The nectar and pollen samples were extracted by polytron blending with methanol and water, concentrated using a roto evaporator, and extract eluted through a ChemElute (CE 1020 column). The pollen samples were passed through a silica gel cleanup column. The samples were then analyzed by High Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry (HPLC-MS/MS). Quantification was accomplished by using weighted (1/x) linear regression from a eight to nine point calibration curve.

The method validation consisted of two control matrix samples, 3 samples spiked at LOQ of 0.5 ppb for TI-435 and 1.0 ppb for imidicloprid and metabolites, two samples at five times LOQ, and two samples at ten times LOQ. The calibration curve values were acceptable for all compounds. The average recoveries for the validation were good for all analytes. TI-435 in pollen was 92% and in nectar 86%. The average for the validation for imidicloprid, hydroxy and olefin metabolites in pollen was 108%, 96% and 96%, respectively. The averages for the validation for imidicloprid, hydroxy and olefin metabolites in nectar was 98%, 92% and 102%, respectively. See **Appendix 4** - Tables 1-4 for validation results and recoveries.

The control samples were verified to be free of interferences at the retention times of interest. There were no perceived problems with cross-talk, matrix interferences, or analytical standard degradation since the recoveries were good for the validation.

There were no detections of TI-435 or imidicloprid metabolites below the LOQ. Some samples had detection of imidicloprid at <LOQ. The LOD was estimated at 0.0003 ppm which was approximately 1/3 of the LOQ and had a s/n of >5:1.

RESULTS

Honey Bee Information (Ontario and Minnesota)

Brood Measurements. An overall comparison of brood measurements at sites in Canada and the U.S. indicate that colony populations in Minnesota were larger than those in Ontario (Table 3). Although colony strength varied between countries, the results indicate that there was no significant difference in the amount of sealed honey bee brood in colonies exposed to any of the canola treatments (Table 5).

Foraging Activities. There were no significant differences in the nectar and pollen foraging activity of colonies exposed to any of the canola treatments in Ontario and Minnesota (Table 6). In Ontario, the number of bees visiting canola blossoms without collecting nectar and pollen, was not significantly different between treatments (Table 6). Honey bees visiting canola blossoms was not recorded in Minnesota because very few bees visited blossoms without foraging for nectar or pollen. The number of nectar and pollen collectors at sites in Minnesota were slightly higher than those in Ontario. This is likely due to the larger colony populations present in the Minnesota colonies. Ontario colonies had more nectar than pollen collectors (Table 6). Colonies with lower brood amounts tends to collect less pollen (ie. less brood = lower protein requirements) and this may have been the case for the test colonies in Ontario.

Table 5. Mean amount of sealed honey bee brood in colonies located at canola test sites in Ontario (measured on 26 June and 27 July, 2000) and Minnesota (measured on 24 July, 2000)

Treatments	Sealed Brood (cm^2) Ontario	Sealed Brood (cm^2) Minnesota
Vitavax RS Flowable®	**3285a***	**5368a****
Gaucho®	**2735a**	**5200a**
TI-435	**2939a**	**5536a**

* Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test).
**Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)).

Table 6. Mean number of honey bees visiting canola blossoms (1 m^2 for 1 min), and collecting nectar and pollen during the entire bloom period in Ontario (26 June-27 July, 2000) and Minnesota (28 June-24 July, 2000).

Treatments	Foraging Activity (No. of Bees)					
	Ontario			Minnesota		
	Nectar	Pollen	Visit	Nectar	Pollen	Visit
Vitavax RS Flowable®	**5.3b***	**0.8b**	**1.1a**	**4.1a****	**3.3a**	**na*****
Gaucho®	**8.4ab**	**0.9b**	**2.8a**	**6.8a**	**2.7a**	**na**
TI-435	**15.3a**	**3.0a**	**2.7a**	**4.0a**	**1.5a**	**na**

*Within columns of Ontario data, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test).

** Within columns of Minnesota data, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Tukey's studentized range test (HSD)).

*** Data not available.

If we do not look at specific foraging activities, the results indicate that there were significantly more honey bees foraging on canola grown from seed treated with TI-435 compared to VITAVAX® in southern Ontario. There was, however, no significant difference between the number of bees foraging on TI-435 versus GAUCHO® (Table 7) or GAUCHO® versus VITAVAX® (Table 7). In Minnesota, there was no significant difference between the number of bees foraging at any of the sites (ie. Only the portions of the GAUCHO® treated site that bloomed normally were observed - see Materials and Methods for Minnesota) (Table 7).

Table 7. Mean number of forager honey bees observed (1 m^2 for 1 min) during the entire canola bloom period in southern Ontario (26 June-27 July, 2000) and Minnesota (28 June-24 July, 2000).

Treatments	Mean Number of Forager Bees Ontario	Mean Number of Forager Bees Minnesota
Vitavax RS Flowable®	**2.4b***	**3.7a****
Gaucho®	**4.0ab**	**4.8a**
TI-435	**7.0a**	**2.7a**

*Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test).

**Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)).

Bee Mortality. The results indicate that bee mortality in Ontario and Minnesota was not significantly different for any of the seed treatments tested (Table 8). Bee mortality was higher in Minnesota compared to Ontario. This may be a result of the larger colony populations present in hives in Minnesota as is indicated from the brood measurements (Table 5).

Table 8. Mean number of dead honey bees collected outside colonies located at canola test sites in southern Ontario and Minnesota (June and July, 2000).

Treatments	Mean Bee Mortality (No. bees) Ontario	Mean Bee Mortality (No. Bees) Minnesota
Vitavax RS Flowable®	**14.0a***	**148.1a****
Gaucho®	**20.6a**	**112.4a**
TI-435	**19.0a**	**101.5a**

*Within each column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test)
**Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)).

Honey Production. The results from Ontario and Minnesota indicate that there was no significant difference in honey yields of colonies exposed to canola treated with any of the test products (Table 9). The mean honey yield for the 11 (Note: One colony at the TI-435 site became queenless during the study due to colony manipulations. Therefore, honey yields are based on N= 12-1) test colonies in Ontario was 40.7 kg for one month (26 June - 27 July). The 5-year (1995-1999) annual honey production average for colonies in Ontario is 51.2 kg. Honey yields in Minnesota were substantially lower than those in Ontario. This may have been a result of lower nectar yield of canola due to unseasonal weather and poor soil conditions. Annual average honey production for colonies in Minnesota is approximately 40.0 kg.

Table 9. Mean honey yields of colonies located in seed treated canola test sites in southern Ontario (26 June-27 July, 2000) and Minnesota (28 June-24 July, 2000) throughout the entire bloom period.

Treatments	Mean Honey Yields of Colonies (kg) Ontario	Mean Honey Yields of Colonies (kg) Minnesota
Vitavax RS Flowable®	**42.5a***	**9.2a****
Gaucho®	**40.8a**	**11.1a**
TI-435	**38.2a**	**8.0a**

*Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test).
**Within this column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)).

Bee Behavior. No aggressive, convulsive, erratic or any other kind of abnormal bee behavior was observed at any of the test sites in southern Ontario and Minnesota throughout the entire duration of the study.

Additional Information

Flea Beetle Damage Assessments. In Ontario, flea beetle damage assessed on the basis of whole plant and new leaf damage was observed to be substantially lower for TI-435 compared to VITAVAX and GAUCHO. VITAVAX appeared to be more effective than GAUCHO in controlling flea beetles (Table 10).

Table 10. Flea beetle damage assessments on seed treated canola sites in southern Ontario during the Summer 2000 field season.

Treatments	Mean Flea Beetle Damage (%)			
	June 11, 2000		June 20, 2000	
	Whole Plant	New Leaf	Whole Plant	New Leaf
Vitavax RS Flowable®	**27.0**	**16.0**	**44.0**	**5.5**
Gaucho®	**57.0**	**14.0**	**64.4**	**13.5**
TI-435	**0.7**	**0.0**	**2.3**	**0.1**

Residue Analysis Results (Ontario and Minnesota)

Residues of TI-435 were detected in the pollen samples from Ontario and Minnesota (Table 11). Samples from the Elora Research Station Site in Ontario had residues of TI-435 at 3.0 ppb (07/05/00) and 1.6 ppb (07/11/00) compared to the control that had no quantifiable residues of the product (<0.5 ppb LOQ). The pollen samples from the Rosemount Site in Minnesota had residues of TI-435 at 2.3 ppb (07/06/00) and 2.8 ppb (07/12/00) compared to the control that had no quantifiable residues of the product (<0.5 ppb LOQ).

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Table 11. Residue results for TI-435 in canola pollen at locations in Ontario (Elora) and Minnesota (Rosemount).

Site	Days After Hive Placement	Results (ppb)
Elora	**7**	**3.0**
Elora	**14**	**1.6**
Control (Cdn.)	**/**	**<0.5**
Rosemount	**7**	**2.3**
Rosemount	**14**	**2.8**
Control (US)	**/**	**<0.5**

Residues of TI-435 were detected in the nectar samples from Ontario and Minnesota (Table 12). Samples from the Elora Research Station Site in Ontario had residues of TI-435 at 3.7 ppb (07/03/00) and 0.9 ppb (07/10/00) compared to the control that had no quantifiable residues of the product (<0.5 ppb LOQ). The nectar samples from the Rosemount Site in Minnesota had residues of TI-435 at 1.1 ppb (07/06/00) and 1.0 ppb (07/12/00) compared to the control that had no quantifiable residues of the product (<0.5 ppb LOQ).

Table 12. Residue results for TI-435 in canola nectar at locations in Ontario (Elora) and Minnesota (Rosemount).

Site	Days After Hive Placement	Results (ppb)
Elora	**7**	**3.7**
Elora	**14**	**0.9**
Control (Cdn.)	**/**	**<0.5**
Rosemount	**7**	**1.1**
Rosemount	**14**	**1.0**
Control (US)	**/**	**<0.5**

None of the samples of pollen from Gaucho treated canola fields in Ontario and Minnesota contained quantifiable levels of the associated hydroxy (<1.0 ppb LOQ) and

olefin (<1.0 ppb LOQ) metabolites (Table 13). The control sample also had no quantifiable residues of the metabolites (<1.0 ppb LOQ). Samples from the Grand Valley Site in Ontario had no quantifiable residues of imidicloprid at either of the collection dates - <1.0 ppb LOQ (07/11/00) and <1.0 ppb LOQ (07/17/00). The pollen samples from the Gaucho field at the Rosemount Site in Minnesota had residues of imidicloprid at 7.6 ppb (07/12/00) and 4.4 ppb (07/20/00) compared to the control sample that had no quantifiable residues of imidicloprid (<1.0 ppb LOQ).

Table 13. Residue results for imidicloprid and associated hydroxy and olefin metabolites in canola pollen in Ontario (GVF) and Minnesota (Rosemount).

Site	Days After Hive Placement	Analyte	Results (ppb)
GVF	7	O,H,I*	<1.0
GVF	14	O,H,I	<1.0
Control (Cdn.)	/	O,H,I	<1.0
Rosemount	7	O,H	<1.0
		I	7.6
Rosemount	14	O,H	<1.0
		I	4.4
Control (US)	/	O,H,I	<1.0

* O = olefin; H = hydroxy; I = imidicloprid

None of the samples of nectar from Gaucho treated canola fields in Ontario and Minnesota contained quantifiable levels of the associated hydroxy (<1.0 ppb LOQ) and olefin (<1.0 ppb LOQ) metabolites (Table 14). In addition, samples from the Grand Valley site in Ontario had no quantifiable residues of imidicloprid at either of the collections dates - <1.0 ppb LOQ (07/11/00) and <1.0 ppb LOQ (07/17/00). Imidicloprid residues were found in the nectar samples from the Gaucho field at the Rosemount Site in Minnesota however the amounts were < LOQ (1.0 ppb) and > LOD (0.3 ppb) - 0.81 ppb (07/12/00) and 0.60 ppb (07/20/00).

Table 14. Residue results for imidicloprid and associated hydroxy and olefin metabolites in canola nectar in Ontario (GVF) and Minnesota (Rosemount).

Site	Days After Hive Placement	Analyte	Results (ppb)
GVF	7	O,H,I*	<1.0
GVF	14	O,H,I	<1.0
Control (Cdn.)	/	O,H,I	<1.0
Rosemount	7	O,H	<1.0
		I	0.81
Rosemount	14	O,H	<1.0
		I	0.60
Control (US)	/	O,H,I	<1.0

*O = olefin; H = hydroxy; I = imidicloprid
< LOQ (1.0 ppb) and > LOD (0.3 ppb)

SUMMARY

Honey Bee Information

It is evident from the results of the large scale commercial study conducted in southern Ontario and Minnesota that none of the canola seed treatments tested, VITAVAX RS FLOWABLE®, GAUCHO® or TI-435, had statistically significant impacts on brood, foraging activity, bee mortality, honey yield or bee behavior.

Residue Analysis Information

Although some samples of pollen and nectar collected from canola seed-treated with TI-435 and GAUCHO (imidicloprid + metabolites = olefin and hydroxy) contained residues of these products, all levels detected were substantially below the NOAEC of 20 ppb. The fact that the residue levels were below the NOAEC supports our results that indicate no negative impact on bee behaviour and hive variables (ie. sealed brood, honey yield) for any of the colonies exposed to canola seedtreated with the test products.

ACKNOWLEDGEMENTS

Ontario

We would like to extend our sincere appreciation to the Ontario Canola Grower's Association and specifically to [redacted] Windy Acre Farms, Grand Valley) for the allowing us the use his land. His assistance throughout the study was definitely an asset. To the technical and farm staff at the University of Guelph - Elora Research Station and the Townsend House Bee Lab we also extend a note of gratitude. We also thank Pioneer Hi-Bred Production Ltd. - Plant Breeding Division, Georgetown, Ontario and Gustafson Partnership, Guelph, Ontario for providing and treating the canola seed, respectively. Last, but certainly not least, to [redacted] hank you for standing and watching the canola grow and the bees buzz.

Minnesota

We would like to thank the staff at the Rosemount Research and Outreach Center, Dakota County, Minnesota for their assistance throughout this project.

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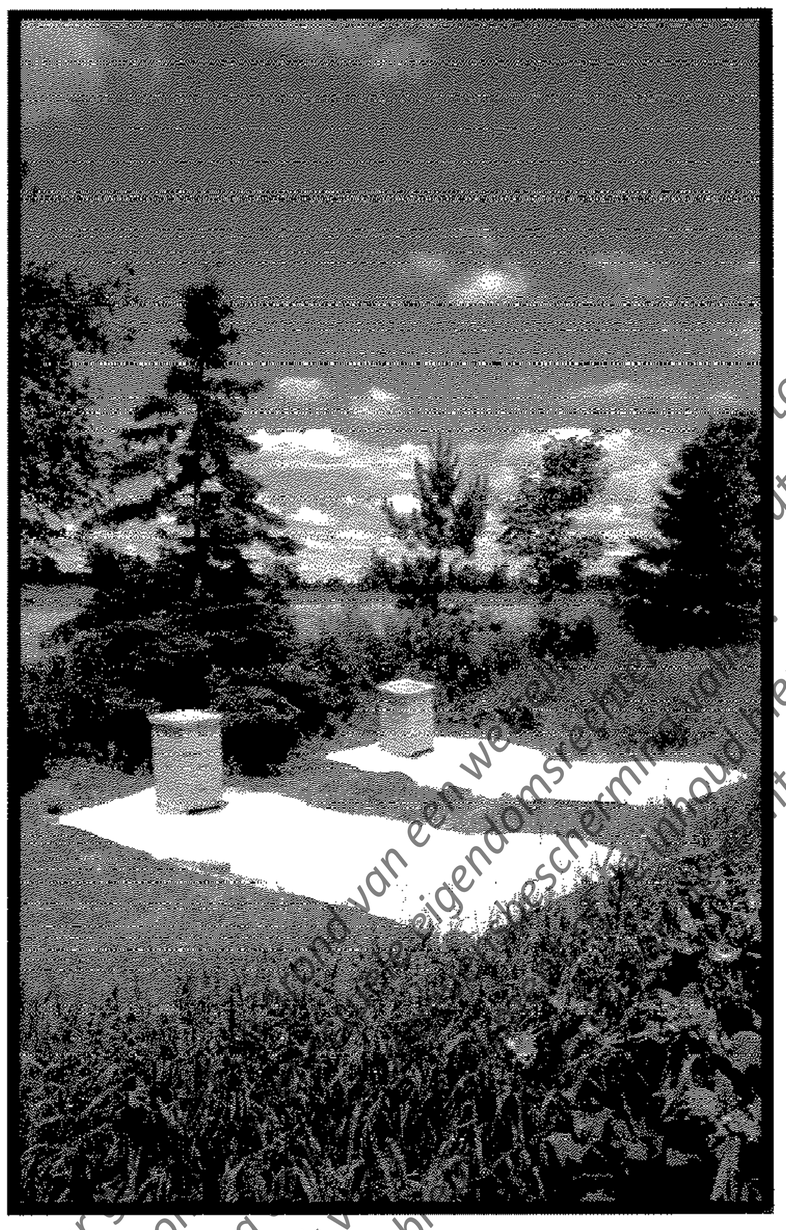

Fig. 1. Each colony was placed on top of a 2 m x 5 m white sheet.

Fig. 2. Colonies were weighed weekly using a tripod scale.

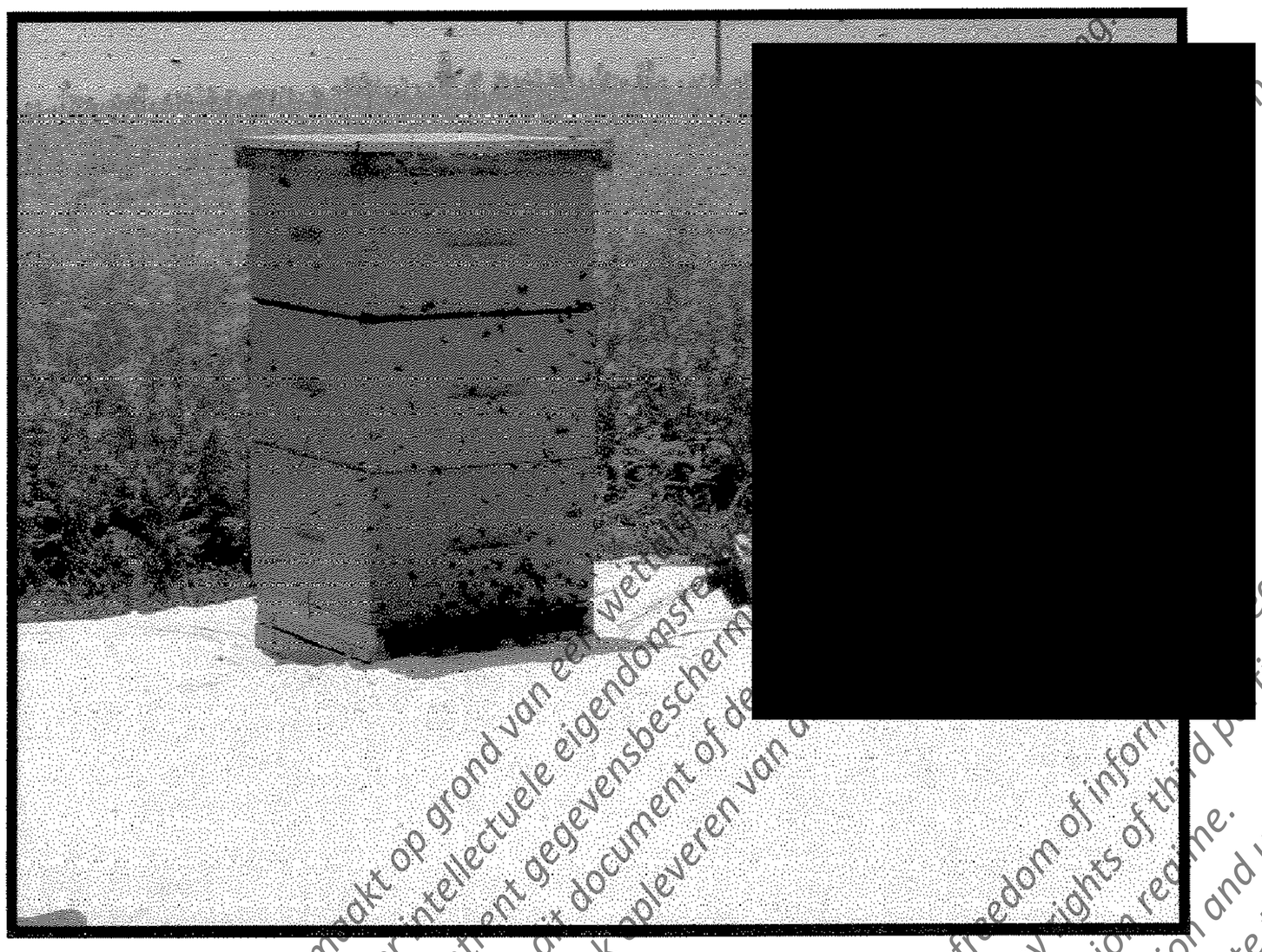

Fig. 3. Dead bees found outside the colonies were counted and removed every other day.

Fig. 4. Nectar was collected from frames using a pipette with a disposable tip.

-APPENDIX 1-

Fergus-Elora Meteorological Records

(See attached)

110403

MONTHLY CLIMATOLOGICAL SUMMARY for MAY. 2000

NAME: Ontario CITY: Fergus STATE: Ontario
ELEV: 303 LAT: 43 40 48 N LONG: 80 19 21 W

TEMPERATURE (°C), RAIN (mm), WIND SPEED (km/h)

DAY	MEAN TEMP	HIGH	TIME	LOW	TIME	HEAT DEG DAYS	COOL DEG DAYS	RAIN	AVG WIND SPEED	HIGH	TIME	DOM DIR
1	9.5	13.8	10:40a	6.3	12:00m	8.2	0.0	3.2	4.2	32.2	9:30p	SW
2	9.5	15.3	3:00p	3.8	12:00m	8.8	0.0	0.0	3.6	20.9	5:40a	NNE
3	11.3	20.3	3:35p	0.2	5:20a	8.1	0.0	0.0	2.3	16.1	9:40a	SW
4	15.6	23.6	3:55p	5.2	4:35a	3.9	0.0	0.0	4.4	30.6	4:55p	SW
5	20.4	26.5	2:40p	15.3	5:40a	0.0	2.6	0.0	3.3	27.4	10:15a	WSW
6	21.3	27.7	2:20p	13.3	5:30a	0.0	2.2	0.0	3.9	27.4	12:15p	SW
7	21.5	26.7	3:10p	16.4	2:30a	0.0	3.3	0.2	4.9	37.0	2:05p	SW
8	20.7	24.4	2:55p	15.3	5:25a	0.0	1.6	0.2	5.0	24.1	6:30p	SW
9	18.0	22.6	12:40p	10.6	12:00m	1.7	0.0	15.2	4.3	35.4	3:35a	SW
10	10.9	15.2	11:10a	4.8	12:00m	8.3	0.0	6.0	3.5	27.4	2:40p	SSW
11	10.2	17.2	1:05p	2.8	5:10a	8.3	0.0	4.4	2.4	20.9	11:45p	SW
12	14.3	23.8	3:50p	9.3	12:10a	1.7	0.0	30.8	5.9	29.0	3:50a	ESE
13	14.5	18.9	3:35p	8.5	11:50p	4.6	0.0	2.2	5.8	38.6	2:40p	SW
14	7.5	9.9	5:10p	4.4	11:00p	11.1	0.0	0.0	5.1	32.2	2:15p	SW
15	7.9	13.3	3:15p	1.9	2:55a	10.7	0.0	0.0	2.6	22.5	12:25p	SW
16	7.4	12.9	1:55p	1.7	4:40a	11.0	0.0	2.8	1.6	32.2	2:00p	SW
17	13.0	20.5	3:40p	6.6	5:45a	4.7	0.0	1.0	2.7	19.3	4:45p	ESE
18	11.2	15.4	7:05a	7.1	10:45p	7.0	0.0	19.2	4.7	35.4	12:50p	NE
19	5.7	10.8	4:10p	2.1	10:00a	11.8	0.0	2.2	8.3	35.4	12:40p	ENE
20	8.7	12.9	3:30p	3.3	12:05a	10.2	0.0	0.0	4.1	22.5	5:00a	ENE
21	12.0	17.3	2:40p	7.0	3:15a	6.1	0.0	0.0	1.0	12.9	1:55p	SSW
22	12.3	19.2	4:30p	6.8	4:15a	5.3	0.0	0.0	1.0	11.3	12:15p	ESE
23	11.4	14.8	8:00p	8.8	12:55a	6.5	0.0	10.2	2.0	16.1	10:15a	ESE
24	14.1	21.8	3:10p	7.8	5:30a	3.5	0.0	9.0	3.8	46.7	4:15p	SW
25	10.4	13.4	3:45p	6.7	11:10p	8.2	0.0	0.0	4.3	29.0	11:35a	SSW
26	10.3	15.9	5:00p	5.2	6:10a	7.7	0.0	0.0	3.4	22.5	12:20a	SSW
27	12.3	19.1	5:00p	5.7	4:45a	5.9	0.0	0.0	3.1	19.3	7:15p	NE
28	10.7	14.9	2:40p	6.9	4:05a	7.4	0.0	0.0	6.0	27.4	3:20p	ENE
29	12.6	18.8	2:45p	6.8	5:00a	5.5	0.0	0.0	3.9	19.3	11:20a	ESE
30	14.7	20.3	4:55p	7.9	5:20a	4.1	0.0	0.0	3.0	16.1	10:35a	SSW
31												
	12.7	27.7	6	0.2	3	180.1	9.7	106.6	3.8	46.7	24	SW

Max >= 32.0: 0
Max <= 0.0: 0
Min <= 0.0: 0
Min <= -18.0: 0
Max Rain: 30.80 ON 5/12/00
Days of Rain: 12 (> .2 mm) 11 (> 2 mm) 1 (> 20 mm)
Heat Base: 18.3 Cool Base: 18.3 Method: (High + Low) / 2

http://www.ontarioweather.com/station/NOAAM

110403

MONTHLY CLIMATOLOGICAL SUMMARY for JUN. 2000

NAME: Ontario CITY: Fergus STATE: Ontario
ELEV: 303 LAT: 43 40 48 N LONG: 80 19 21 W

TEMPERATURE (°C), RAIN (mm), WIND SPEED (km/h)

DAY	MEAN TEMP	HIGH	TIME	LOW	TIME	HEAT DEG DAYS	COOL DEG DAYS	RAIN	AVG WIND SPEED	HIGH	TIME	DOM DIR
1	15.4	20.5	6:25p	10.2	6:30a	2.9	0.0	0.0	0.7	9.7	9:15p	E
2	14.3	20.9	12:40p	9.6	11:50p	3.0	0.0	2.0	4.2	27.4	10:25a	SSW
3	12.9	19.2	4:50p	6.8	6:25a	5.3	0.0	0.0	3.2	24.1	2:00a	NE
4	13.4	19.8	3:05p	8.0	3:00a	4.4	0.0	0.0	2.5	19.3	8:10p	NE
5	10.2	12.6	5:35p	6.5	5:50a	8.8	0.0	0.8	6.9	29.0	12:00p	ENE
6	12.4	18.3	5:55p	8.6	12:05a	4.8	0.0	5.2	5.8	29.0	10:45a	ENE
7	15.9	23.3	4:00p	7.5	5:40a	2.9	0.0	0.0	2.5	30.6	2:55p	SSW
8	18.8	25.7	5:55p	13.6	6:00a	0.0	1.3	0.0	4.2	29.0	9:30a	SSW
9	19.2	25.8	6:05p	15.9	1:55a	0.0	2.6	2.8	2.1	22.5	1:10p	SSW
10	22.4	29.8	4:45p	13.3	5:40a	0.0	3.3	0.0	4.5	27.4	2:50p	SSW
11	20.3	25.0	12:30p	10.4	11:35p	0.6	0.0	7.6	3.8	24.1	3:05a	SSW
12	12.6	15.9	5:50p	10.0	3:10a	5.3	0.0	0.4	2.9	14.5	2:50a	E
13	12.6	15.2	3:30p	10.3	6:10a	5.6	0.0	42.0	5.9	24.1	6:50a	E
14	20.1	28.7	4:05p	13.3	1:45a	0.0	2.7	3.6	3.3	19.3	12:10a	SSW
15	19.6	23.3	3:05p	13.8	12:00m	0.0	0.2	0.8	5.5	37.0	1:00p	SSW
16	19.3	26.4	3:55p	12.8	4:10a	0.0	1.3	13.6	4.4	33.8	1:00p	SW
17	15.6	18.7	12:50p	9.8	10:50p	4.0	0.0	0.0	2.1	19.3	3:40p	SW
18	12.4	19.0	5:55p	8.7	9:00a	4.4	0.0	7.0	1.2	11.3	3:55p	ENE
19	15.7	22.3	2:35p	7.9	4:45a	3.2	0.0	0.8	2.2	20.9	12:35p	SSW
20	18.3	25.7	3:55p	11.4	5:40a	0.0	0.2	0.2	1.0	17.7	3:55p	W
21	20.1	24.9	4:05p	14.8	12:00m	0.0	1.6	5.4	4.4	27.4	4:15p	SSW
22	16.5	20.9	3:15p	12.7	2:45a	1.4	0.0	2.4	4.6	32.2	1:05p	SSW
23	17.8	24.0	4:35p	10.8	5:40a	0.9	0.0	0.0	1.3	11.3	12:05a	SSW
24	18.3	21.8	5:50p	14.1	4:45a	0.3	0.0	0.8	0.7	14.5	6:25p	ESE
25												
26												
27												
28												
29												
30												
	16.4	29.8	10	6.5	5	57.8	13.4	95.4	3.3	37.0	15	SSW

Max >= 32.0: 0
Max <= 0.0: 0
Min <= 0.0: 0
Min <= -18.0: 0
Max Rain: 42.00 ON 6/13/00
Days of Rain: 15 (> .2 mm) 9 (> 2 mm) 1 (> 20 mm)
Heat Base: 18.3 Cool Base: 18.3 Method: (High + Low) / 2

MONTHLY CLIMATOLOGICAL SUMMARY for JUL. 2000

NAME: Ontario CITY: Fergus STATE: Ontario
ELEV: 303 LAT: 43 40 48 N LONG: 80 19 21 W

TEMPERATURE (°C), RAIN (mm), WIND SPEED (km/h)

DAY	MEAN TEMP	HIGH	TIME	LOW	TIME	HEAT DEG DAYS	COOL DEG DAYS	RAIN	AVG WIND SPEED	HIGH	TIME	DOM DIR
1	18.9	25.4	3:25p	11.9	4:10a	0.0	0.4	0.0	2.9	24.1	2:50p	SSW
2	20.1	25.8	4:35p	13.5	5:25a	0.0	1.4	0.0	3.4	22.5	9:45a	SSW
3	20.2	24.4	3:50p	18.0	6:35a	0.0	2.9	2.0	0.9	12.9	4:30p	SSW
4	19.5	24.1	3:35p	12.4	12:00m	0.1	0.0	0.0	2.1	17.7	7:00a	ENE
5	17.2	23.6	3:00p	10.8	2:25a	1.1	0.0	0.0	3.3	29.0	3:25p	NE
6	15.6	21.9	1:55p	11.1	2:05a	1.8	0.0	0.0	2.7	19.3	5:45p	NE
7	15.2	20.9	4:50p	8.9	6:20a	3.3	0.0	0.0	3.6	25.7	12:05p	NE
8	16.4	22.8	3:35p	9.4	1:30a	2.2	0.0	0.0	0.8	16.1	10:45a	SSW
9	17.4	20.7	11:40p	13.3	5:25a	1.3	0.0	5.6	2.8	22.5	2:35p	SSW
10	19.5	23.4	3:10p	14.6	11:55p	0.0	0.7	0.2	2.6	19.3	3:25p	NE
11	17.7	23.5	1:50p	11.4	5:45a	0.8	0.0	0.0	2.8	27.4	1:25p	NE
12	19.1	25.6	3:40p	12.1	4:45a	0.0	0.6	0.0	1.9	11.3	5:55a	NNE
13	19.5	26.9	4:25p	13.6	3:25a	0.0	1.9	0.0	0.1	16.1	4:50p	SW
14	17.8	22.4	12:10p	14.3	1:00p	0.0	0.1	11.8	1.4	30.6	12:20p	SSW
15	17.2	23.2	1:40p	14.3	2:25a	0.0	0.5	3.6	1.8	16.1	6:00p	SW
16	17.6	22.4	4:10p	14.4	4:40a	0.0	0.1	0.2	1.6	20.9	2:55p	E
17	20.3	26.8	5:00p	16.6	12:05a	0.0	3.4	6.8	2.3	22.5	5:45p	SSW
18	13.6	17.8	12:05a	10.7	5:15a	4.0	0.0	0.0	3.7	22.5	9:00a	SSW
19	14.7	20.6	6:35p	10.6	2:35a	2.7	0.0	0.0	2.1	16.1	9:50a	NE
20	15.6	22.2	3:15p	7.9	5:55a	3.3	0.0	0.0	1.8	17.7	3:50p	SSW
21	15.3	20.1	12:55p	9.8	12:00m	3.4	0.0	0.0	2.1	16.1	12:15p	SSW
22	13.5	19.4	3:55p	7.8	5:15a	4.7	0.0	0.0	1.9	17.7	10:30a	SSW
23	14.3	20.6	3:15p	7.3	6:15a	4.3	0.0	0.2	1.6	17.7	12:40p	S
24												
25												
26												
27												
28												
29												
30												
31												
	17.2	26.9	13	7.3	23	32.8	12.0	30.4	2.2	30.6	14	SSW

Max >= 32.0: 0
Max <= 0.0: 0
Min <= 0.0: 0
Min <= -18.0: 0
Max Rain: 11.80 ON 7/14/00
Days of Rain: 5 (> .2 mm) 4 (> 2 mm) 0 (> 20 mm)
Heat Base: 18.3 Cool Base: 18.3 Method: (High + Low) / 2

MONTHLY CLIMATOLOGICAL SUMMARY for AUG. 2000

NAME: Ontario CITY: Fergus STATE: Ontario
ELEV: 303 LAT: 43 40 48 N LONG: 80 19 21 W

TEMPERATURE (°C), RAIN (mm), WIND SPEED (km/h)

DAY	MEAN TEMP	HIGH	TIME	LOW	TIME	HEAT DEG DAYS	COOL DEG DAYS	RAIN	AVG WIND SPEED	HIGH	TIME	DOM DIR
1	19.5	22.1	5:40p	17.4	12:00p	0.0	1.5	18.2	0.8	11.3	11:30a	S
2	20.2	23.6	11:55a	17.3	10:30p	0.0	2.2	2.2	2.9	20.9	4:10p	SSW
3	16.4	20.1	3:05p	11.3	12:00m	2.6	0.0	0.0	2.6	19.3	8:10a	SSW
4	15.2	22.4	3:15p	9.0	3:35a	2.6	0.0	0.2	1.5	20.9	11:30a	WSW
5	17.0	24.7	2:55p	10.1	6:55a	0.9	0.0	0.0	1.6	14.5	2:15p	SSW
6	15.9	17.3	4:35p	14.3	12:55a	2.4	0.0	0.6	1.2	12.9	10:30a	SSW
7	20.8	25.9	4:10p	16.9	12:05a	0.0	3.2	2.2	2.7	24.1	2:55p	SW
8	20.4	27.6	3:45p	14.5	6:30a	0.0	2.7	1.0	2.4	32.2	5:05p	SSW
9	19.9	24.4	4:15p	16.1	11:55p	0.0	2.0	1.6	2.6	35.4	1:20a	SSW
10	18.9	24.8	5:05p	15.1	2:50a	0.0	1.6	0.2	1.9	17.7	2:55p	SSW
11	18.9	23.9	3:20p	15.6	3:20a	0.0	1.4	0.0	2.9	29.0	5:45p	NE
12	17.8	23.4	5:15p	11.9	5:50a	0.6	0.0	0.0	3.2	20.9	8:25a	ENE
13	18.8	25.7	4:20p	12.1	6:30a	0.0	0.6	0.2	0.3	11.3	2:05p	E
14	19.8	27.6	4:20p	14.9	1:50a	0.0	3.0	0.0	0.9	12.9	11:40a	SW
15	20.6	26.3	2:15p	13.3	5:10a	0.0	1.5	0.2	2.4	19.3	2:20p	SW
16	16.2	19.7	1:30p	12.8	6:40a	2.0	0.0	0.0	3.9	27.4	9:45a	S
17	13.5	19.4	4:30p	7.3	6:50a	4.9	0.0	0.0	0.4	16.1	1:20a	SSW
18	14.4	18.7	5:55p	11.2	5:15a	3.3	0.0	1.2	1.4	25.7	5:15p	SSW
19	13.3	18.8	3:25p	7.4	12:00m	5.2	0.0	0.2	2.1	19.3	1:10p	NE
20	11.9	18.3	4:50p	5.9	6:40a	6.2	0.0	0.0	2.0	20.9	11:30a	NE
21	14.3	21.6	4:45p	7.4	6:25a	3.8	0.0	0.2	1.1	14.5	11:05a	ESE
22	16.7	22.7	5:20p	9.5	6:45a	2.2	0.0	0.2	1.4	11.3	4:15p	ESE
23	17.6	22.3	3:10p	13.9	12:00m	0.2	0.0	16.0	1.4	16.1	4:30p	SSW
24	17.1	24.4	2:15p	9.5	7:05a	1.3	0.0	0.2	1.2	16.1	3:30p	NE
25	17.8	25.4	2:35p	11.3	6:25a	0.0	0.1	0.2	0.2	9.7	5:45p	NE
26	17.3	21.4	12:25p	12.6	4:05a	1.3	0.0	0.2	0.8	12.9	11:30a	SW
27	18.2	22.0	4:55p	15.2	11:55p	0.0	0.3	0.8	4.0	19.3	12:20p	ENE
28	18.1	22.8	3:45p	14.2	7:15a	0.0	0.2	0.0	4.6	25.7	11:00a	E
29	20.0	25.3	4:50p	15.9	12:10a	0.0	2.4	0.2	2.6	12.9	9:30a	SSW
30												
31												
	17.5	27.6	8	5.9	20	39.6	22.7	46.0	2.0	35.4	9	SSW

Max >= 32.0: 0
Max <= 0.0: 0
Min <= 0.0: 0
Min <- -18.0: 0
Max Rain: 18.20 ON 1/08/00
Days of Rain: 9 (> .2 mm) 4 (> 2 mm) 0 (> 20 mm)
Heat Base: 18.3 Cool Base: 18.3 Method: (High + Low) / 2

-APPENDIX 2-

Mean Soil Moisture Measurement for Study Sites in Southern Ontario
July and August, 2000

Site 1 - VITAVAX RS FLOWABLE® (Windy Acre Farms, Grand Valley, Ontario)

10 July = 4.9%
27 July = 3.3%

Site 2 - GAUCHO® (Windy Acre Farms, Grand Valley, Ontario)

10 July = 4.6%
27 July = 3.0%

Site 3 - TI-435 (University of Guelph - Elora Research Station, Elora, Ontario)

10 July = 5.5%
11 August = 5.9%

-APPENDIX 3-

Rosemount Research and Outreach Centre
Meteorological Data
Minnesota, U.S.A.

(See attached)

110403

U MN DEPT ENTO F&W

003

10/11/00

(Logical) Mississippi Research & Outreach Ctr (River Station, if different) | MONTH June | 2000

COUNTY Dakota | RIVER Mississippi

TIME (local) OF OBSERVATION RIVER | TEMP. 0500 0800 | PRECIPITATION | STANDARD TIME IN USE CD ST

TYPE OF RIVER GAGE | ELEVATION OF RIVER GAGE ZERO Ft. | FLOOD STAGE Ft. | NORMAL POOL STAGE Ft.

WS FORM B-91 (W)

U.S. DEPARTMENT OF COMMERCE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NATIONAL WEATHER SERVICE
NWS

RECORD OF RIVER AND CLIMATOLOGICAL OBSERVATIONS

21a

DATE	TEMPERATURE F. 24 HRS. ENDING AT OBSERVATION MAX.	MIN.	AT OBSN.	PRECIPITATION 24-HR AMOUNTS Rain, melted snow, etc. (ins. and hundredths)	Snow, ice pellets, (ins. and tenths)	At Ob. Snow, ice pellets, hail, ice on ground (ins.)	Draw a straight line (——) through hours precipitation was observed, and a wavy line (~~~) through hours precipitation probably occurred unobserved. A.M. NOON P.M.	WEATHER (Calendar Day) Mark 'X' for all types occurring each day. Fog	Ice Pellets	Glaze	Thunder	Hail	Damaging Winds	Time of observation if different from above	RIVER STAGE CONDITION	GAGE READING AT ___ A.M.	TENDENCY	REMARKS (Special observations, etc.)
1	69	50	66	.08													0800 .68"
2	66	49	63														
3	64	45	56														Reading change 1700 to 0800
4	68	45	54	.35													
5	54	41	51	1.18													
6	66	45	54														
7	72	54	63														
8	83	60	70														
9	94	67	74														
10	91	74	75														
11	89	56	62	—													windy
12	80	57	61	—													
13	73	58	59	.10													
14	76	59	67	.53													
15	71	54	58	.06													
16	68	55	56	.33													
17	62	43	52	.01													Ck bar No. CK2105
18	66	52	58	.06													
19	75	56	64	.01													windy
20	80	64	68	.62													Calm No records
21	[redacted]	57	63	.02													
22		61	65	—													Calm. Cloudy Humid
23		59	63	.02													
24		62	69	.12													1045 Reading
25		57	67	.00													
26		59	63	.17													
27		50	67														
28		56	61	0													
29	74	51	61	.03													
30	77	57	65														77.1
31																	
Sum			SUM	4.29													

CHECK BAR (For wire-weight) NORMAL CK. BAR

READING	DATE

CONDITION OF RIVER AT GAGE

A. Obstructed by rough ice.
B. Frozen, but open at gage.
C. Upper surface of smooth ice.
D. Ice gorge above gage.
E. Ice gorge below gage
F. Shore ice.
G. Floating ice.
H. Pool stage.

[redacted]

110403

21b

0004

MN DEPT ENTO F&W

10/11/00

STATION (Climatological) ROSEMOUNT RESEARCH + OUTREACH CENTER (River Station, if different)

MONTH JULY 2000

STATE MN

COUNTY DAKOTA

RIVER MISSISSIPPI

TIME (local) OF OBSERVATION

TEMP. 0800

PRECIPITATION 0800

STANDARD TIME IN USE CDST

TYPE OF RIVER GAGE

ELEVATION OF RIVER GAGE ZERO Ft.

FLOOD STAGE Ft.

NORMAL POOL STAGE Ft.

WS FORM B-91 (12-93)

U.S. DEPARTMENT OF COMMERCE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NATIONAL WEATHER SERVICE

RECORD OF RIVER AND CLIMATOLOGICAL OBSERVATIONS

DATE	TEMPERATURE F. 24 HRS. ENDING AT OBSERVATION MAX.	MIN.	AT OBSN.	PRECIPITATION 24-HR AMOUNTS Rain, melted snow, etc. (ins. and hundredths)	Snow, ice pellets, (ins. and tenths)	At Ob. Snow, ice pellets, hail, ice on ground (ins.)	WEATHER (Calendar Day) Mark "X" for all types occurring each day. Fog	Ice Pellets	Glaze	Thunder	Hail	Damaging Winds	Time of observation if different from above	RIVER STAGE CONDITION	GAGE READING AT ___ A.M.	TENDENCY	REMARKS (Special observations, etc.)
1	83	62	70	.00													CLEAR · BREEZY · HUMID
2	88	66	26	.65													RAIN ENDING
3	77	63	65														
4	73	69	69														
5	79	66	68														
6	81	64	64	.44													
7	81	64	66	0													
8	79	66	78	4.40													CLEAR · BREEZY · VERY HUMID
9	91	65	68	3.15													
10	82	68	73	.17													
11	85	63	71	0													
12	85	63	70	.01													
13	85	63	73	0													
14	88	64	73														
15	95	63	72	.00													CLEAR · CALM
16	82	65	69	.00													CLOUDY · CALM
17	85	60	67														
18	74	53	54	.00													
19	56	52	56	.05													
20	68	56	62	.01													
21	73	50	61														
22	75	54	63	.00													
23	75	58	64	.00													
24	79	60	65	.01													SUNNY - CLEAR
25	79	60	66	.09													WINDY
26																	
27																	
28																	
29																	
30																	
31																	
SUM			SUM														

Draw a straight line (——) through hours precipitation was observed, and a waved line (~~~) through hours precipitation probably occurred unobserved.

A.M. NOON P.M. 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11

CHECK BAR (For wire-weight) NORMAL CK. BAR

READING DATE

CONDITION OF RIVER AT GAGE

A. Obstructed by rough ice.
B. Frozen, but open at gage.
C. Upper surface of smooth ice.
E. Ice gorge below gage
F. Shore ice.
G. Floating ice.

[redacted]

[redacted]

110403

AGRICULTURAL EXPERIMENT STATION

ROSEMOUNT MINNESOTA

Site 2

M1 58.0 | C13,4 13.3 | C13,3 13.5 | C13,2 13.9 | C13,1 13.8 | C1 65.0

M2 41.0

A1HN 13.7 | A15 13.7 | A20 34.0 | P3 51.0 | C11 19.3 | C3 17.1

A11,2 55.0 | R21,2 20.0 | C10 10.3 | C9N 4.6 | C9E 6.7 | C8 56.3 | C7 45.0

A21,1 20.0 | G17 40.5 | G18 43.0

A1 | A2 | A3 | A4 | A5 | A55 | A9 | A10 22.6 | A8 | A7 | G19 10.0

Forestry OFFICE

D1 15.3 | D1,1 2.3 | D2 30.5 | D3 23.5 | D6 8.0 | D5 17.0 | DHN | DHS | D7 38.8

G22 42.0 | G21,2 30.5 | G21,1 18.0 | G13+14 | G23 15.5

Private Owned 160 ac. Hoth Bios.

BG2+3 16.9 | BJ2+3 20.2

U.S. Plant Pathology

Site 1

G26 36.0

V3W 22.0 | V3E 19.0 | V1 10.0 | V4 6.0 | V3N 30.0 | V7 9.0 | V2 | V6 14.0 | V5 25.0 | V8 11.0 | V9 9.0 | V10 19.0 | G25 10.0 | V11 7.0 | V8N 6.0 | V12 6.0 | G24 10.5 | V13 15.0 | V14 27.0 | V15W 17.0 | V15E 13.0 | V17 21.4 | I3 26.0

I1 100H.0 | I7+8 37.0 | I9 12.0 | I10 66.0 | I11 54.0 | I12 51.1 | V18 22.8 | V19W 20.0 | V19E 24.0 | V20 10.0

Tulip Farms

S1HA 19.0 | S1HB 21.0 | S13 20.0 | S15 16.0 | T5 14.0 | T1 16.0 | S7 26.0 | S6 22.0

Bonanza Shrine Terrace

Agronomy Forage

Trapp Farms Gun Club.

R170 15.0 | R130 27.0 | R100 35.7 | R80 W.0 | R40 17.0

LEGEND

A AGRONOMY
B PLANT PATHOLOGY
C BEEF-SHEEP-SWINE
D DAIRY
E AGRICULTURAL ENGINEERING
F FORESTRY
G GENERAL FARM
H GENERAL FARM
P POULTRY
R AGRONOMY FORAGE
S SOILS
V VETERINARY MEDICINE
WINDBREAK
NON-CULTIVATED AREAS

SCALE 0 300 FEET

N

Site 3 off map

-APPENDIX 4-

FINAL REPORT ON RESIDUE DATA

submitted by

ENVIRO-TEST LABORATORIES (ETL)

Study Title: Validation and Analysis of Residues of TI-435, Imidicloprid, Hydroxy Metabolite, and Olefin Metabolite in Nectar and Pollen

(See attached)

ENVIRO-TEST LABORATORIES (ETL)
9936 - 67 AVENUE
EDMONTON, ALBERTA T6E 0P5
CANADA

STUDY TITLE: Validation and Analysis of Residues of TI-435, Imidacloprid, Hydroxy Metabolite, and Olefin Metabolite in Nectar and Pollen

AUTHORS: [redacted]

STUDY MONITOR: [redacted]
Bayer Corporation
[redacted]

SPONSOR: Bayer Corporation
Agriculture Division
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Stilwell, KS 66085-9104
[redacted]

STUDY DIRECTOR: [redacted]
Enviro-Test Laboratories
[redacted]

PERFORMING LABORATORY: Enviro-Test Laboratories
9936 - 67 Avenue
Edmonton, Alberta T6E 0P5 Canada
[redacted]

STUDY DATES: Study Initiation Date: Sepl.7, 2000
Experimental Initiation Date: Dec.11, 2000
Experimental Termination Date: Dec.21, 2000

PROTOCOL NO.: ETL00BAY02

ETL REFERENCE NO.: 01BAY09.REP

REPORT DATE: February 13, 2001

TOTAL PAGES: 95

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

We, the undersigned, hereby certify that the attached study was generated by Enviro-Test Laboratories in compliance with Good Laboratory Practices according to EPA-FIFRA section 40 CFR part 160 (Oct. 16, 1989).

[redacted]

Feb. 13, 2001
Date

[redacted]

3/01
Date

Exceptions to GLP Guidelines:

1) TI-435d_3 and olefin standards expired November 2000. The analytical work was completed December 21, 2000. This did not affect the quantitation of residues.

STATEMENT OF THE QUALITY ASSURANCE UNIT

The quality assurance unit of Enviro-Test Laboratories has inspected and/or audited the analytical phase of this study and the report, and has reported its findings to the Study Director and to ETL Management. The raw data is complete, consistent, well-documented and accurately reflects the method in which the study was conducted.

AUDIT DATES	REPORTED TO STUDY MONITOR	REPORTED TO ETL MNGMT.	REPORTED TO STUDY DIRECTOR
In-phase:			
Dec. 6-8, 11-13/00	Jan. 30/01	Jan. 18/01	Jan. 8/01
Final Report:			
Jan. 5,8, 9, 2001	Feb. 13, 2001	Feb. 13, 2001	Feb. 13, 2001

[redacted]

Feb. 13, 2001
Date

REF.#: QAD1.OLN

STUDY TEAM APPROVAL PAGE

[redacted]

MAY 15 2001
Date

Date

TABLE OF CONTENTS

APPENDICES:

1. SUMMARY

The objective of this study was to determine the limit of quantitation (LOQ) for all analytes, the limit of detection (LOD) for Imidacloprid, validate the methods and analyze the pollen and nectar samples using the following Bayer Methods: 1) "Residue Analytical Method 00554 for the Determination of Residues of TI-435 in Nectar, Honey, Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS-detection", Bayer Report Number MR-812/98, and 2) "Residue Analytical Method for the Determination of Residues of Imidacloprid, Hydroxy-Metabolite and Olefin-Metabolite in Nectar, Honey, Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS-detection", Bayer Report Number MR-551/98.

The nectar and pollen samples were extracted by polytron blending with methanol and water, concentrated using a roto evaporator, and the extract eluted through a ChemElute (CE 1020) column. The pollen samples were passed through a silica gel cleanup column. The samples were then analyzed by High Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry (HPLC-MS/MS). Quantitation was accomplished by using weighted (1/x) linear regression from a eight to nine point calibration curve.

The method validation consisted of two control matrix samples, 3 samples spiked at LOQ of 0.5 ppb for TI-435 and 1.0 ppb for imidacloprid and metabolites, two samples at five times LOQ, and two samples at ten times LOQ. The calibration curve values were acceptable for all compounds. The average recoveries for the validation were good for all analytes. TI-435 in pollen was 92% and in nectar 86%. The averages for the validation for Imidacloprid, hydroxy and olefin metabolites in pollen was 108%, 96% and 96% respectively. The averages for the validation for Imidacloprid, hydroxy and olefin metabolites in nectar was 98%, 92% and 102% respectively. See Tables 1-4 for validation results and recoveries.

The control samples were verified to be free of interferences at the retention times of interest. There were no perceived problems with cross-talk, matrix interferences, or analytical standard degradation since the recoveries were good for the validation.

1. SUMMARY cont'd

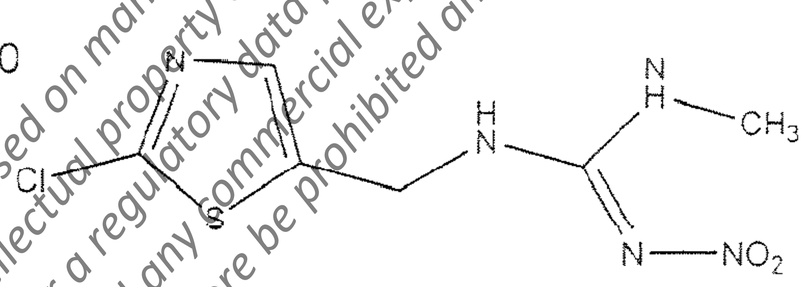
There were no detections of TI-435 or imidacloprid metabolites below the LOQ. Some samples had detection of imidacloprid at <LOQ. The LOD was estimated at 0.0003 ppm which was approximately 1/3 the LOQ and had a s/n of >5:1 (see Appendix 1, sample E0-08-003-10A for an example of this estimation).

2. MATERIALS

2.1 Reference Substances and Chemical Structures:

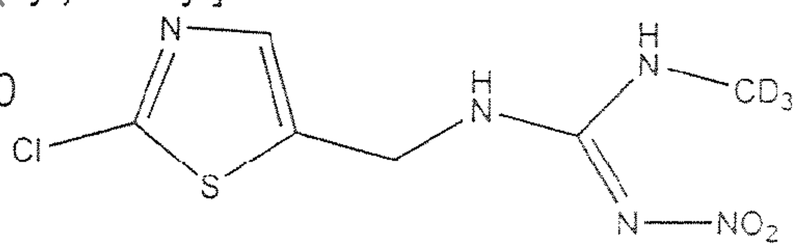
2.1.1 Characterization Information

Standard Name: TI-435
ID#: K-814
Chemical Name: N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N"-nitroguanidine
Date of Receipt: September 12, 2000
Reference No.: M00343
CAS No.: 210880-92-5
Expiration Date: August 31, 2001



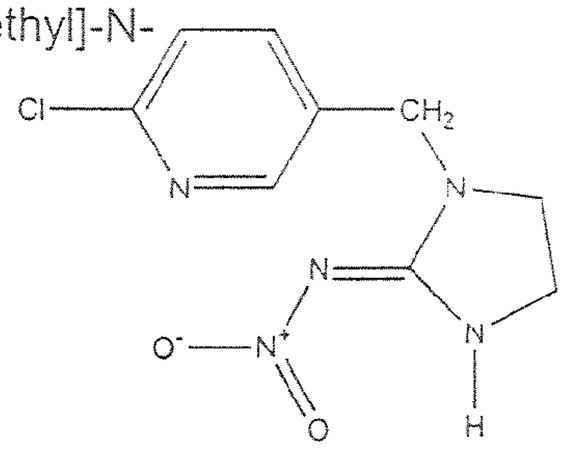
TI 435

Standard Name: TI-435-methyl-D_3
ID#: K-833
Chemical Name N-[(2-chloro-5-thiazolyl)methyl]
N'-methyl-N"-nitroguanidine
Date of Receipt: September 12, 2000
Reference No.: 30061814
Expiration Date: November 1, 2000



TI 435-D3

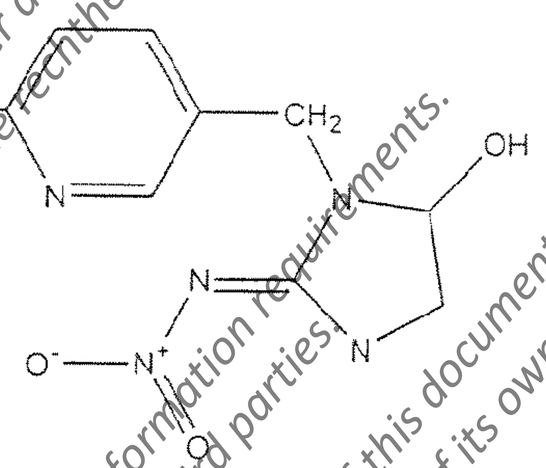
Standard Name: Imidacloprid (NTN 33893)
ID#: K-664
Chemical Name: 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine
Date of Receipt: September 12, 2000
Reference No.: 93R-008-140
CAS No.: 138261-41-3
Expiration Date: June 19, 2003



Imidacloprid (NTN 33893)

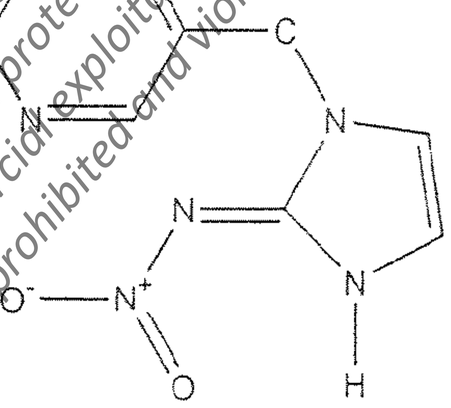
2.1 Reference Substances and Chemical Structures: contd

Standard Name: Hydroxy NTN 33893
ID#: K-527
Chemical Name: 3-[(6-chloro-3-pyridinyl)methyl]-2-nitroimino)-5-imidazolidnol
Date of Receipt: Sep. 12, 2000
Reference No.: 98r83-144
Expiration Date: July 19, 2004



Hydroxy NTN 33893

Standard Name: Olefin NTN 33893
ID#: K-770
Chemical Name: 1-[(6-chloro-3-pyridinyl)methyl]-1,3-dihydro-N-nitro-2H-imidazol-2-imine
Date of Receipt: September 12, 2000
Reference No.: 930323ELB02
Expiration Date: October 31, 2000



Olefin NTN 33893

2.1.2 Standard Solution Preparation:

All standard solutions were stored at -15°C to -20°C with teflon lined screw caps and the absence of light when possible.

2.1.2.1 Native Analyte Stock Solutions

1000 ppm (mg/L) stock solutions of each native were prepared by dissolving 10 mg of reference material in a corresponding volume of acetonitrile. For compound purities, a correction in the weight was made in order to calculate the concentration. The native olefin NTN 33893 reference material expired during the course of the study. Replacement standards were ordered from the Sponsor and will be analyzed against the expired standard solutions to verify no degradation has occurred.

2.1.2.2 Native Analyte Fortification Solutions

Secondary standards were prepared for TI-435 individually by serial dilutions of the stock standard to 10mL final volumes in 2:8 acetonitrile:water. Concentrations made for TI-435 were 100ppm, 10ppm, 1ppm, 0.25ppm, 0.10ppm, 0.05ppm, 0.025ppm, 0.01ppm, 0.005ppm, 0.0025ppm, 0.001ppm, 0.0005ppm, and 0.00025ppm.

Secondary standards were prepared for Imidacloprid and its metabolites by serial dilutions of the stock standard to 10mL final volumes in 2:8 acetonitrile:water. Concentrations made for the mix were approximately 10ppm, 1ppm, 0.5ppm, 0.25ppm, 0.10ppm, 0.05ppm, 0.025ppm, 0.01ppm, 0.005ppm, 0.0025ppm, 0.001ppm.

2.1.2.3 Internal Standard (I.S.) Stock Solutions

The TI-435-methyl-d_3 stock solution was prepared by dissolving 2-3 mg of the reference material in a corresponding volume of acetonitrile. For compound purities, a correction in the weight was made in order to calculate the concentration. The TI-435-methyl-d_3 native reference expired during the course of the study. Replacement standards were ordered from the Sponsor and will be diluted and the analysis compared to the expired standard solutions to prove no degradation has occurred.

2.1.2.4 I.S. Fortification Solutions

TI-435-methyl-d_3 secondary standards were made by diluting 0.036mL of the stock standard to 10mL final volume in 2:8 acetonitrile:water. Further dilutions of the 1ppm TI-435-methyl-d_3 were made by diluting to 10mL final volume in 2:8 acetonitrile:water for concentrations of 0.1ppm and 0.001ppm.

2.1.2.5 Calibration Curve Standard Solutions for TF-435

Concentration of native spiking solution (ppm)	Volume of native spiking solution (mL)	Volume of internal standard 0.1ppm (mL)	Native Standard Conc. (ppm)	I.S. Conc. (ppm)
100	0.10	0.10	1.00	0.00100
10.0	0.25	0.10	0.250	0.00100
10.0	0.10	0.10	0.100	0.00100
10.0	0.050	0.10	0.0500	0.00100
1.03	0.243	0.10	0.0250	0.00100
1.03	0.098	0.10	0.0101	0.00100
1.03	0.049	0.10	0.00505	0.00100
0.100	0.25	0.10	0.00250	0.00100
0.100	0.10	0.10	0.00100	0.00100
0.100	0.050	0.10	0.000500	0.00100
0.100	0.025	0.10	0.0000250	0.00100

All solutions were brought to 10mL final volume with 20:80 acetonitrile:water.

2.1.2.5 Calibration Curve Standard Solutions for Imidacloprid, Hydroxy Met. And Olefin Met.

Conc./Vol. of Imidicloprid spiking solution (ppm)/(mL)	Conc./Vol. of Hydroxy Met. (ppm)/(mL)	Conc./Vol. of Olefin Met. standard (ppm)/(mL)	Imidacloprid Standard Conc. (ppm)	Hydroxy Met. Standard Conc. (ppm)	Olefin Met. Standard Conc. (ppm)
1122/0.090	1053/0.095	993/0.101	10.1	10.0	10.0
10.1/1.0	10.0/1.0	10.0/1.0	1.01	1.00	1.00
10.1/0.50	10.0/0.50	10.0/0.50	0.505	0.500	0.500
10.1/0.25	10.0/0.25	10.0/0.25	0.253	0.250	0.250
10.1/0.10	10.0/0.10	10.0/0.10	0.101	0.100	0.100
10.1/0.050	10.0/0.050	10.0/0.050	0.0505	0.0500	0.0500
1.01/0.25	1.00/0.25	1.00/0.25	0.0253	0.0250	0.0250
1.01/0.10	1.00/0.10	1.00/0.10	0.0101	0.0100	0.0100
1.01/0.050	1.00/0.050	1.00/0.050	0.00505	0.00500	0.00500
0.101/0.25	0.100/0.25	0.100/0.25	0.00253	0.00250	0.00250
0.101/0.10	0.100/0.10	0.100/0.10	0.00101	0.00100	0.00100

All solutions were brought to 10mL final volume with 20:80 acetonitrile:water and protected from light by covering the standard tubes with foil and keeping them in the WIC at 4°C with the light off.

2.2 Reagents

Acetonitrile, HPLC grade, EM Science, OmniSolv®

Cyclohexane, EM Science, OmniSolv®

Dichloromethane, EM Science, OmniSolv®

Ethyl Acetate, HPLC grade, Burdick and Jackson

Methanol, HPLC grade, EM Science, OmniSolv®

Nitrogen, Pre Pure, Praxair

Toluene, pesticide grade, Caledon Labs, distilled in glass

Water, HPLC grade, EM Science, OmniSolv®

Methanol:water 3:1. Add 3000 mL of methanol to 1000 mL of water.

2.2 Reagents cont'd

Cyclohexane:ethyl acetate 1:1. Add 2000 mL of cyclohexane to 2000 mL of Ethyl acetate.

Acetonitrile:water 20:80. Add 800 mL of acetonitrile to 3200 mL of water.

Toluene:Ethyl acetate 85:15. Add 850 mL of toluene to 150 mL of ethyl acetate.

Toluene:Ethyl acetate 70:30. Add 700 mL of toluene to 300 mL of ethyl acetate.

Mobile Phase A. 0.1% acetic acid/H_2O. Add 4 mL of acetic acid to 4 L of water.

Mobile Phase B. Acetonitrile.

2.3 Laboratory Materials and Apparatus:

Balance, analytical, model ER-182A, AND (accuracy to the nearest 0.00001 g)

Balance, top-loading, model FX-4000, AND (accuracy to the nearest 0.01 g)

Bottles, centrifuge, 250mL, polypropylene

Bottles, sample, amber, 1 L, EPA Certified Clean Ltd.

ChemElut (CE 1020) columns, 20mL, Extube®, Part # 12198008

Flasks, volumetric, Kimax®, 50 and 100 mL, Kimble Glass Inc.

Filters, syringe, Acrodisc® CR PTFE,0.45µm x 13mm, Gelman Sciences Inc. (4422T)

Guard Column: Phenomex C_{18}

HPLC column, Keystone Betasil (C_{18}), 100cm x 4.6mm (5µm)

Oven, Thelco, model 27, Precision Scientific Co.

Pipets, 50 µL - 1 mL, Gilson

Pipets, pasteur, 9" VWR Scientific Products

Pipet, Socorex, 0.5-5 mL, Swiss

Polytron Homogenizer, Kinematica tissuemizer, CH-6010

Rotoevaporators, Janke & Kunkel, IKA®-Labortechnik, RV 06-ML

Round Bottom Flasks, 250mL to 500mL

Scoopula, stainless steel, 165 mm, VWR Scientific Products

Silica Gel columns, BondElut®, Part # 1210-2037

2.3 Laboratory Materials and Apparatus: (cont'd)

Syringes, single use, 3cc, Luer Lok®, Becton Dickinson
Tubes, culture, 10 mL, Kimax®, Kimble Glass Inc.
Ultrasonic Bath, Solid State/Ultrasonic, FS-28
Vials, autosampler, clear, National Scientific Co.
Vials, collection, clear, Dionex Corporation (048784)

2.4 Instrumentation, Conditions and Parameters:

2.4.1 Instrumentation:

Data acquisition system: Apple Power Mac G3
Autosampler: Perkin-Elmer Series 200 Autosampler
Solvent delivery system: Perkin-Elmer Series 200 Micropump
Mass analyzer: PE Sciex, model API 3000 LC/MS/MS
Printer: Hewlett Packard, Laser Jet 2100TN

2.4.2 HPLC Operating Conditions:

Column: Phenomenex Columbus C_{18}, 2 x 100mm (5µm)
Mobile Phase A: 0.1% acetic acid in water
Mobile Phase B: Acetonitrile

2.4.2.1 Analysis of TI-435

Injection Volume: 25 µL
Flow Rate: 1 ml/min

Time (min.)	Mobile Phase	
	A (%)	B (%)
0	75	25
5.0	75	25
5.5	10	90
8.0	10	90
8.1	75	25
16.0	75	25

2.4.2.2 Analysis of Imidicloprid, Hydroxy Met., Olefin Met.

Injection Volume: 25 µL
Flow Rate 1 ml/min

Time (min.)	Mobile Phase	
	A (%)	B (%)
0	75	25
6.0	75	25
6.5	10	90
9.0	10	90
9.1	75	25
17.0	75	25

2.4.3 Mass Spectrometer and Interface Operating Parameters:

Interface heater temperature: 60°C

Nebulizer temperature: 475°C

Nebulizer gas pressure: 80 psi (N_2)

Curtain gas flow rate: 1.2 L/min. (N_2)

Auxillary gas flow rate: 8.0 L/min. (N_2)

Collision gas thickness: approximately 2.75×10^{15} molec./cm^2 (Ar)

2.4.3.1 Positive Ion Analysis

Ion spray voltage: 5700 V

Orifice potential: 40 V

Collision energy: -10 V

Dwell time: 250 ms

Analyte	Mass Transition (m/z)	Approx. Retention Time (min.)
TI-435	250.0→169.0	3.36
TI-435-methyl-d_3	253.0→172.0	3.35
Imidacloprid	256.0→209.0	4.04
Hydroxy Metabolite	272.0→191.0	2.46
Olefin Metabolite	254.0→206.0	2.28

2.5 Sample Management:

Untreated control pollen and nectar samples were obtained from a known control source at ETL in Edmonton, Alberta. These samples (ETL sample no. E0-08-010-01A and -02A) were stored in the freezer at -20 ±5°C until subsampling. The sample was received already processed and did not require any further processing.

For the Method Validation of TI-435, nine 2 g subsamples of pollen control were weighed out on December 1, 2000, extracted on December 7-8, 2000 and analyzed on December 9, 2000. Nine 1 g subsamples of honey control were weighed out on December 1,2000, extracted on December 6-7,2000, and analyzed on December 9,2000.

For the Method Validation of Imidacloprid and metabolites, nine 2 g subsamples of pollen control were weighed out on December 1, 2000, extracted on December 11-13, 2000 and analyzed on December 20, 2000. Nine 1 g subsamples of honey control were weighed out on December 1,2000, extracted on December 11-12,2000, and analyzed on December 13,2000.

Sample preparation of pollen included grinding with a coffee grinder in the presence of dry ice. For sample analysis, 2 g subsamples of pollen and 1 g subsamples of honey were weighed out and extracted and analyzed on various dates. See Table 13 for exact dates.

3. ANALYTICAL PROCEDURES

The analytical method used for the analysis of TI-435 in this study was the Bayer method entitled "Residue Analytical Method 00554 for the Determination of Residues of TI-435 in Nectar, Honey, Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS-detection".

The analytical method used for the analysis of Imidacloprid and its metabolites in this study was the Bayer method entitled "Residue Analytical Method for the Determination of Residues of Imidacloprid, Hydroxy-Metabolite and Olefin-Metabolite in Nectar, Honey, Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS-detection".

3.1 Method Modifications

Standards were made to a 10mL final volume instead of the 100mL final volume suggested in Bayer methods 00554 and 00537. In the initial extraction of TI-435 and Imidacloprid and it's metabolites, instead of vacuum filtering and washing the solids with 20mL of 3:1 methanol:water, the samples were centrifuged at 5000 RPM for approximately 5 minutes and the pellet was shaken by hand with the 20 mL rinse of 3:1 methanol:water and re-centrifuged.

In the silica gel clean-up of the pollen samples for both methods, the compounds were eluted off the column using 10mL of acetonitrile instead of 5mL listed in each method. This step was verified before the extraction the validations.

TI-435 results were calculated by a weighted (1/x) linear regression curve instead of the internal bracketting standard procedure.

Imidacloprid and it's metabolites were calculated by a weighted (1/x) linear regression curve instead of the external bracketing standard procedure.

3.2 Flow Diagram of the Analysis of TI-435 in Nectar/Pollen

1.0 g of **Nectar** and 10 mL water

Sonicate

Add 20 mL methanol and polytron

Centrifuge and decant

Rinse with 20 mL 3:1 methanol:H_2O

Combine filtrates and evaporate to aqueous

Adjust to 15 mL with H_2O

2.0 g of **Pollen** and 30 mL 3:1 methanol:H_2O

Soak 30 min. and polytron

Centrifuge and decant

Rinse with 30 mL of 3:1 methanol:H_2O

Combine filtrates and transfer 50% to flask

Evaporate to aqueous
Adjust to 15 mL with H_2O

Transfer to ChemElut column and wait 15 min.

Elute with 60 mL of 1:1 cyclohexane:ethyl acetate into a 250 mL rbf

Evaporate to dryness

NECTAR dissolve in
1.0 mL of 0.001 ppm TI-435 d_3

Analyze by LC/MS/MS

POLLEN
Dissolve in 2 mL 85:15 toluene:ethyl acetate

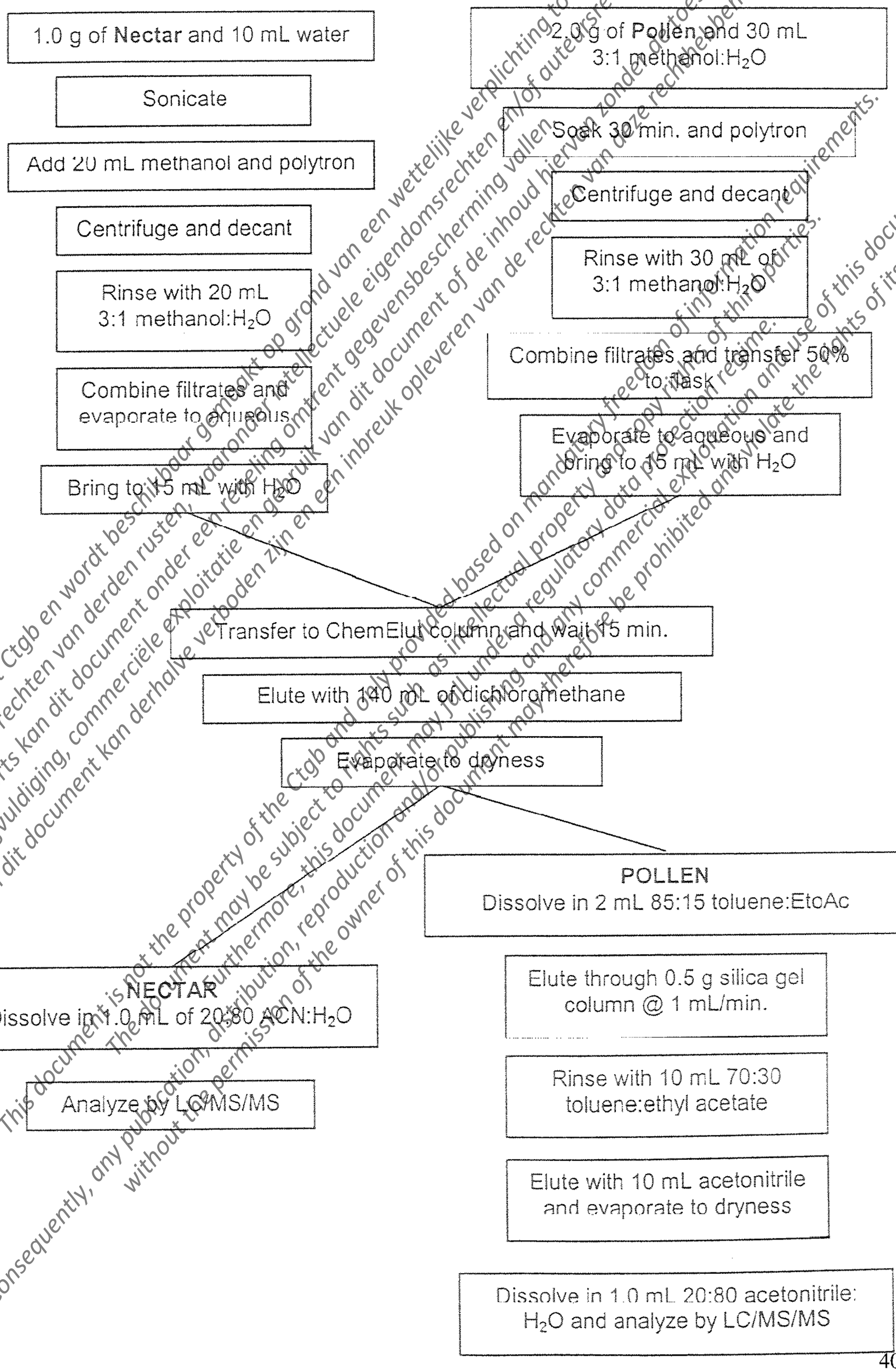
Elute through 0.5 g silica column @ 1 mL/min.

Rinse flask with 10 mL 70:30 toluene:ethyl acetate

Elute with 10 mL acetonitrile and evaporate to dryness

Dissolve in 1.0 mL of 0.001 ppm TI-435 d_3 and analyze by LC/MS/MS

3.3 Flow Diagram of the Analysis of Imidacloprid and Metabolites in Nectar/Pollen



3.4 Calculation Procedures

Quantitation by LC/MS/MS was performed using a linear regression plot of calibration standards injected intermittenly between samples of an analytical sample. The best fit regression curve for all compounds was a weighted 1/x plot.

3.4.1 Concentration of Analyte

$$\text{Conc.(ug/g)} = \frac{\left[\frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}\right] \times \left[\frac{\text{Final Volume}}{\text{Sample Mass}}\right] \times \text{Aliquot Factor}}{1000}$$

Where:

All volumes are in milliliters (mL)

Sample Mass is in grams (g)

Regression: Linear - weighted 1/x

3.4.2 Percent Recovery

$$\%\ \text{Recovery} = \frac{\text{Amount Recovered (ppm)}}{\text{Amount Fortified (ppm)}} \times 100$$

3.4.3 Example of a Field Sample Calculation

Compound: Imidacloprid
Sample I.D.: E0-08-003-06A (Nectar Gaucho A11 12/07/00)
Matrix: Nectar
Analysis Date: Dec. 21/00
File Name: NM122100A016 (See Appendix 1)

$$\frac{\left[\frac{2442 - (-898)}{4118}\right] \times \left[\frac{1.0\,\text{mL}}{1.0\,\text{g}}\right] \times 1.0}{1000} = 0.00081\ \text{ug/g(ppm)}$$

3.4.4 Example of a Recovery Calculation

Compound: Imidacloprid
Sample I.D.: E0-08-001-11A+1 (GVS Road Nectar 07/19/00)
Matrix: Nectar
Analysis Date: Dec. 21/00
File Name: NM122100A005 (See Appendix 1)

$$\frac{\left[\frac{3949 - (-898)}{4118}\right] \times \left[\frac{1.0\,mL}{10\,g}\right] \times 1.0}{1000} = 0.00118\,ppm$$

$$\%\,Recovery = \frac{0.00118\,ppm}{0.00101\,ppm} \times 100 = 117\,\%$$

4. RECOVERY AND RESULTS DATA

Table 1: Validation Results for TI-435 in Pollen

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-010-01A-3	Pollen UTC	TI-435	---	<0.000500	---
E0-08-010-01A-4	Pollen UTC	TI-435	---	<0.000500	---
E0-08-010-01A+5	Pollen UTC	TI-435	0.000505	0.000416	82
E0-08-010-01A+6	Pollen UTC	TI-435	0.000505	0.000453	90
E0-08-010-01A+7	Pollen UTC	TI-435	0.000505	0.000393	78
E0-08-010-01A+8	Pollen UTC	TI-435	0.00250	0.00246	98
E0-08-010-01A+9	Pollen UTC	TI-435	0.00250	0.00247	99
E0-08-010-01A+10	Pollen UTC	TI-435	0.00500	0.00473	95
E0-08-010-01A+11	Pollen UTC	TI-435	0.00500	0.00522	104
			Average Recovery=		92%
			SD=		± 9.5
			RSD=		10%

Table 2: Validation Results for TI-435 in Nectar

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-010-02A-3	Honey UTC	TI-435	---	<0.000500	-
E0-08-010-02A-4	Honey UTC	TI-435	---	<0.000500	-
E0-08-010-02A+5	Honey UTC	TI-435	0.000505	0.000411	81
E0-08-010-02A+6	Honey UTC	TI-435	0.000505	0.000406	80
E0-08-010-02A+7	Honey UTC	TI-435	0.000505	0.000379	75
E0-08-010-02A+8	Honey UTC	TI-435	0.00250	0.00225	90
E0-08-010-02A+9	Honey UTC	TI-435	0.00250	0.00213	85
E0-08-010-02A+10	Honey UTC	TI-435	0.00500	0.00490	98
E0-08-010-02A+11	Honey UTC	TI-435	0.00500	0.00460	92
			Average Recovery=		86%
			SD=		± 7.9
			RSD=		9.2%

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4. RECOVERY AND RESULTS DATA

Table 3: Validation Results for Imidacloprid, Hydroxy and Olefin metabolite in Pollen

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-010-01A-1	Pollen UTC	Olefin metabolite	---	<0.00100	-
		Hydroxy metabolite	---	<0.00100	-
		Imidacloprid	---	<0.00100	-
E0-08-010-01A-2	Pollen UTC	Olefin metabolite	---	<0.00100	-
		Hydroxy metabolite	---	<0.00100	-
		Imidacloprid	---	<0.00100	-
E0-08-010-01A+1	Pollen UTC	Olefin metabolite	0.00100	0.000972	97
		Hydroxy metabolite	0.00100	0.000793	79
		Imidacloprid	0.00101	0.00119	118
E0-08-010-01A+2	Pollen UTC	Olefin metabolite	0.00100	0.000818	82
		Hydroxy metabolite	0.00100	0.000951	95
		Imidacloprid	0.00101	0.00113	112
E0-08-010-01A+3	Pollen UTC	Olefin metabolite	0.00100	0.00100	100
		Hydroxy metabolite	0.00100	0.00112	112
		Imidacloprid	0.00101	0.00123	122
E0-08-010-01A+4	Pollen UTC	Olefin metabolite	0.00500	0.00468	94
		Hydroxy metabolite	0.00500	0.00424	85
		Imidacloprid	0.00505	0.00489	97
E0-08-010-01A+5	Pollen UTC	Olefin metabolite	0.00500	0.00430	86
		Hydroxy metabolite	0.00500	0.00453	91
		Imidacloprid	0.00505	0.00467	92
E0-08-010-01A+6	Pollen UTC	Olefin metabolite	0.0100	0.00950	95
		Hydroxy metabolite	0.0100	0.0102	102
		Imidacloprid	0.0101	0.0105	104
E0-08-010-01A+7	Pollen UTC	Olefin metabolite	0.0100	0.0115	115
		Hydroxy metabolite	0.0100	0.0108	108
		Imidacloprid	0.0101	0.0112	111
		Olefin metabolite	Average Recovery=		96%
			SD=		±11
			RSD=		11%
		Hydroxy metabolite	Average Recovery=		96%
			SD=		±12
			RSD=		13%
		Imidacloprid	Average Recovery=		108%
			SD=		±11
			RSD=		10%

4. RECOVERY AND RESULTS DATA

Table 4: Validation Results for Imidacloprid, Hydroxy and Olefin metabolite in Nectar

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-010-02A-1	Honey UTC	Olefin metabolite	---	<0.00100	-
		Hydroxy metabolite	---	<0.00100	-
		Imidacloprid	---	<0.00100	-
E0-08-010-02A-2	Honey UTC	Olefin metabolite	---	<0.00100	-
		Hydroxy metabolite	---	<0.00100	-
		Imidacloprid	---	<0.00100	-
E0-08-010-02A+1	Honey UTC	Olefin metabolite	0.00100	0.00114	114
		Hydroxy metabolite	0.00100	0.000733	73
		Imidacloprid	0.00101	0.00107	106
E0-08-010-02A+2	Honey UTC	Olefin metabolite	0.00100	0.001 1	111
		Hydroxy metabolite	0.00100	0.00106	106
		Imidacloprid	0.00101	0.00103	102
E0-08-010-02A+3	Honey UTC	Olefin metabolite	0.00100	0.00112	112
		Hydroxy metabolite	0.00100	0.00107	107
		Imidacloprid	0.00101	0.00109	108
E0-08-010-02A+4	Honey UTC	Olefin metabolite	0.00500	0.00449	90
		Hydroxy metabolite	0.00500	0.00383	77
		Imidacloprid	0.00505	0.00449	89
E0-08-010-02A+5	Honey UTC	Olefin metabolite	0.00500	0.00424	85
		Hydroxy metabolite	0.00500	0.00420	84
		Imidacloprid	0.00505	0.00430	85
E0-08-010-02A+6	Honey UTC	Olefin metabolite	0.0100	0.0100	100
		Hydroxy metabolite	0.0100	0.00935	94
		Imidacloprid	0.0101	0.00957	95
E0-08-010-02A+7	Honey UTC	Olefin metabolite	0.0100	0.00998	100
		Hydroxy metabolite	0.0100	0.00999	100
		Imidacloprid	0.0101	0.0105	104
		Olefin metabolite	Average Recovery=		102%
			SD=		±11
			RSD=		11%
		Hydroxy metabolite	Average Recovery=		92%
			SD=		±14
			RSD=		15%
		Imidacloprid	Average Recovery=		98%
			SD=		±8.9
			RSD=		9.1%

4. RECOVERY AND RESULTS DATA

Table 5: In-Phase Results for TI-435 in Pollen

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-001-10A-1	GVS Road Pollen 07/11/00	TI-435	---	<0.000500	---
E0-08-001-10A+1	GVS Road Pollen 07/11/00	TI-435	0.000505	0.000432	86
E0-08-001-10A+2	GVS Road Pollen 07/11/00	TI-435	0.00250	0.00289	116
			Average Recovery=		101%

Table 6: In-Phase Results for TI-435 in Nectar

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-001-09A-1	GVS Road Nectar 07/10/00	TI-435	---	<0.000500	---
E0-08-001-09A+1	GVS Road Nectar 07/10/00	TI-435	0.000505	0.000396	78
E0-08-001-09A+2	GVS Road Nectar 07/10/00	TI-435	0.00250	0.00272	109
			Average Recovery=		94%

4. RECOVERY AND RESULTS DATA

Table 7: In-Phase Results for Imidacloprid, Hydroxy and Olefin metabolite in Pollen

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-001-12A-2	GVS Road Pollen 07/21/00	Olefin metabolite	---	<0.00100	---
		Hydroxy metabolite	---	<0.00100	---
		Imidacloprid	---	<0.00100	---
E0-08-001-12A+2	GVS Road Pollen 07/21/00	Olefin metabolite	0.00500	0.00437	87
		Hydroxy metabolite	0.00500	0.00498	100
		Imidacloprid	0.00505	0.00478	95
E0-08-001-12A+1	GVS Road Pollen 07/21/00	Olefin metabolite	0.00100	0.00101	101
		Hydroxy metabolite	0.00100	0.000949	95
		Imidacloprid	0.00101	0.00120	119
		Olefin metabolite	Average Recovery=		94%
		Hydroxy metabolite	Average Recovery=		98%
		Imidacloprid	Average Recovery=		107%

Table 8: In-Phase Results for Imidacloprid, Hydroxy and Olefin metabolite in Nectar

Lab Sample No.	Client I.D.	Analyte	Spike Level (ppm)	Results (ppm)	% Recovery
E0-08-001-11A-2	GVS Road Nectar 07/19/00	Olefin metabolite	---	<0.00100	-
		Hydroxy metabolite	---	<0.00100	-
		Imidacloprid	---	<0.00100	-
E0-08-001-11A+1	GVS Road Nectar 07/19/00	Olefin metabolite	0.00100	0.00105	105
		Hydroxy metabolite	0.00100	0.00110	110
		Imidacloprid	0.00101	0.00118	117
E0-08-001-11A+2	GVS Road Nectar 07/19/00	Olefin metabolite	0.00500	0.00399	80
		Hydroxy metabolite	0.00500	0.00455	91
		Imidacloprid	0.00505	0.00460	91
		Olefin metabolite	Average Recovery=		93%
		Hydroxy metabolite	Average Recovery=		101%
		Imidacloprid	Average Recovery=		104%

4. RECOVERY AND RESULTS DATA

Table 9: Results for TI-435 in Pollen

Lab Sample No.	Client I.D.	Analyte	Results (ppm)
E0-08-001-02A	Elora Pollen 07/05/00	TI-435	0.0030
E0-08-001-04A	Elora Pollen 07/11/00	TI-435	0.0016
E0-08-001-12A	GVS Road Pollen 07/21/00	TI-435	<0.000500
E0-08-003-11A	Pollen TI-435 ALL 06/07/00	TI-435	0.0023
E0-08-003-13A	Pollen Control ALL 06/07/00	TI-435	<0.000500
E0-08-003-17A	Comp Pollen D10-12 12/07/00	TI-435	0.0028
E0-08-003-25A	Comp Pollen CTL D8,D13,O12 12/07/00	TI-435	<0.000500
E0-08-003-35A	Comp Pollen CTL D8,D13,D14,O12 20/07/00	TI-435	<0.000500

Table 10: Results for TI-435 in Nectar

Lab Sample No.	Client I.D.	Analyte	Results (ppm)
E0-08-001-01A	Elora Nectar 07/03/00	TI-435	0.0037
E0-08-001-03A	Elora Nectar 07/10/00	TI-435	0.00094
E0-08-001-11A	GVS Road Nectar 07/19/00	TI-435	<0.000500
E0-08-003-01A	Nectar TI-435 ALL 06/07/00	TI-435	0.0011
E0-08-003-02A	Nectar Control ALL 06/07/00	TI-435	<0.000500
E0-08-003-04A	Nectar TI-435 ALL 12/07/00	TI-435	0.0010
E0-08-003-05A	Nectar Control ALL 12/07/00	TI-435	<0.000500
E0-08-003-09A	Comp Nectar Control D13-D8	TI-435	<0.000500

4. RECOVERY AND RESULTS DATA

Table 11: Results for Imidacloprid ,Hydroxy and Olefin metabolite in Pollen

Lab Sample No.	Client I.D.	Analyte	Results (ppm)
E0-08-001-06A	GVH Farm Pollen 07/11/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-001-07A	GVH Farm Pollen 07/11/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-001-10AB	GVS Farm Pollen 07/11/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-12A	Pollen GAUCHO ALL	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	0.0015
E0-08-003-13AB	Pollen Control ALL	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-21A	Comp-Pollen GAUCHO D1,D3,O22 12/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	0.0076
E0-08-003-25AB	Comp-Pollen CTL D8,D13,O12 12/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-30A	Comp-Pollen GAUCHO D1,D3,D9,O22 20/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	0.0044
E0-08-003-35AB	Comp-Pollen CTL D8,D13,D14,O12 20/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100

4. RECOVERY AND RESULTS DATA

Table 12: Results for Imidacloprid Olefin and Hydroxy metabolite in Nectar

Lab Sample No.	Client I.D.	Analyte	Results (ppm)
E0-08-001-05A	GVH Farm Nectar 07/10/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-001-08A	GVH Farm Nectar 07/19/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-001-09AB	GVS Farm Nectar 07/10/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-02A	Nectar Control ALL 06/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-03A	Nectar GAUCHO ALL 06/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	(0.00075)
E0-08-003-05AB	Nectar Control ALL 12/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-06A	Nectar GAUCHO ALL 12/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	(0.00081)
E0-08-003-09AB	Comp-Nectar CTL D8,D13 20/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	<0.00100
E0-08-003-10A	Nectar GAUCHO ALL 20/07/00	Olefin metabolite	<0.00100
		Hydroxy metabolite	<0.00100
		Imidacloprid	(0.00060)

(J) - Result is < LOQ (0.00100) but ≥ LOD (0.0003).

4. RECOVERY AND RESULTS DATA

Table 13: Dates for sample processing

Lab Sample No.	Client I.D.	Date Processed (m/d/y)	Date Weighed (m/d/y)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)
E0-08-001-10A-1	GVS Road Pollen 07/11/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-001-10A+1	GVS Road Pollen 07/11/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-001-10A+2	GVS Road Pollen 07/11/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-001-02A	ELORA Pollen 07/05/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-001-04A	ELORA Pollen 07/11/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-001-12A	GVS Road Pollen 07/21/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-003-11A	Pollen TI-435 ALL 06/07/00	12/12/00	12/12/00	12/14/00	12/20/00
E0-08-003-13A	Pollen Control ALL 06/07/00	12/12/00	12/12/00	12/14/00	12/20/00
E0-08-003-17A	Comp Pollen D10-12 12/07/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-003-25A	Comp Pollen CTL D8,D13,O12 12/07/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-003-35A	Comp Pollen CTL D8,D13,D14,O12 20/07/00	12/11/00	12/12/00	12/14/00	12/20/00
E0-08-001-09A-1	GVS Road Nectar 07/10/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-001-09A+1	GVS Road Nectar 07/10/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-001-09A+2	GVS Road Nectar 07/10/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-001-01A	ELORA Nectar 07/03/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-001-03A	ELORA Nectar 07/10/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-001-11A	GVS Road Nectar 07/19/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-003-01A	Nectar TI-435 ALL 06/07/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-003-02A	Nectar Control ALL 06/07/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-003-04A	Nectar TI-435 ALL 12/07/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-003-05A	Nectar Control ALL 12/07/00	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-003-09A	Comp Nectar Control D13-D8	12/12/00	12/12/00	12/13-14/00	12/20/00
E0-08-001-12A-2	GVS Road Pollen 07/21/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-001-12A+2	GVS Road Pollen 07/21/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00

4. RECOVERY AND RESULTS DATA

Table 13: Dates for sample processing cont'd

Lab Sample No.	Client I.D.	Date Processed (d/m/y)	Date Weighed (d/m/y)	Date Extracted (d/m/y)	Date Analyzed (d/m/y)
E0-08-001-12A+1	GVS Road Pollen 07/21/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-001-06A	GVH Farm Pollen 07/11/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-001-07A	GVH Farm Pollen 07/17/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-001-10AB	GVS Farm Pollen 07/11/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-003-12A	Pollen GAUCHO ALL 06/07/00	12/12/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-003-13AB	Pollen Control ALL 06/07/00	12/12/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-003-21A	Comp-Pollen GAUCHO D1,D3,O22 12/07/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-003-25AB	Comp-Pollen CTL D8,D13,O12 12/07/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-003-30A	Comp-Pollen GAUCHO D1,D3,D9,O22 20/07/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-003-35AB	Comp-Pollen CTL D8,D13,D14,O12 20/07/00	12/11/00	12/12/00	12/12-19/00	12/21-22/00
E0-08-001-11A-2	GVS Road Nectar 07/19/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-001-11A+1	GVS Road Nectar 07/19/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-001-11A+2	GVS Road Nectar 07/19/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-001-05A	GVH Farm Nectar 07/10/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-001-08A	GVH Farm Nectar 07/19/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-001-09AB	GVS Farm Nectar 07/10/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-003-02A	Nectar Control ALL 06/07/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-003-03A	Nectar GAUCHO ALL 06/07/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-003-05AB	Nectar Control ALL 12/07/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-003-06A	Nectar GAUCHO ALL 12/07/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-003-09AB	Comp-Nectar CTL D8,D13 20/07/00	12/12/00	12/12/00	12/18-19/00	12/21/00
E0-08-003-10A	Nectar GAUCHO ALL 20/07/00	12/12/00	12/12/00	12/18-19/00	12/21/00

4.1 Recovery Results

The methods for TI-435 and Imidacloprid, Hydroxy-metabolite, and Olefin-metabolite worked well. The average validation recoveries and standard deviations (SD) for TI-435 in pollen and nectar were 92 ±9.5% and 86 ±7.9% respectively. See Tables 1-2 for results and recoveries.

The average validation recoveries and SD for Imidacloprid, Hydroxy-metabolite, and Olefin-metabolite in pollen were 96 ±11%, 96 ±12%, and 108 ±11%, respectively. The average validation recoveries and SD for Imidacloprid, Hydroxy-metabolite, and Olefin-metabolite in nectar were 102 ±11%, 92 ±14%, and 98 ±8.9% respectively. See Tables 3-4 for results and recoveries.

The procedural recoveries for TI-435 were 86% and 116% for pollen and 79% and 109% for nectar. See Tables 5-6 for TI-435 results.

The procedural recoveries for Imidacloprid, Hydroxy-metabolite, and Olefin-metabolite in pollen were 94%, 98%, and 107%, respectively. The procedural recoveries for Imidacloprid, Hydroxy-metabolite, and Olefin-metabolite in nectar were 93%, 101% and 104% respectively. See Tables 7-8 for recoveries.

4.2 Discussion of Results

Residues of TI-435 were found in Elora pollen at 0.0030 ppm and 0.0016 ppm and in Elora nectar at 0.0037 ppm and 0.00094 ppm. Imidacloprid residues were found in Gaucho pollen at 0.0015 ppm, 0.0076 ppm, and 0.0044 ppm and Gaucho nectar at 0.00075 ppm, 0.00081 ppm and 0.00060 ppm. See Tables 5-8 for detailed results.

Appendix I contains a complete analytical data set, and Appendix II contains examples of spreadsheets for the analysis and linearity curves.

4.3 Conclusion

The method worked well and gave good recoveries at levels near the LOQ.

There were no detectable residues in any of the controls with the exception of two pollen controls which were < LOD of 0.0003 ppm.

APPENDIX I

COPY OF A COMPLETE ANALYTICAL DATA SET

Set: Honey#1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Honey — Analysis Date: December 21, 2000

SITE: — Analytical Method: MS224.00 — Weight = 1g

ETL Sample/Standards I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
blank	solvent	Olefin metabolite	0	-	-	-	28	267	-	-	-	NM122100A001
Thu, Dec 21, 2000 14:01		Hydroxy metabolite	0	-	-	-	-720	2946	-	-	-	NM122100A001
		Imidacloprid	0	-	-	-	-898	4118	-	-	-	NM122100A001
1.00 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	323	-	-	-	28	267	-	-	-	NM122100A002
Thu, Dec 21, 2000 14:18		Hydroxy metabolite	2668	-	-	-	-720	2946	-	-	-	NM122100A002
		Imidacloprid	3713	-	-	-	-898	4118	-	-	-	NM122100A002
2.50 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	700	-	-	-	28	267	-	-	-	NM122100A003
Thu, Dec 21, 2000 14:36		Hydroxy metabolite	5795	-	-	-	-720	2946	-	-	-	NM122100A003
		Imidacloprid	8458	-	-	-	-898	4118	-	-	-	NM122100A003
E0-08-001-11A-2, 1mL FV	GVS ROAD NECTAR 07/19/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A004
Thu, Dec 21, 2000 14:54		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A004
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A004
E0-08-001-11A+1, 1mL FV	GVS ROAD NECTAR 07/19/00 0.001ppm spike	Olefin metabolite	308	1.00	1.0	1.0	28	267	0.00105	0.00100	105	NM122100A005
Thu, Dec 21, 2000 15:12		Hydroxy metabolite	2508	1.00	1.0	1.0	-720	2946	0.00110	0.00100	110	NM122100A005
		Imidacloprid	3949	1.00	1.0	1.0	-898	4118	0.00118	0.00100	117	NM122100A005
E0-08-001-11A+2, 1mL FV	GVS ROAD NECTAR 07/19/00 0.005ppm spike	Olefin metabolite	1093	1.00	1.0	1.0	28	267	0.00399	0.00500	80	NM122100A006
Thu, Dec 21, 2000 15:30		Hydroxy metabolite	1268?	1.00	1.0	1.0	-720	2946	0.00455	0.00500	91	NM122100A006
		Imidacloprid	18036	1.00	1.0	1.0	-898	4118	0.00460	0.00505	91	NM122100A006
5.00 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	1263	-	-	-	28	267	-	-	-	NM122100A007
Thu, Dec 21, 2000 15:47		Hydroxy metabolite	14015	-	-	-	-720	2946	-	-	-	NM122100A007
		Imidacloprid	18585	-	-	-	-898	4118	-	-	-	NM122100A007
E0-08-001-05A, 1mL FV	GVH FARM NECTAR 07/10/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A008
Thu, Dec 21, 2000 16:05		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A008
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A008

Comments:

Calibration Filename: NM122100ACal

Regression type: Weighted (1/x)

Quantitation for [redacted] rcept)/slope x Final Volume x Aliquot factor / (Sample Wt) * 1000 **

Data Entered By [redacted] Date: Jan. 9. 2001

QC'ed by audited NB Jan. 5, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

34

56

110403

Set: Honey#1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Honey — Weight = 1g

STE: — Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-001-08A, 1mL FV	GVH FARM NECTAR 07/19/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A009
Thu, Dec 21, 2000 16:23		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A009
		Imidacloprid	809	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A009
E0-08-001-09AB, 1mL FV	GVS FARM NECTAR 07/19/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A010
Thu, Dec 21, 2000 16:41		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A010
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A010
10.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	2486	-	-	-	28	267	-	-	-	NM122100A011
Thu, Dec 21, 2000 16:59		Hydroxy metabolite	29346	-	-	-	-720	2946	-	-	-	NM122100A011
		Imidacloprid	40554	-	-	-	-898	4118	-	-	-	NM122100A011
E0-08-003-02A, 1mL FV	NECTAR CONTROL ALL 06/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A012
Thu, Dec 21, 2000 17:16		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A012
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A012
E0-08-003-03A, 1mL FV	NECTAR GAUCHO ALL 06/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A013
Thu, Dec 21, 2000 17:34		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A013
		Imidacloprid	2195	1.00	1.0	1.0	-898	4118	0.00075	-	-	NM122100A013
E0-08-003-05AB, 1mL FV	NECTAR CONTROL ALL 12/07/00	Olefin metabolite	0	1.01	1.0	1.0	28	267	<0.00100	-	-	NM122100A014
Thu, Dec 21, 2000 17:52		Hydroxy metabolite	0	1.01	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A014
		Imidacloprid	0	1.01	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A014
25.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	6810	-	-	-	28	267	-	-	-	NM122100A015
Thu, Dec 21, 2000 18:10		Hydroxy metabolite	73515	-	-	-	-720	2946	-	-	-	NM122100A015
		Imidacloprid	102590	-	-	-	-898	4118	-	-	-	NM122100A015
E0-08-003-06A, 1mL FV	NECTAR GAUCHO ALL 12/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A016
Thu, Dec 21, 2000 18:28		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A016
		Imidacloprid	2442	1.00	1.0	1.0	-898	4118	0.00081	-	-	NM122100A016

Comments:

Calibration Filename: NM122100ACal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppm)=(Peak area - y-Intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.) ÷1000

Data Entered By: [redacted] Date: Jan. 5, 2001

QC'ed by audited 08 JB Jan 5, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

35

57

110403

Set: Honey#1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ET_00BAY02.PRO — Matrix: Honey — Weight = 1g

SITE: — Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-003-09AB, 1mL FV	COMP-NECTAR CTL D8,D13 20/07/00	Olefin metabolite	0	1.01	1.0	1.0	28	267	<0.00100	-	-	NM122100A017
Thu, Dec 21, 2000 18:45		Hydroxy metabolite	0	1.01	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A017
		Imidacloprid	0	1.01	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A017
E0-08-003-10A, 1mL FV	NECTAR GAUCHO ALL 20/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A018
Thu, Dec 21, 2000 19:03		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A018
		Imidacloprid	1561	1.00	1.0	1.0	-898	4118	0.00060	-	-	NM122100A018
50.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	13763	-	-	-	28	267	-	-	-	NM122100A019
Thu, Dec 21, 2000 19:21		Hydroxy metabolite	138968	-	-	-	-720	2946	-	-	-	NM122100A019
		Imidacloprid	211721	-	-	-	-898	4118	-	-	-	NM122100A019
100 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	26467	-	-	-	28	267	-	-	-	NM122100A020
Thu, Dec 21, 2000 19:39		Hydroxy metabolite	283177	-	-	-	-720	2946	-	-	-	NM122100A020
		Imidacloprid	401857	-	-	-	-898	4118	-	-	-	NM122100A020
250 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	66639	-	-	-	28	267	-	-	-	NM122100A021
Thu, Dec 21, 2000 19:57		Hydroxy metabolite	758133	-	-	-	-720	2946	-	-	-	NM122100A021
		Imidacloprid	1031795	-	-	-	-898	4118	-	-	-	NM122100A021

Comments:

Calibration Filename: NM122100ACal

Regression type: Weighted (1/x)

Quantitation formula: [redacted]ot)'slope x Final Volume x Aliquot factor / (Sample Wt) ÷1000

Data Entered By: [redacted] Date: Jan. 5, 2001

QC'ed by [redacted] Jan. 5, 2001

Protocol No.: ET_00BAY02
ETL Report No.:01BAY09.REP

EnviroTest Laboratories

Analysis of Imidacloprid, Hydroxy- and Olefin-Metabolite in/on Nectar and Pollen by LC/MS/MS

Method Ref.: MS 224.00
Protocol no.: ETL00BAY02.PRO
Method Ref.: Bayer 00537/E001

II. Samples Weighed

Analyst: [redacted] Storage Location: WEH 2 Container: 250mL dark bottles
Balance I.D.: #60
Set No.: Honey Set 1 Date: Dec. 12/2000

III. Sample Fortification

Sample ID (ETL#)	Client ID	Analyte	STD. Conc. (ppm)	Fort. Vol. (mL)	Fort.Lev. (ppm)	Final Vol. (mL)
E0-08-001-11A+1	G.V.S.ROAD NECTAR 07/19/00	Imidacloprid	0.0101		0.00001	1.00
		Hydroxy Metab.	0.0100	0.100	0.00100	
		Olefin Metab.	0.0200		0.00200	
E0-08-001-11A+2	G.V.S.ROAD NECTAR 07/19/00	Imidacloprid	0.101		0.00505	1.00
		Hydroxy. Metab.	0.100	0.050	0.00500	
		Olefin Metab.	0.200		0.00500	0.00500

CB (EE) Dec. 12, 2000

Spiking Std. ID: D-379E, D-379H Prep. Date: Oct. 30 100, Oct. 3-00 Analyst: [redacted]
Name of Witness for Fortification: [redacted] Initials: LH Date: Dec 12/00

IV. Samples Extracted

Sample ID (ETL#)	Client ID	Weight (g)	Extraction Vol. (mL)	Aliquot Vol (mL)	Final Vol. (mL)
E0-08-001-11A-2	G.V.S.ROAD NECTAR 07/19/00	1.00	50.0		1.00
E0-08-001-11A+1	G.V.S.ROAD NECTAR 07/19/00	1.00	50.0		1.00
E0-08-001-11A+2	G.V.S.ROAD NECTAR 07/19/00	1.00	50.0		1.00
E0-08-001-05A	G.V.H.FARM NECTAR 07/10/00	1.00	50.0		1.00
E0-08-001-08A	G.V.H.FARM NECTAR 07/19/00	1.00	50.0		1.00
E0-08-001-09AB	G.V.S.ROAD NECTAR 07/10/00	1.00	50.0		1.00
E0-08-003-02AB	NECTAR CONTROL ALL 06/07/00	1.00	50.0		1.00
E0-08-003-03A	NECTAR GAUCHO ALL 06/07/00	1.00	50.0		1.00
E0-08-003-05AB	NECTAR CONTROL ALL 12/07/00	1.01	50.0		1.00
E0-08-003-06A	NECTAR GAUCHO ALL 12/07/00	1.00	50.0		1.00
E0-08-003-09AB	COMP-NECTAR CTL D8,D13 20/07/00	1.01	50.0		1.00
E0-08-003-10A	NECTAR GAUCHO ALL 20/07/00	1.00	50.0		1.00

(EE) LH Dec. 19/2000

EnviroTest Laboratories

Analysis of Imidacloprid, Hydroxy- and Olefin-Metabolite in/on Nectar and Pollen by LC/MS/MS

Method Ref.: MS 224.00
Protocol no.: ETL00BAY02.PRO
Method Ref.: Bayer 00537/E001

Page 2 of 2

V. Method Summary

Nectar Extraction

1 Weigh 1.0 g of nectar (2 g of pollen) sample into a 250mL centrifuge bottle.
2 Fortify recovery sample prior to the extraction and allow sample to equilibrate.
3 Add 10 mL of water. Place in ultrasonic bath for 2 min.
4 Add 20mL of MeOH, and homogenize with Polytron blender.
5 Centrifuge, and transfer filtrate to a 250mL round-bottom flask.
6 Rinse the centrifuge bottle with 20mL 3:1 MeOH:H2O. Centrifuge.
7 Combine rinse into original 250mL round-bottom flask.
8 Evaporate the sample to aqueous on a rotary evaporator (~50°C).
9 Take aqueous sample to 15mL final volume in water.

Initials:	Date:
1 LHR	Dec. 12/2000
2 LM	Dec. 18/2000
3 LM	"
4 LM	"
5 LM	"
6 LM	"
7 LM	"
8 LB/LM	Dec 18/2000
9 LB/LM	Dec 19/2000

Polen Extraction

1 Weigh 2.0 g of pollen sample into a 250mL centrifuge bottle.
2 Fortify recovery sample prior to the extraction and allow sample to equilibrate.
3 Add 30 mL of 3:1 methanol/water, allow th sample to soak for 30 min.
4 Homogenize with Polytron blender for about 1 min.
5 Centrifuge, and transfer filtrate to a 250mL round-bottom flask.
6 Rinse the centrifuge bottle with 30mL 3:1 MeOH:H2O. Centrifuge.
7 Transfer the filtrate to a 100 mL grad. cylinder and measure total volume. Mix well and transfer 50 % (1.0 g sample equiv.). To a round bottom flask.
8 Evaporate sample to aqueous.
9 Take aqueous sample to 15mL final volume in water.

Initials:	Date:
1	
2	
3	
4	
5	
6	
7	
8	
9	

Chem Elute Cleanup

10 Transfer aqueous sample from Step 9 to a ChemElut (CE 1020) column.
11 Wait for approximately 15 minutes, to achieve an uniform distribution.
12 Elute residues with 140 mL of CH_2Cl_2 into 250ml flask.
13 Evaporate the sample to dryness on a rotary evaporator (~40°C).
14 For nectar dissolve residues in 1.0mL 20:80 ACN:H_2O and analyze by LC/MS/MS.
15 For polen dissolve residues in 2.0mL 85:15 Toluene/ EtOAc, and proceed to SIOH cleanup.

Initials:	Date:
10 LB	Dec 19/2000
11 "	"
12 "	"
13 "	"
14 "	"
15 —	—

Silica Gel (SIOH) Cleanup (Polen)

16 This procedure is performed using gravity flow. Do not allow column to go dry between elutions.
17 Transfer extracts from Step 15 to a 0.5g SiOH column.
18 Allow solution to pass through the column at a flow rate of 1 mL / min.
19 Rinse the roundbottom flask with 10mL 70:30 toluene:ethyl acetate.
20 Add rinse to column, and discard.
21 Elute residues with 5 mL of Acetonitrile, collect the eluate.
22 Evaporate the Acetonitrile to dryness. (~40°C)
23 Dissolve final residues in 1 mL of 20:80 Acetonitrile:Water.

Initials:	Date:
16	
17	
18	
19	
20	
21	
22	
23	

Analysis

24 Analysis is done by HPLC-MS/MS (SIM).

24 —	—

Deviations from Method:

Comments:

MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100AC.al, Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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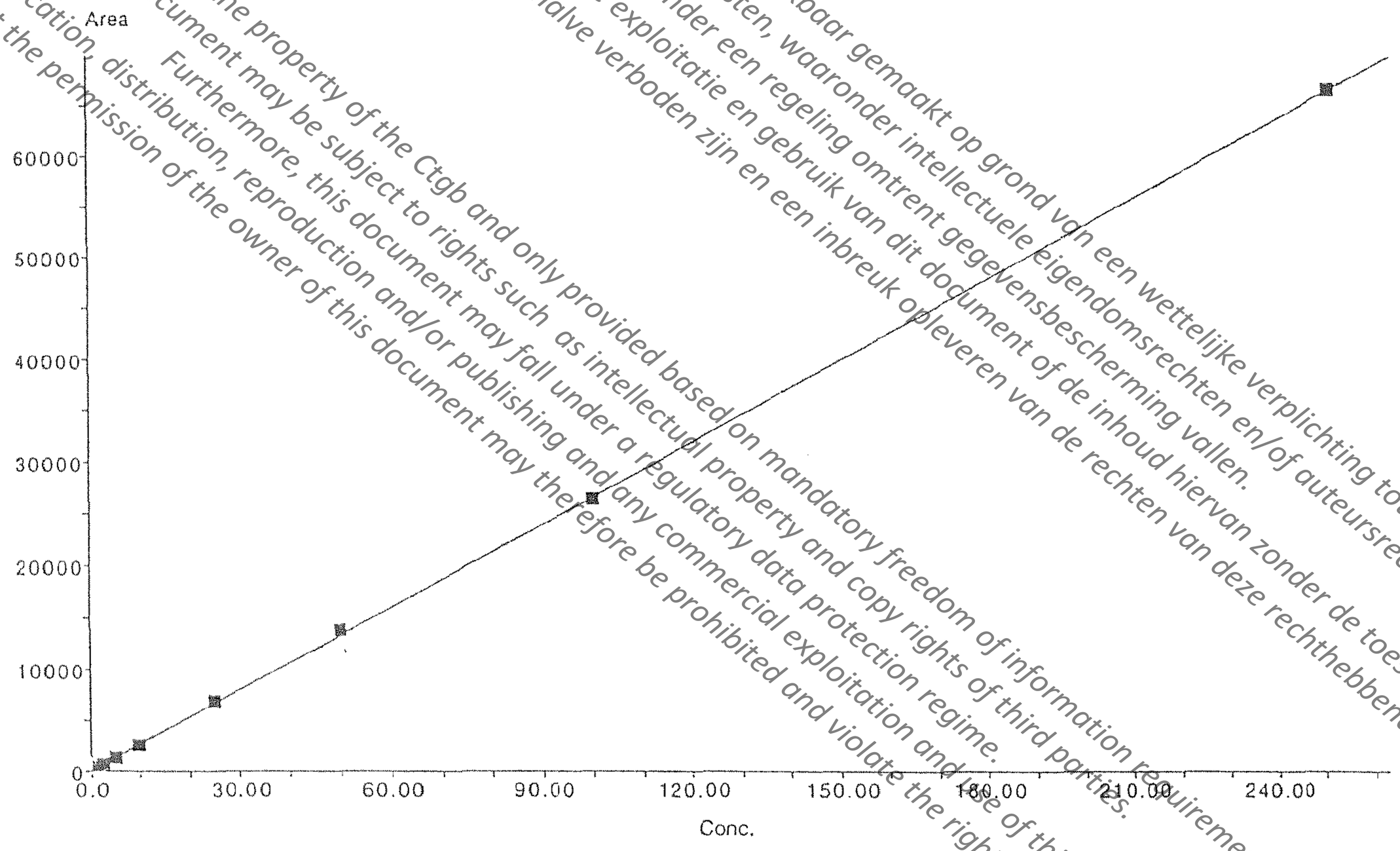
Imidaclcprid Olefin metabolite 254.0->208.0, No Internal Standard
Weighted (1/x)

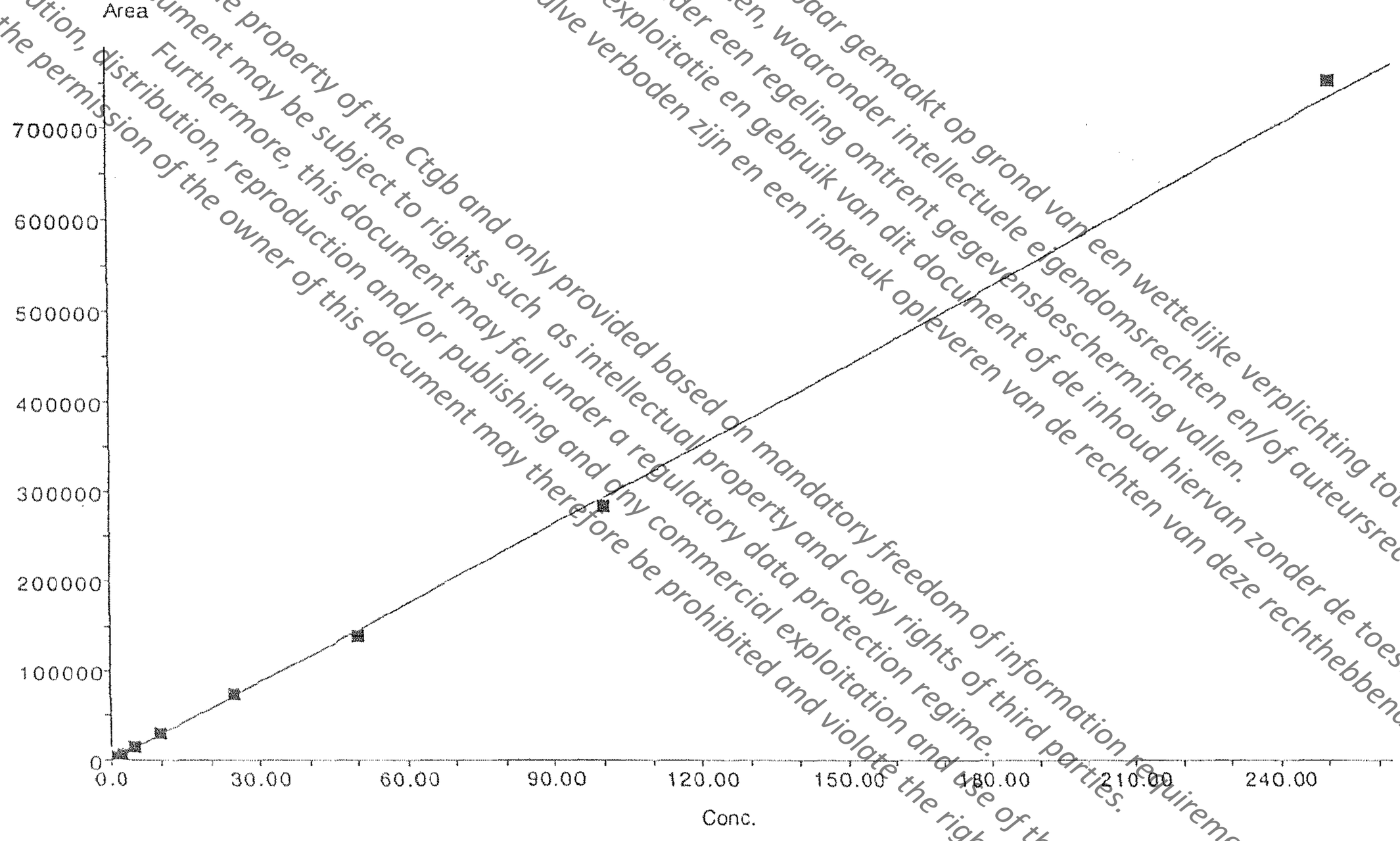
Intercept = 28
Slope = 267
Correlation Coeff. = 0.9998



Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

Imidacloprid Hydroxy metabolite 272.0->191.0 No Internal Standard
Weighted (1/x)
Intercept = -720
Slope = 2946
Correlation Coeff. = 0.9994



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal, Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO / Imidicloprid and metabolites - Honey

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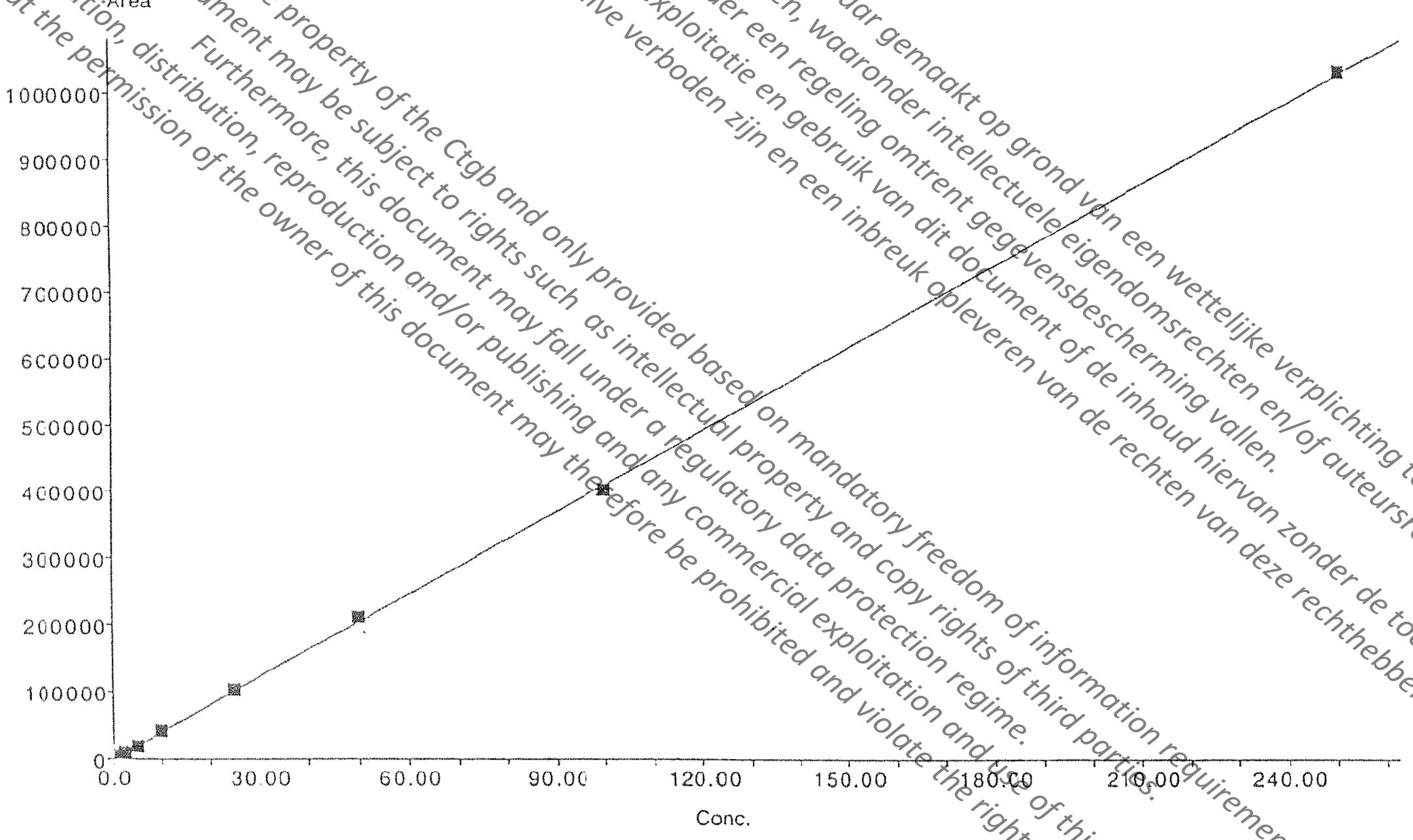
Imidacloprid 256.0->209.0 No Internal Standard
Weighted (1/x)

Intercept = -898
Slope = 4118
Correlation Coeff. = 0.9998



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Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

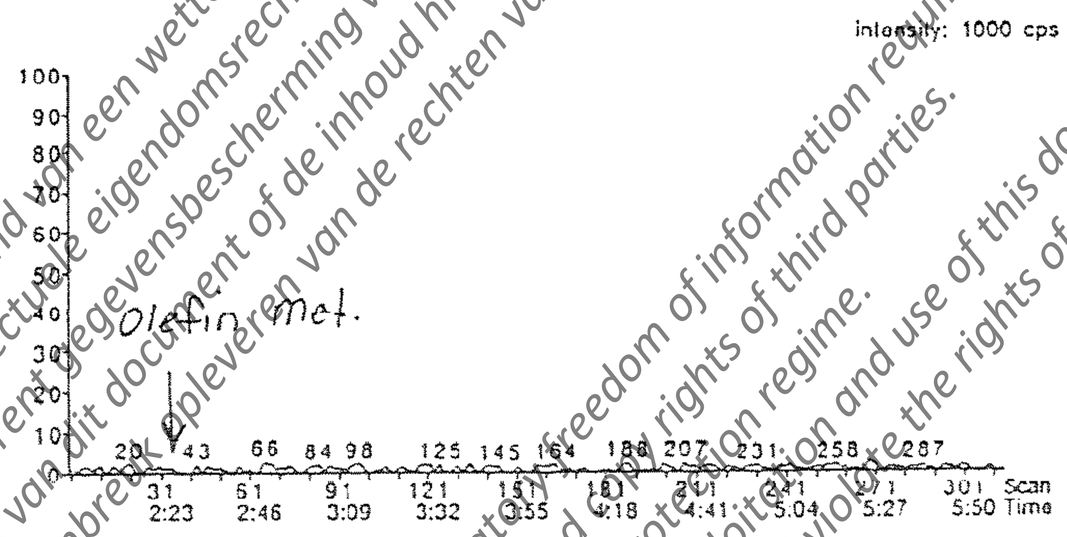
---

NM122100A001 blank Thu, Dec 21, 2000 14:01
solvent

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

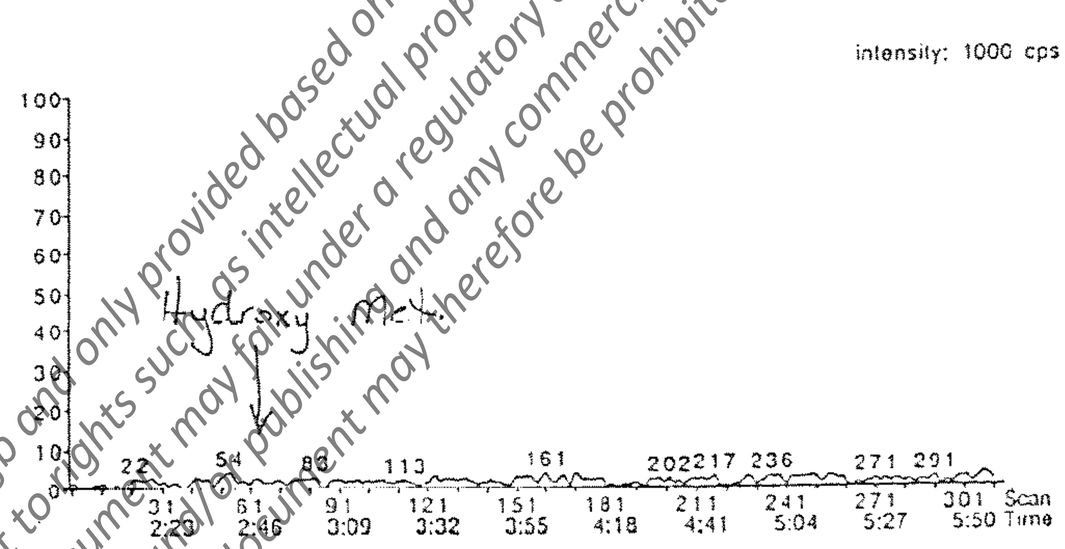


NM122100A001 blank Thu, Dec 21, 2000 14:01
solvent

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	6
Mult. Width	4
Base Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

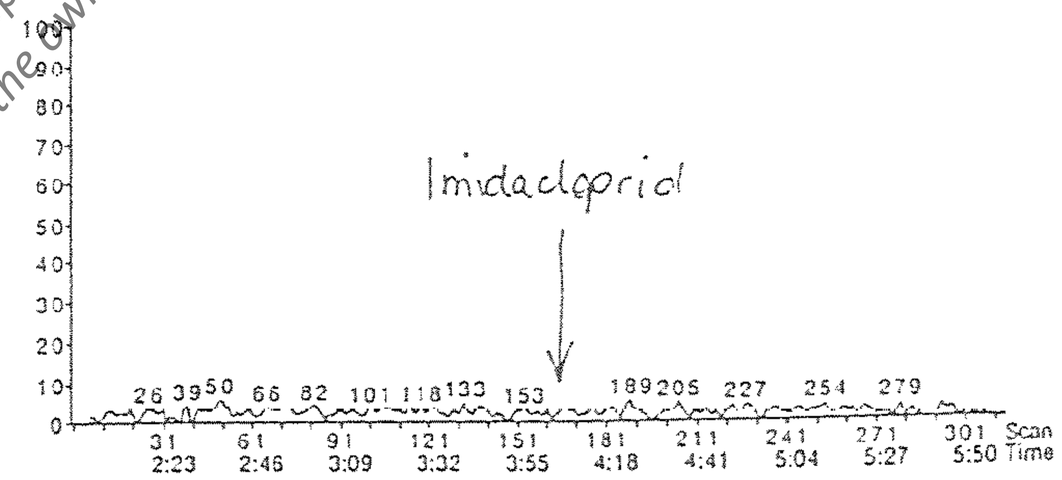


NM122100A001 blank Thu, Dec 21, 2000 14:01
solvent

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



CB Jan. 3, 2001

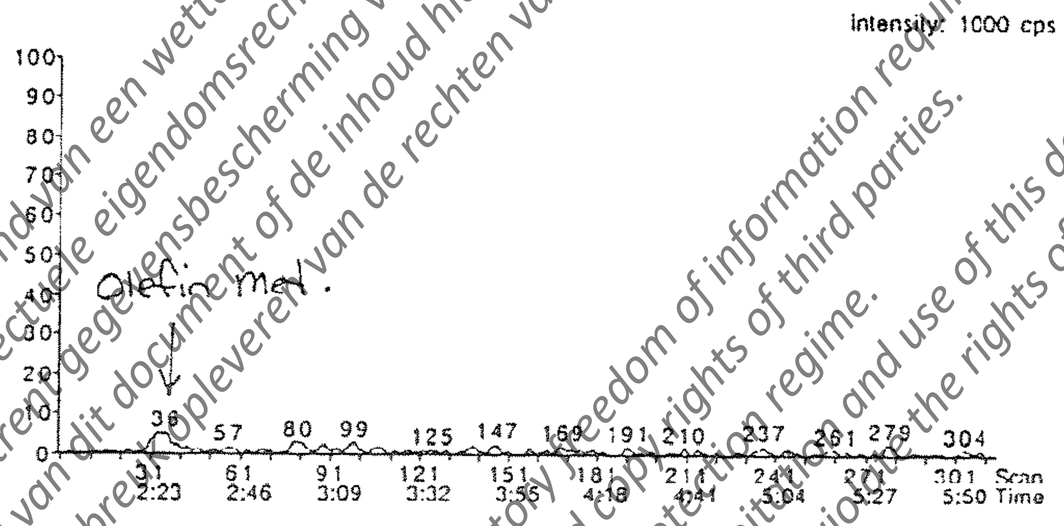
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A002 1.00 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 14:18
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->209.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	323
Height	52
Start Time	2:19
End Time	2:31
Integration Width	0:12.2
Retention Time	2:27
Integration Type	A - BV

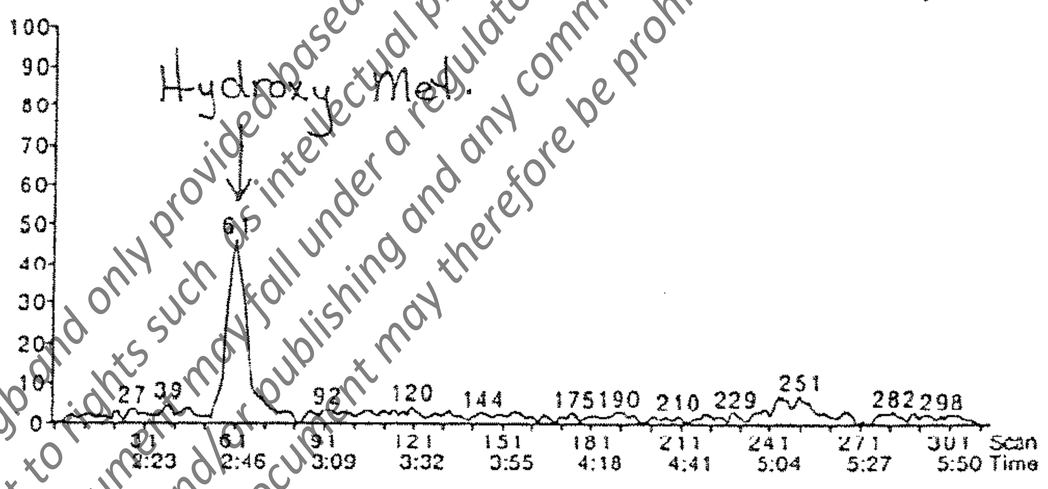


NM122100A002 1.00 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 14:18
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	2568
Height	456
Start Time	2:40
End Time	2:57
Integration Width	0:16.8
Retention Time	2:46
Integration Type	A - VV

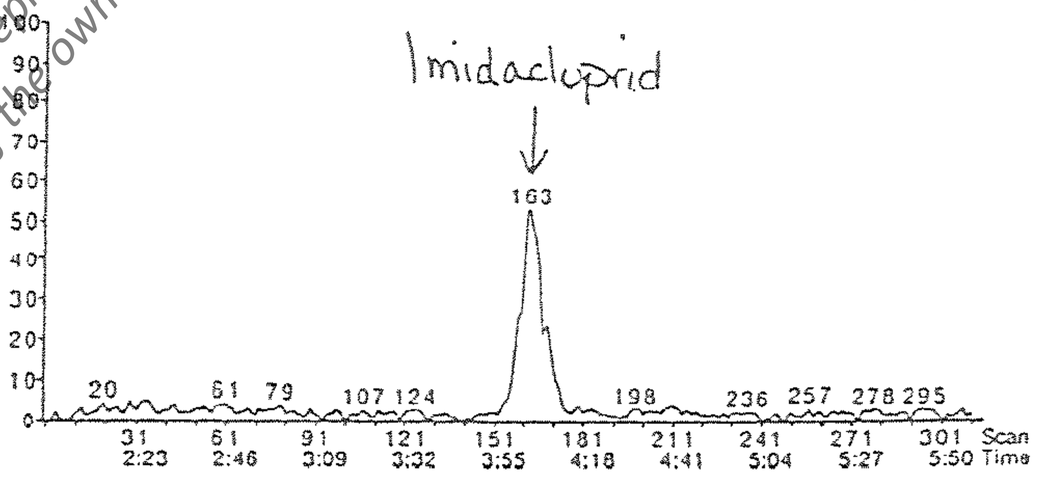


NM122100A002 1.00 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 14:18
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	3713
Height	523
Start Time	3:56
End Time	4:13
Integration Width	0:16.4
Retention Time	4:04
Integration Type	A - VV



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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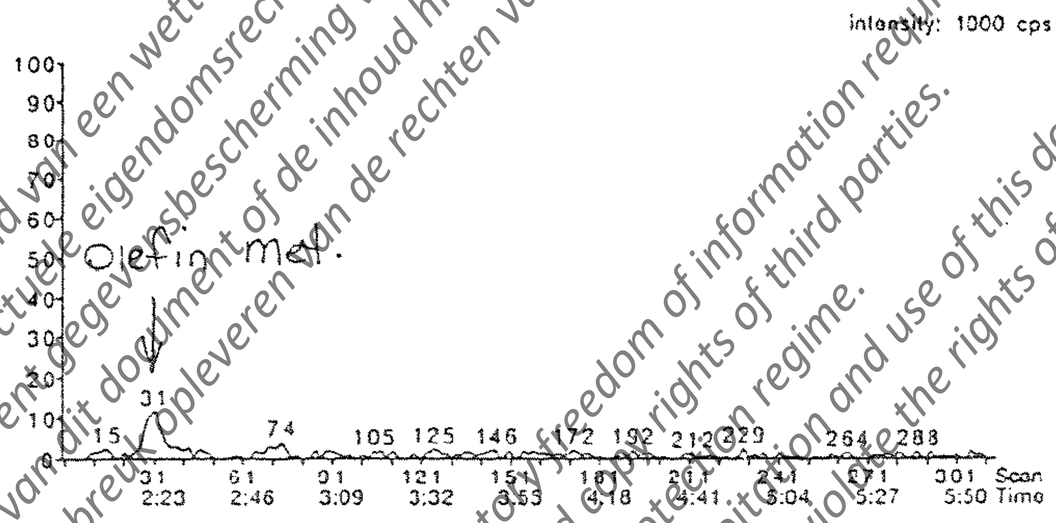
NM122100A003 2.50 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 14:36
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	700
Height	116
Start Time	2:17
End Time	2:30
Integration Width	0:13.0
Retention Time	2:23
Integration Type	A - VV

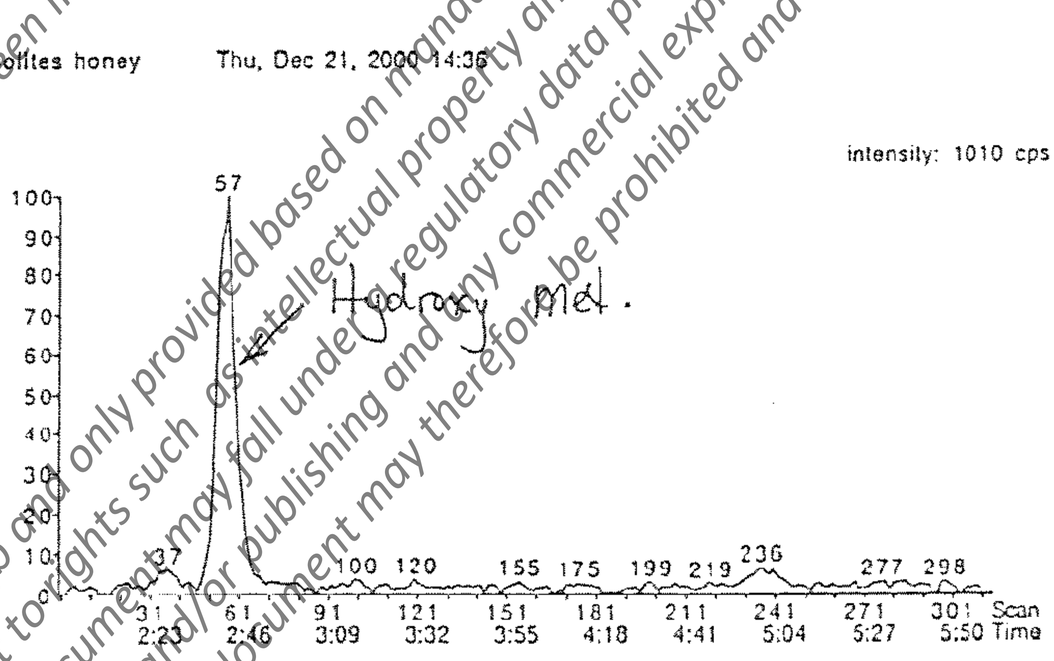


NM122100A003 2.50 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 14:36
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	3
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	5795
Height	1003
Start Time	2:36
End Time	2:52
Integration Width	0:16.8
Retention Time	2:43
Integration Type	A - VV

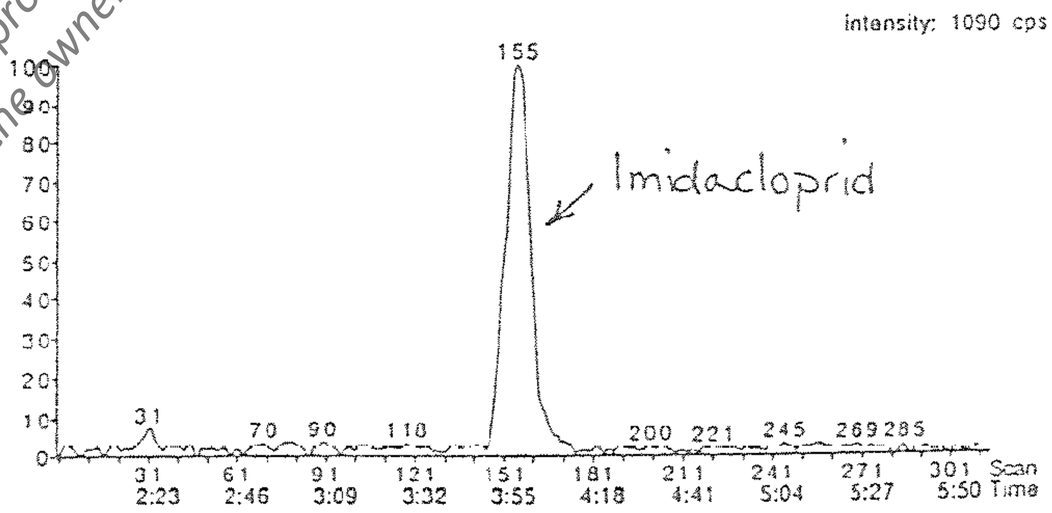


NM122100A003 2.50 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 14:36
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	8458
Height	1083
Start Time	3:51
End Time	4:13
Integration Width	0:22.2
Retention Time	3:58
Integration Type	A - VB



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A

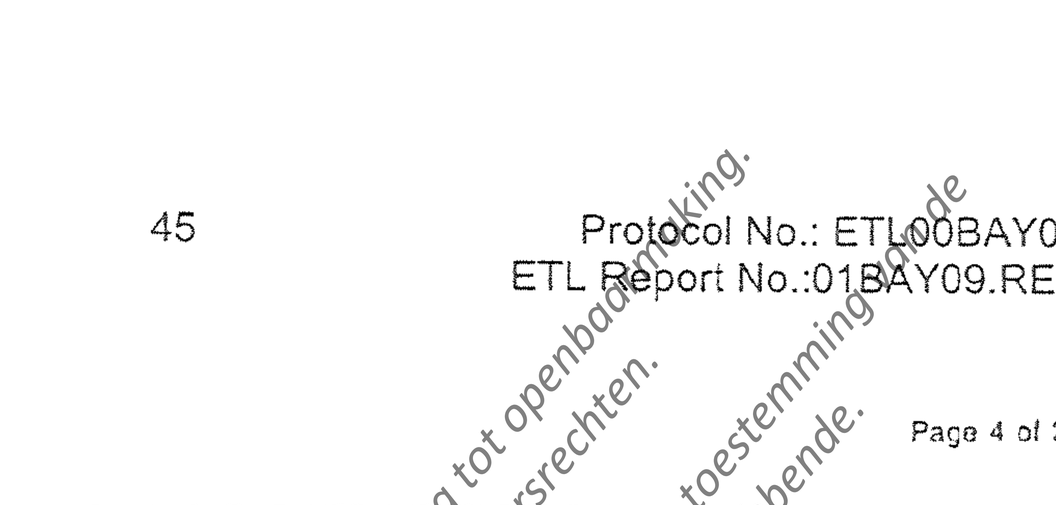
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A004 E0-08-001-11A-2, 1mL FV Thu, Dec 21, 2000 14:54
GVS ROAD NECTAR 07/19/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

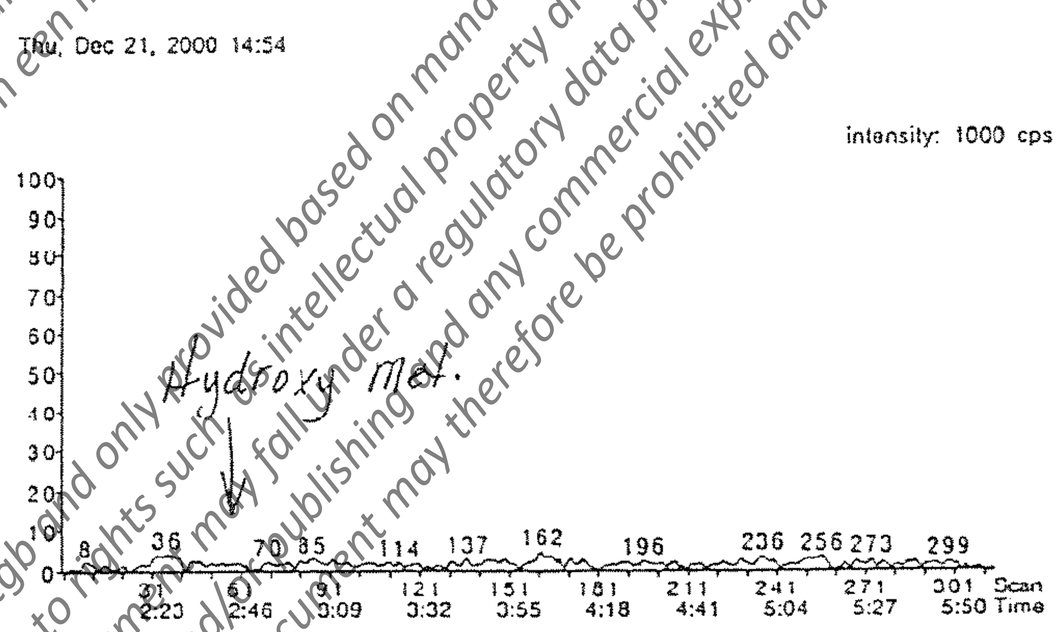


NM122100A004 E0-08-001-11A-2, 1mL FV Thu, Dec 21, 2000 14:54
GVS ROAD NECTAR 07/19/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

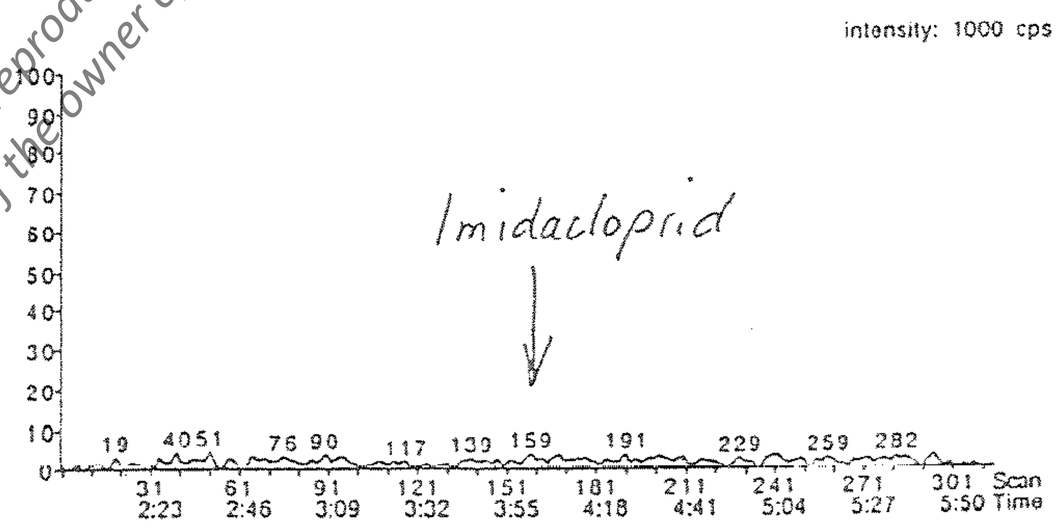


NM122100A004 E0-08-001-11A-2, 1mL FV Thu, Dec 21, 2000 14:54
GVS ROAD NECTAR 07/19/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	6
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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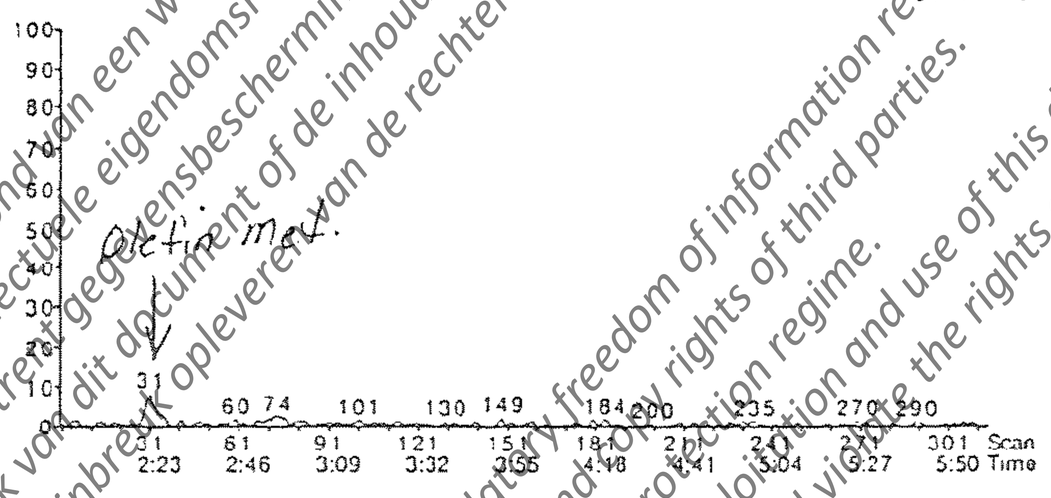
NM122100A005 E0-08-001-11A+1, 1mL FV Thu, Dec 21, 2000 15:12
GVS ROAD NECTAR 07/19/00 0.001ppm spike

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3.59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	308
Height	83
Start Time	2:17
End Time	2:29
Integration Width	0:11.5
Retention Time	2:23
Integration Type	A - BB



NM122100A005 E0-08-001-11A+1, 1mL FV Thu, Dec 21, 2000 15:12
GVS ROAD NECTAR 07/19/00 0.001ppm spike

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->190.0

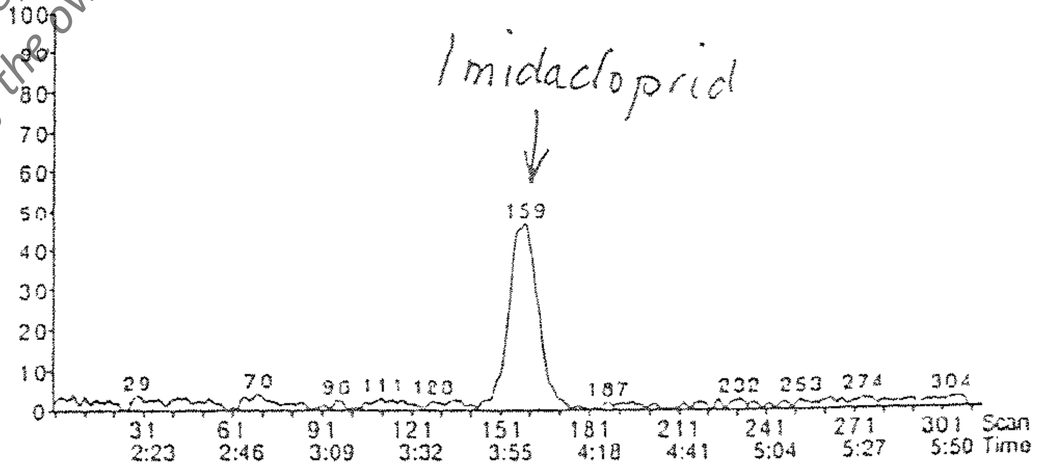
Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	6
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	2508
Height	454
Start Time	2:37
End Time	2:50
Integration Width	0:13.0
Retention Time	2:43
Integration Type	A - BV

NM122100A005 E0-08-001-11A+1, 1mL FV Thu, Dec 21, 2000 15:12
GVS ROAD NECTAR 07/19/00 0.001ppm spike

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	6
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	3949
Height	460
Start Time	3:53
End Time	4:10
Integration Width	0:17.6
Retention Time	4:01
Integration Type	M



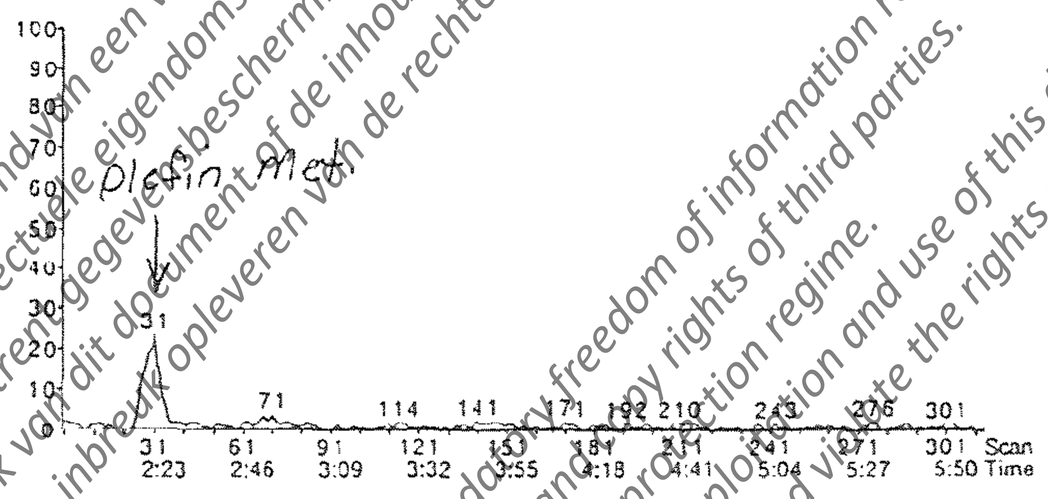
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A006 E0-08-001-11A+2, 1mL FV Thu, Dec 21, 2000 15:30
GVS ROAD NECTAR 07/19/00 0.005ppm spike

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	1093
Height	233
Start Time	2:17
End Time	2:27
Integration Width	0:09.9
Retention Time	2:23
Integration Type	A - BV

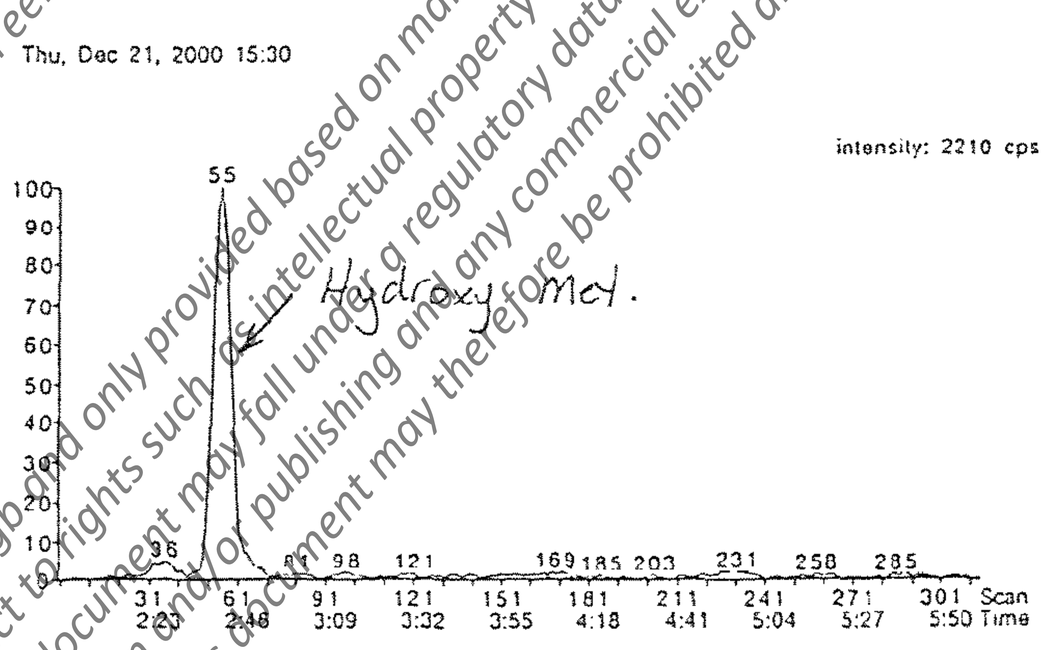


NM122100A006 E0-08-001-11A+2, 1mL FV Thu, Dec 21, 2000 15:30
GVS ROAD NECTAR 07/19/00 0.005ppm spike

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:40
Area	12687
Height	2204
Start Time	2:33
End Time	2:54
Integration Width	0:20.7
Retention Time	2:42
Integration Type	A - VV

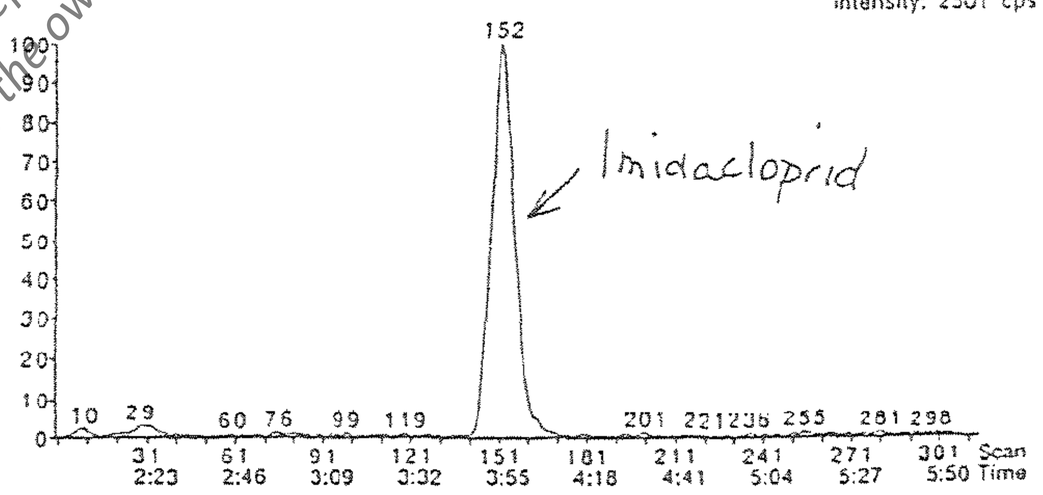


NM122100A006 E0-08-001-11A+2, 1mL FV Thu, Dec 21, 2000 15:30
GVS ROAD NECTAR 07/19/00 0.005ppm spike

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	18036
Height	2497
Start Time	3:41
End Time	4:11
Integration Width	0:00.6
Retention Time	3:56
Integration Type	A - BB



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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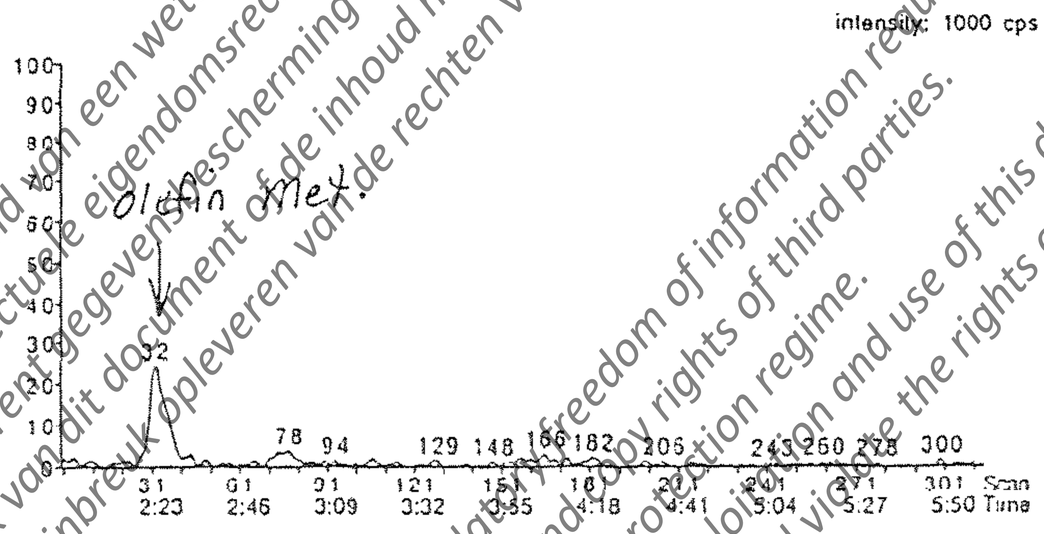
NM122100A007 5.00 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 15:47
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	1263
Height	245
Start Time	2:19
End Time	2:30
Integration Width	0:11.5
Retention Time	2:24
Integration Type	A - VV

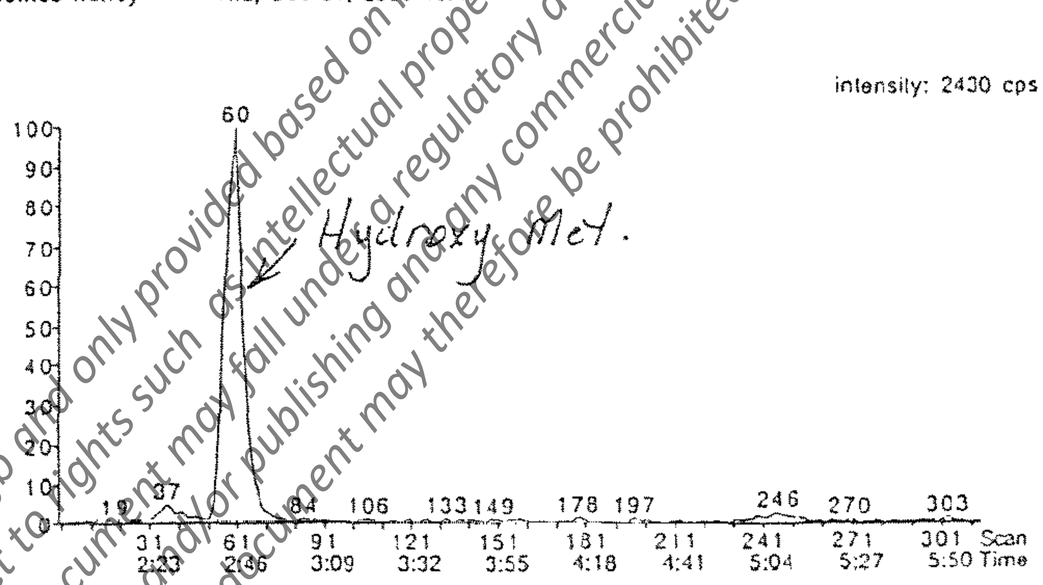


NM122100A007 5.00 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 15:47
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	14015
Height	2423
Start Time	2:30
End Time	2:59
Integration Width	0:21.4
Retention Time	2:45
Integration Type	A - VV

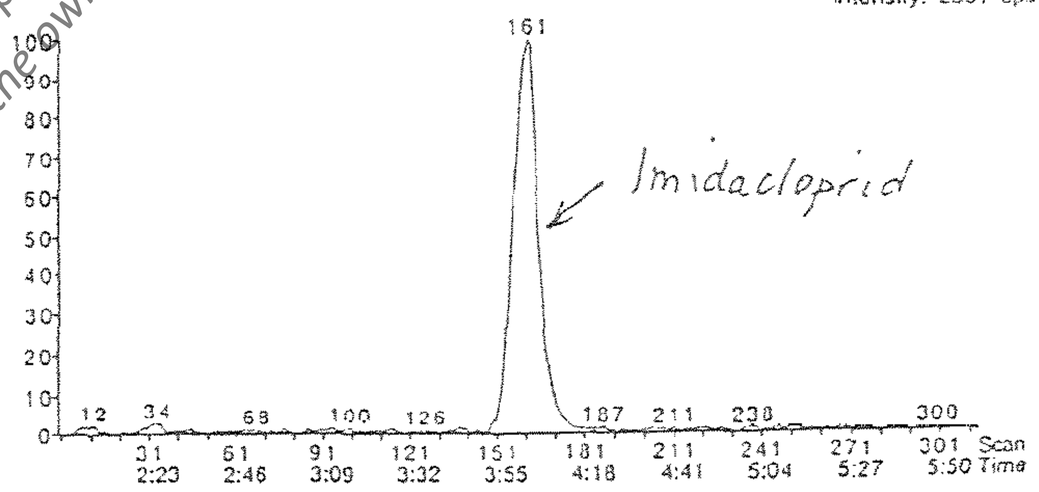


NM122100A007 5.00 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 15:47
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	18585
Height	2327
Start Time	3:52
End Time	4:28
Integration Width	0:36.7
Retention Time	4:03
Integration Type	A - BB



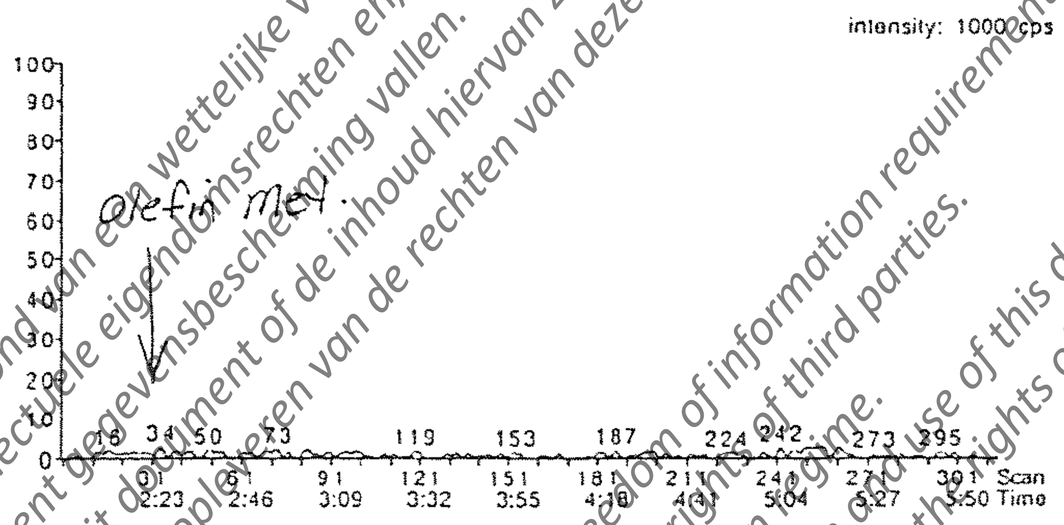
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A008 E0-08-001-05A, 1mL FV Thu, Dec 21, 2000 16:05
GVH FARM NECTAR 07/10/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

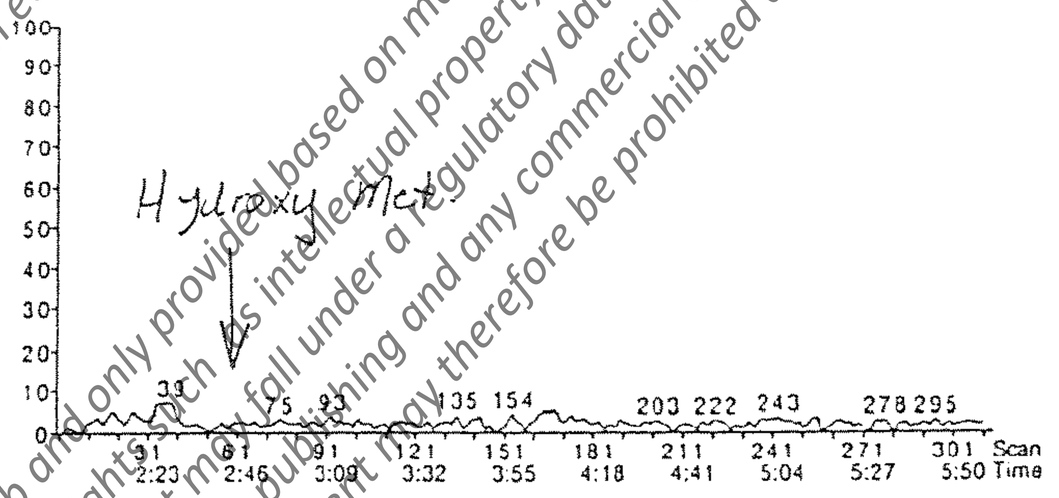


NM122100A008 E0-08-001-05A, 1mL FV Thu, Dec 21, 2000 16:05
GVH FARM NECTAR 07/10/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

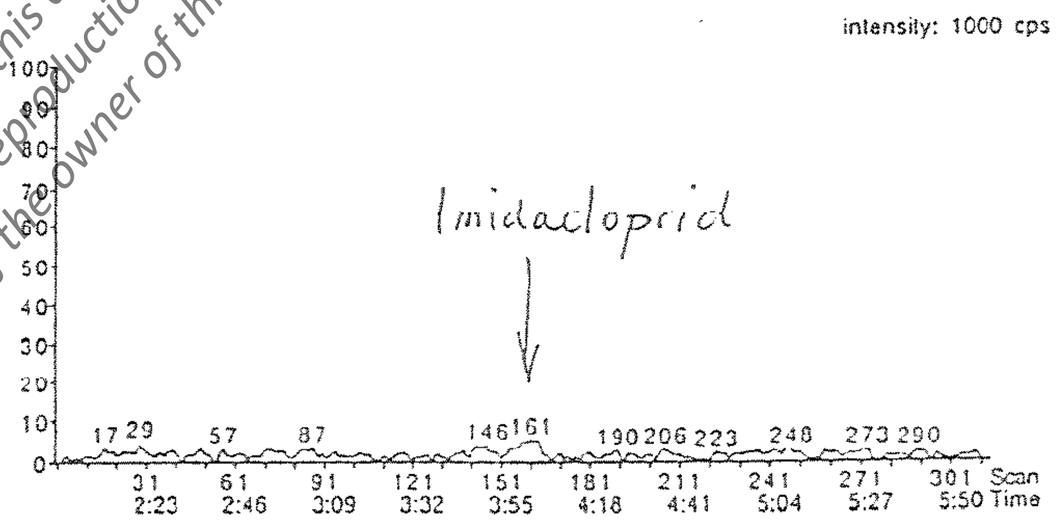


NM122100A008 E0-08-001-05A, 1mL FV Thu, Dec 21, 2000 16:05
GVH FARM NECTAR 07/10/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	1:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



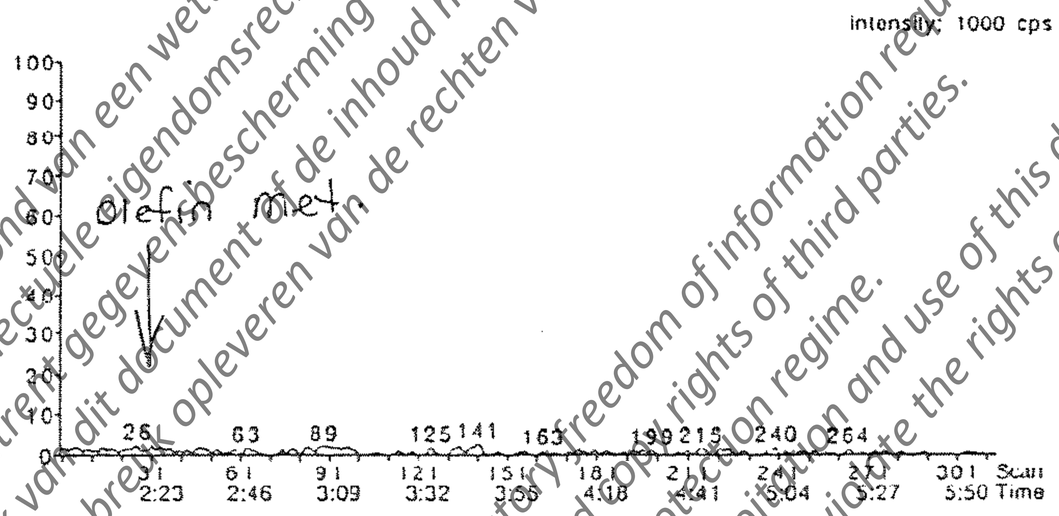
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A009 E0-08-001-08A, 1mL FV Thu, Dec 21, 2000 16:23
GVH FARM NECTAR 07/19/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

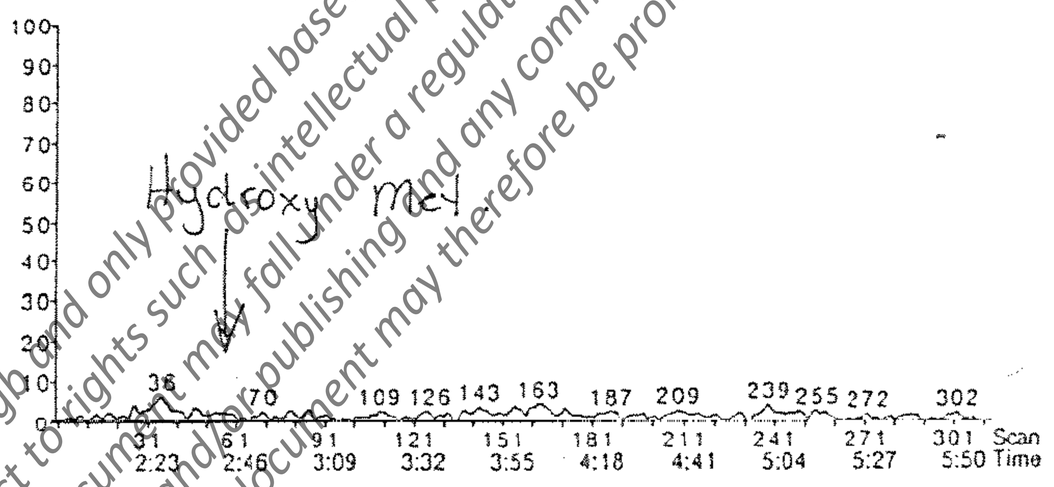


NM122100A009 E0-08-001-08A, 1mL FV Thu, Dec 21, 2000 16:23
GVH FARM NECTAR 07/19/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

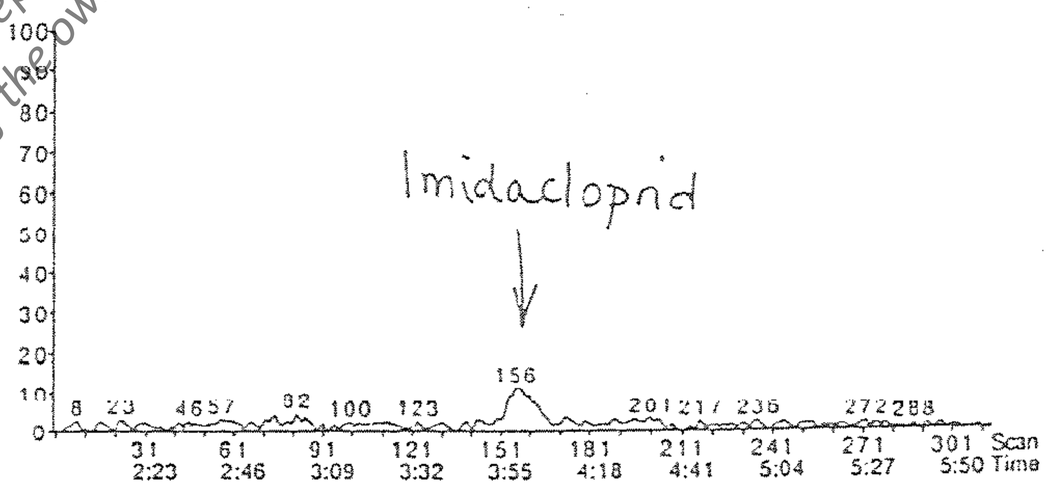


NM122100A009 E0-08-001-08A, 1mL FV Thu, Dec 21, 2000 16:23
GVH FARM NECTAR 07/19/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	2
Mult. Width	3
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	809
Height	100
Start Time	3:53
End Time	4:08
Integration Width	0:15.0
Retention Time	3:59
Integration Type	M



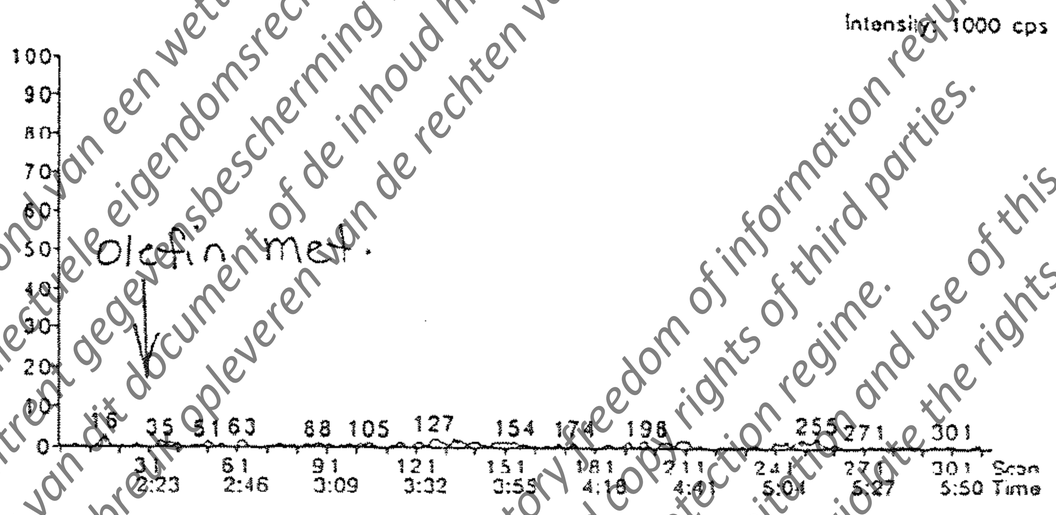
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A010 E0-08-001-09AB, 1mL FV Thu, Dec 21, 2000 16:41
GVS FARM NECTAR 07/10/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

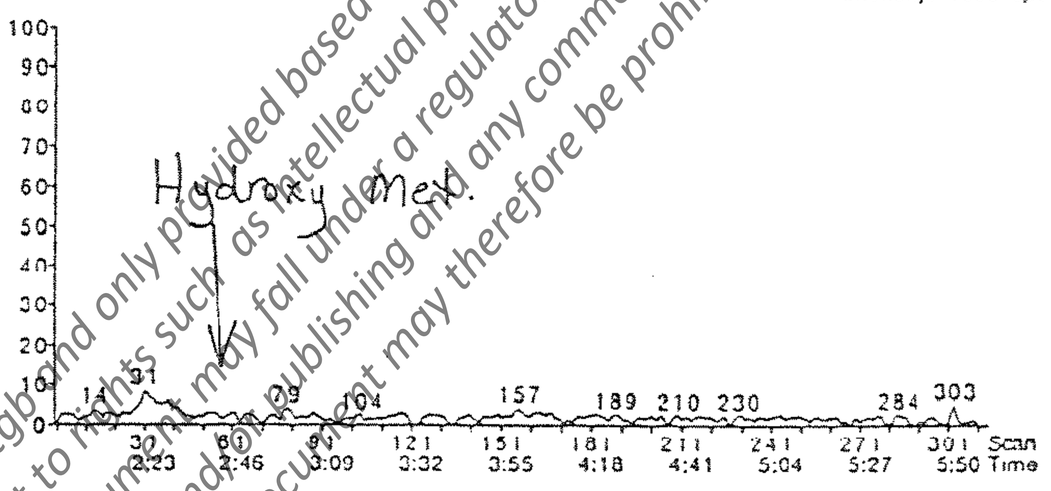


NM122100A010 E0-08-001-09AB, 1mL FV Thu, Dec 21, 2000 16:41
GVS FARM NECTAR 07/10/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

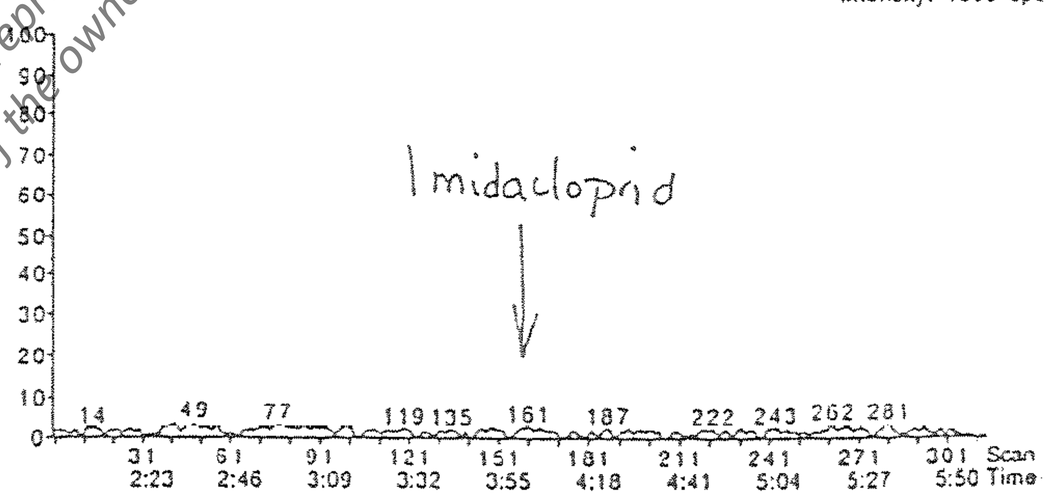


NM122100A010 E0-08-001-09AB, 1mL FV Thu, Dec 21, 2000 16:41
GVS FARM NECTAR 07/10/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



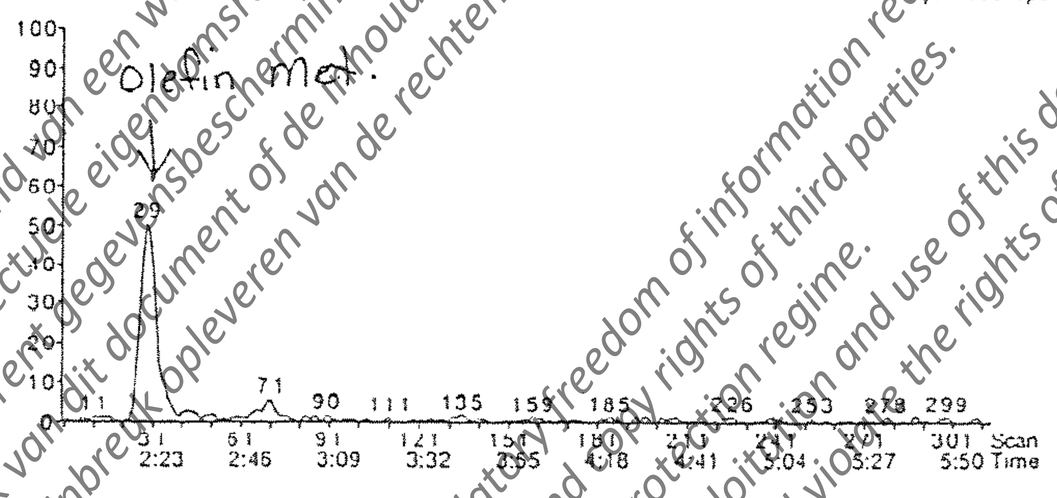
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A011 10.0 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 16:59
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	2486
Height	502
Start Time	2:16
End Time	2:29
Integration Width	0:13.8
Retention Time	2:22
Integration Type	A - BV

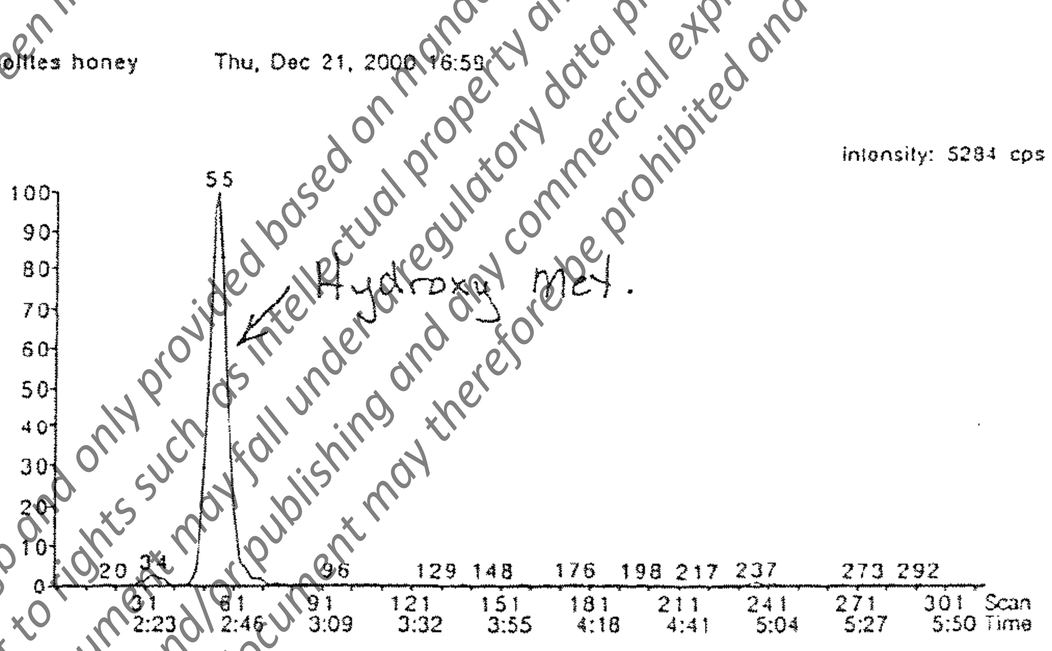


NM122100A011 10.0 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 16:59
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	29346
Height	5278
Start Time	2:33
End Time	2:55
Integration Width	0:21.4
Retention Time	2:42
Integration Type	A - VV

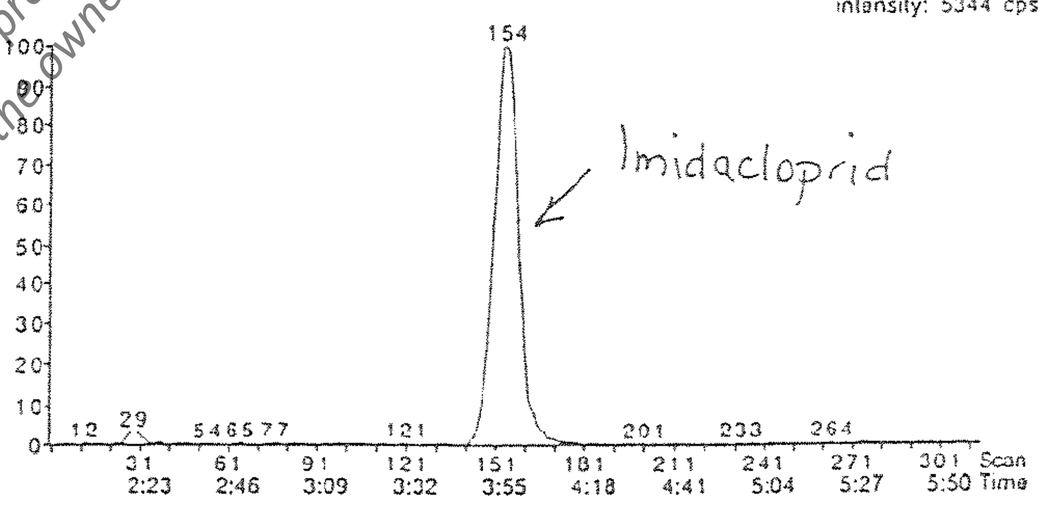


NM122100A011 10.0 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 16:59
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	8
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	40554
Height	5342
Start Time	3:44
End Time	4:17
Integration Width	0:33.0
Retention Time	3:57
Integration Type	A - BB



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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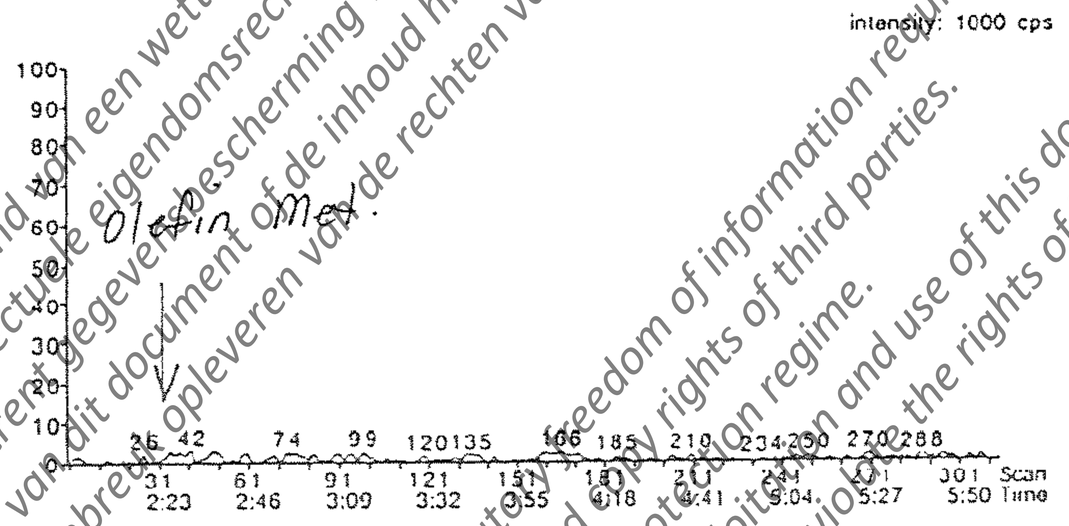
NM122100A012 E0-08-003-02A, 1mL FV Thu, Dec 21, 2000 17:16
NECTAR CONTROL ALL 06/07/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	M

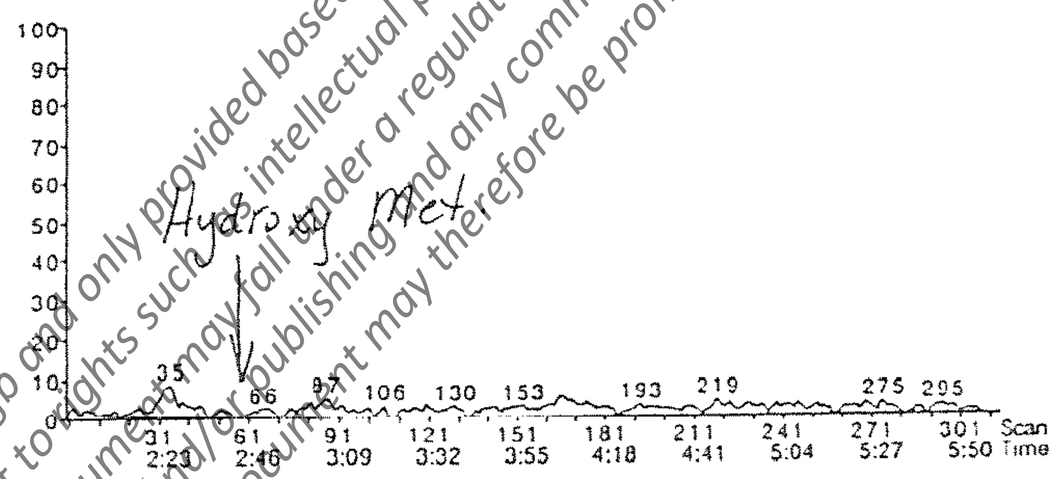


NM122100A012 E0-08-003-02A, 1mL FV Thu, Dec 21, 2000 17:16
NECTAR CONTROL ALL 06/07/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

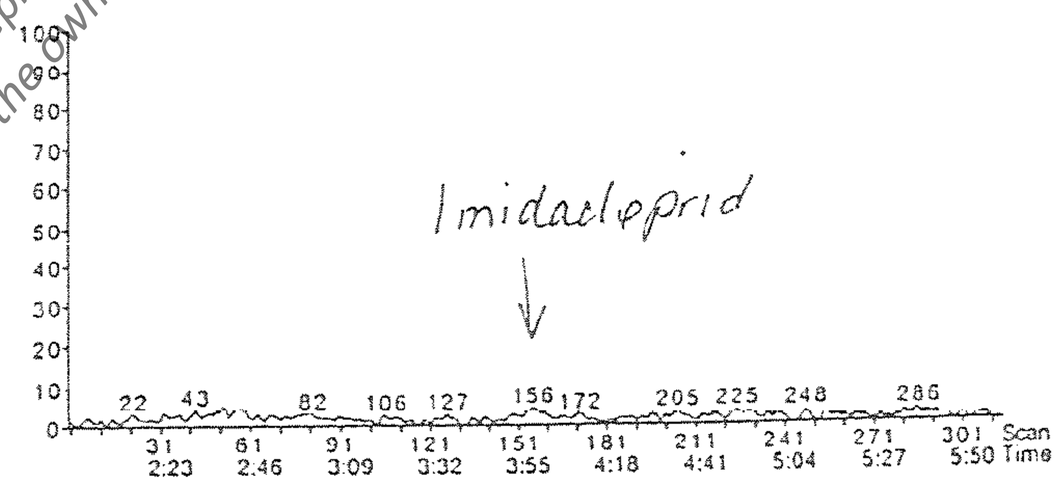


NM122100A012 E0-08-003-02A, 1mL FV Thu, Dec 21, 2000 17:16
NECTAR CONTROL ALL 06/07/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



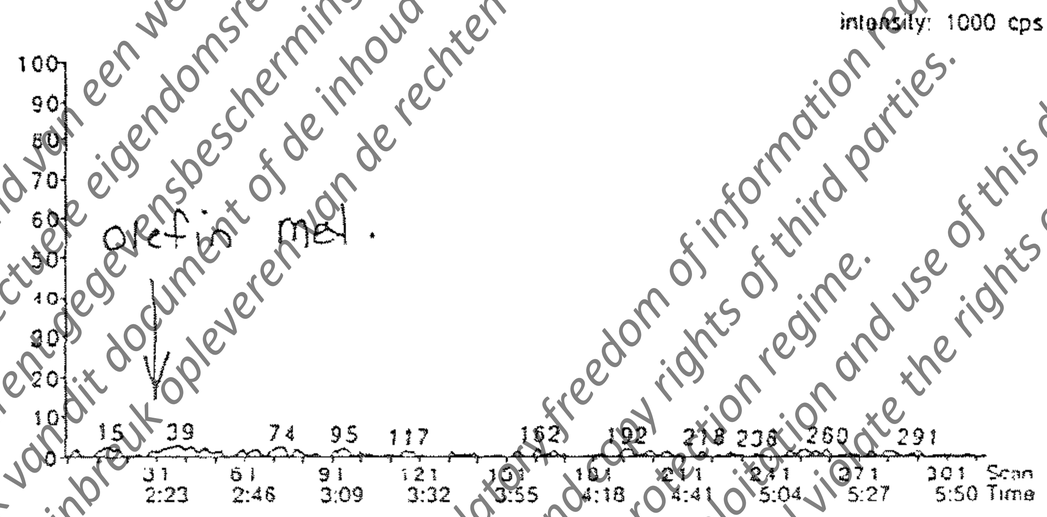
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A013 E0-08-003-03A, 1mL FV Thu, Dec 21, 2000 17:34
NECTAR GAUCHO ALL 06/07/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0
Noise Thres. 1.0
Quant Thres. 0.1
Min. Width 8
Mult. Width 2
Base. Width 100
RT Win. (secs) 10
Smooth 1
Expected RT 2:23

Area 0
Height 0
Start Time 0:00.0
End Time 0:00.0
Integration Width 0:00.0
Retention Time 0:00.0
Integration Type M

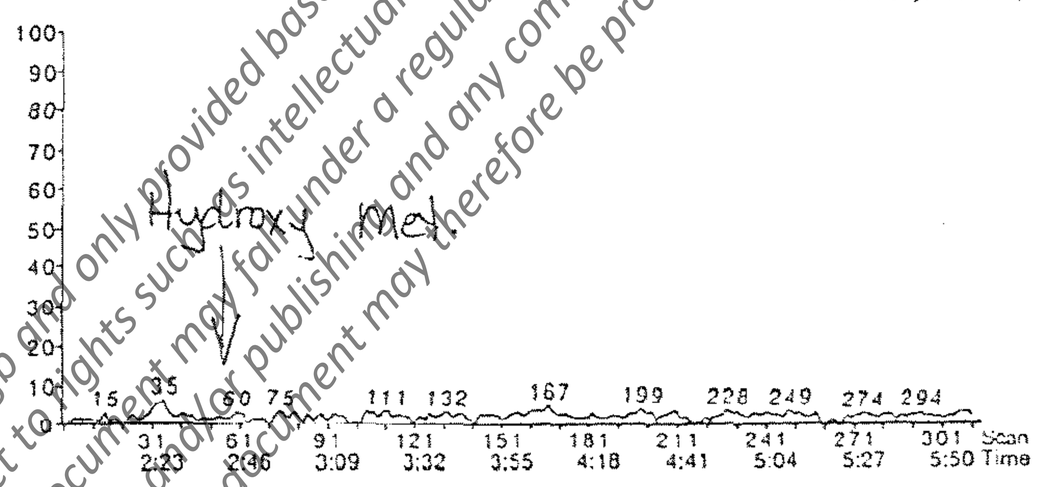


NM122100A013 E0-08-003-03A, 1mL FV Thu, Dec 21, 2000 17:34
NECTAR GAUCHO ALL 06/07/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0
Noise Thres. 5.0
Quant Thres. 0.5
Min. Width 5
Mult. Width 4
Base. Width 100
RT Win. (secs) 6
Smooth 1
Expected RT 2:43

Area 0
Height 0
Start Time 0:00.0
End Time 0:00.0
Integration Width 0:00.0
Retention Time 0:00.0
Integration Type

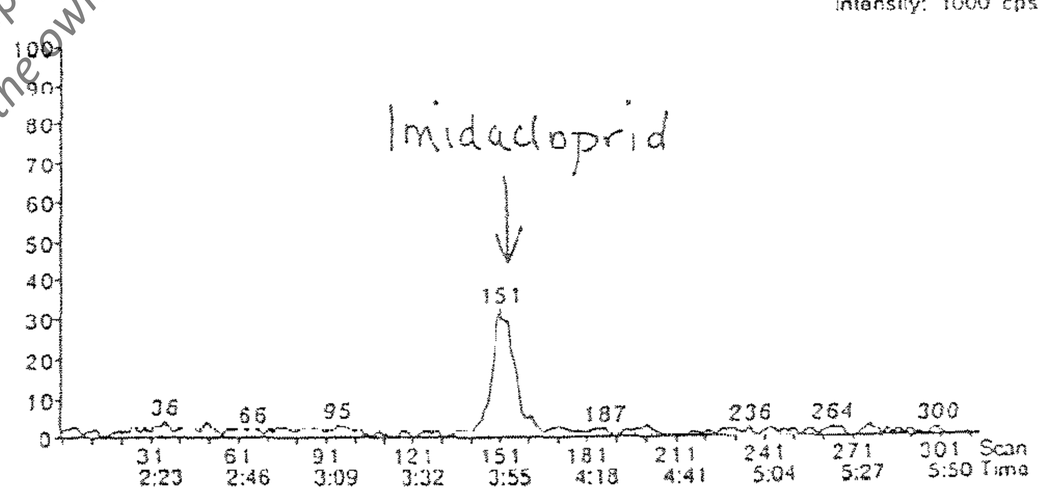


NM122100A013 E0-08-003-03A, 1mL FV Thu, Dec 21, 2000 17:34
NECTAR GAUCHO ALL 06/07/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0
Noise Thres. 10.0
Quant Thres. 0.5
Min. Width 12
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 3:58

Area 2195
Height 313
Start Time 3:45
End Time 4:04
Integration Width 0:19.1
Retention Time 3:55
Integration Type M



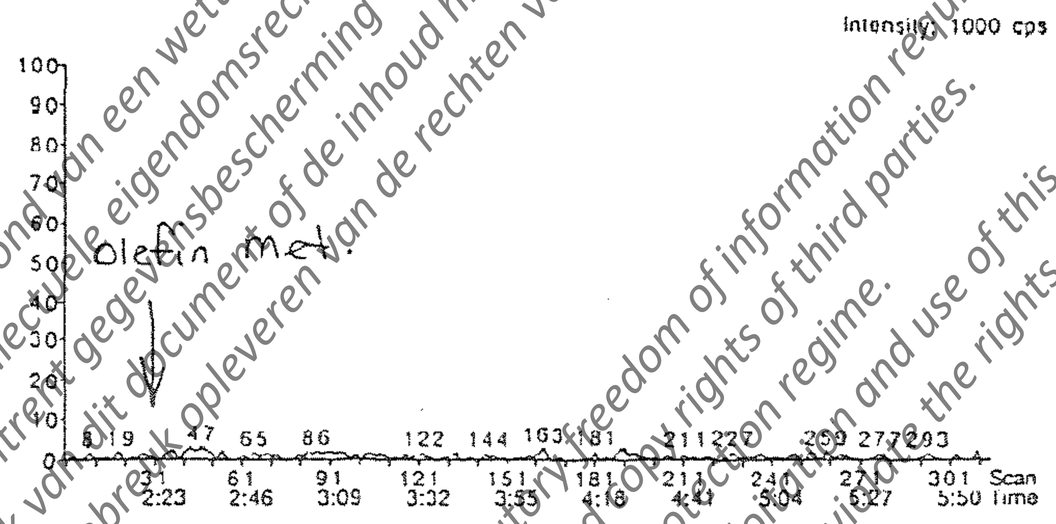
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A014 E0-08-003-05AB, 1mL FV Thu, Dec 21, 2000 17:52
NECTAR CONTROL ALL 12/07/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

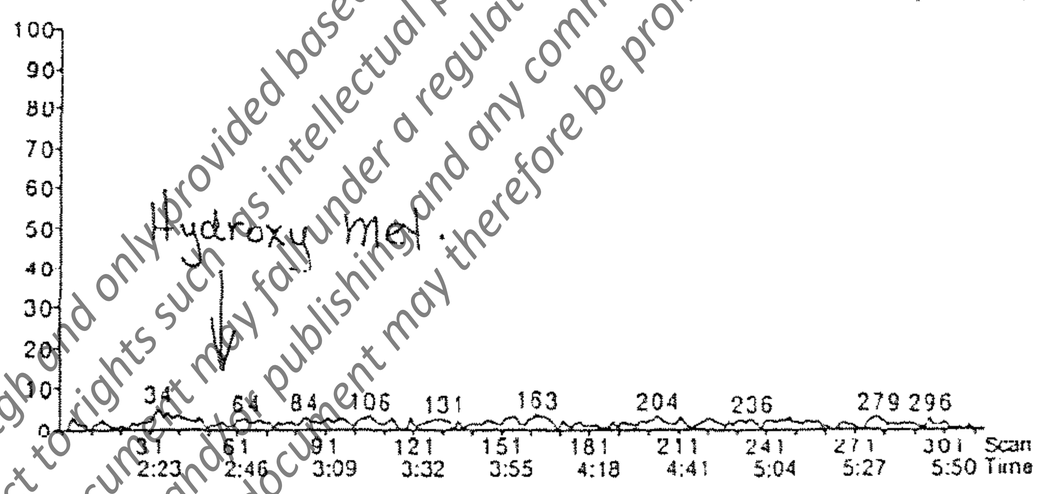


NM122100A014 E0-08-003-05AB, 1mL FV Thu, Dec 21, 2000 17:52
NECTAR CONTROL ALL 12/07/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->190.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

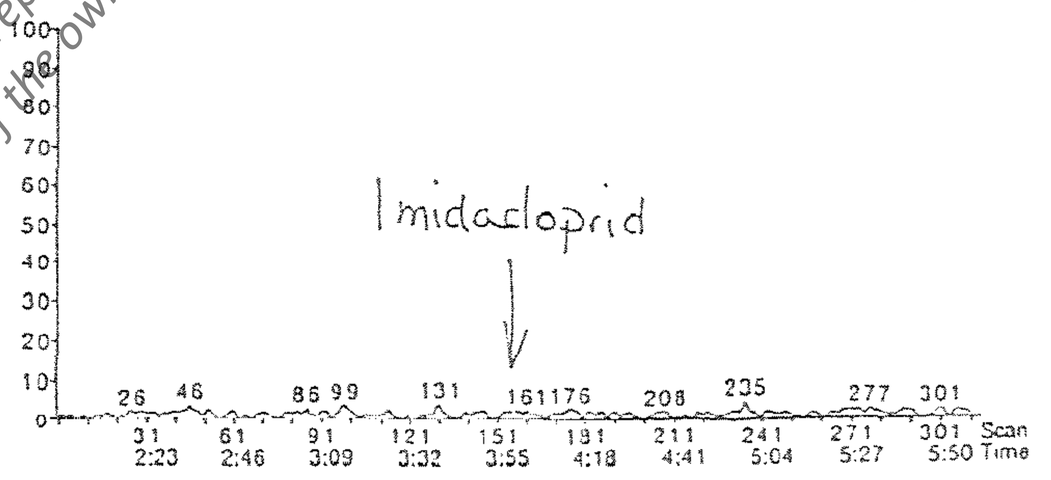


NM122100A014 E0-08-003-05AB, 1mL FV Thu, Dec 21, 2000 17:52
NECTAR CONTROL ALL 12/07/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	6
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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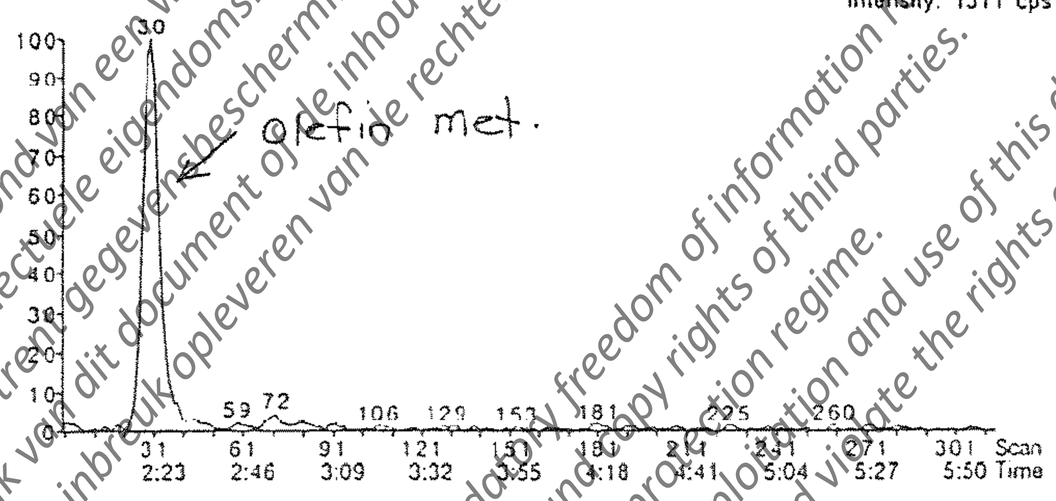
NM122100A015 25.0 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 18:10
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	6810
Height	1310
Start Time	2:16
End Time	2:31
Integration Width	0:14.5
Retention Time	2:23
Integration Type	A - VV

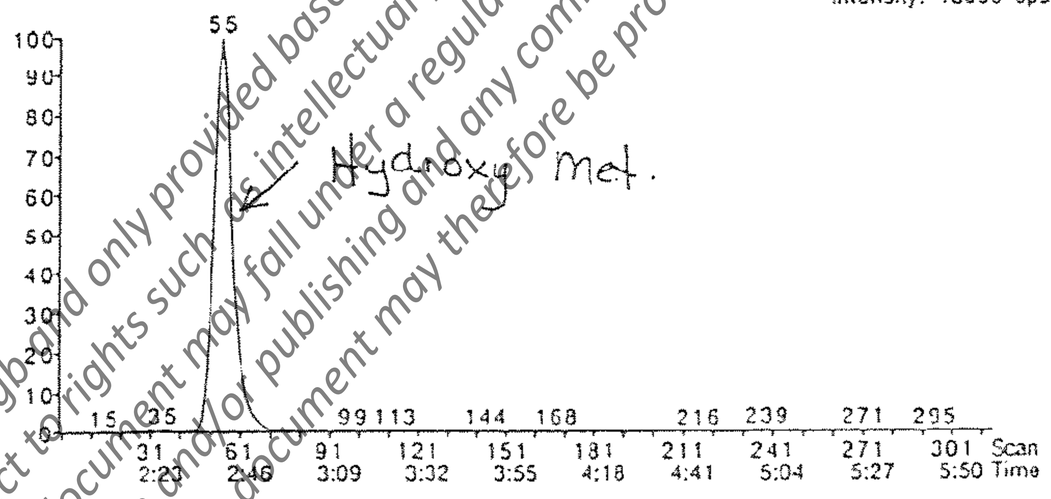


NM122100A015 25.0 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 18:10
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	6
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	73515
Height	13058
Start Time	2:33
End Time	3:04
Integration Width	0:30.6
Retention Time	2:42
Integration Type	A - VB

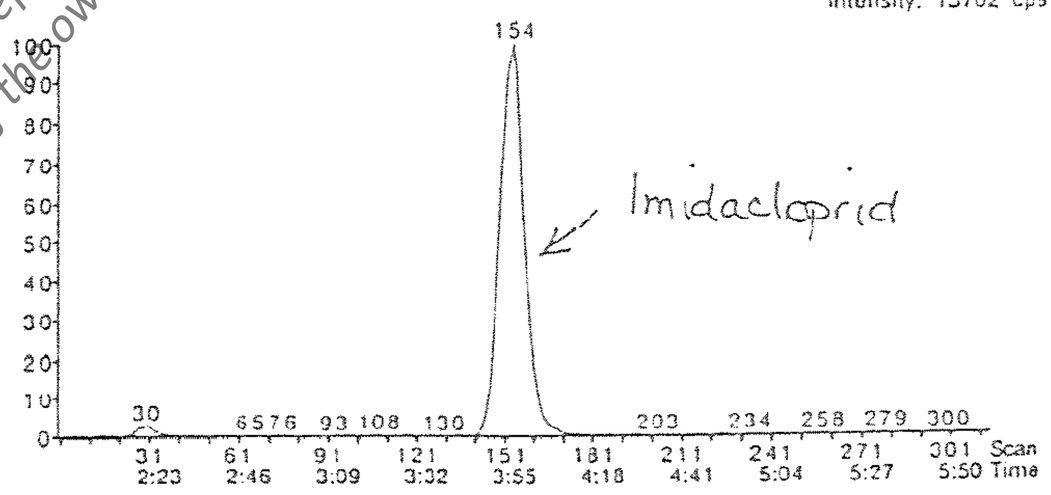


NM122100A015 25.0 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 18:10
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	102590
Height	13696
Start Time	3:43
End Time	4:18
Integration Width	0:35.2
Retention Time	3:57
Integration Type	A - VV



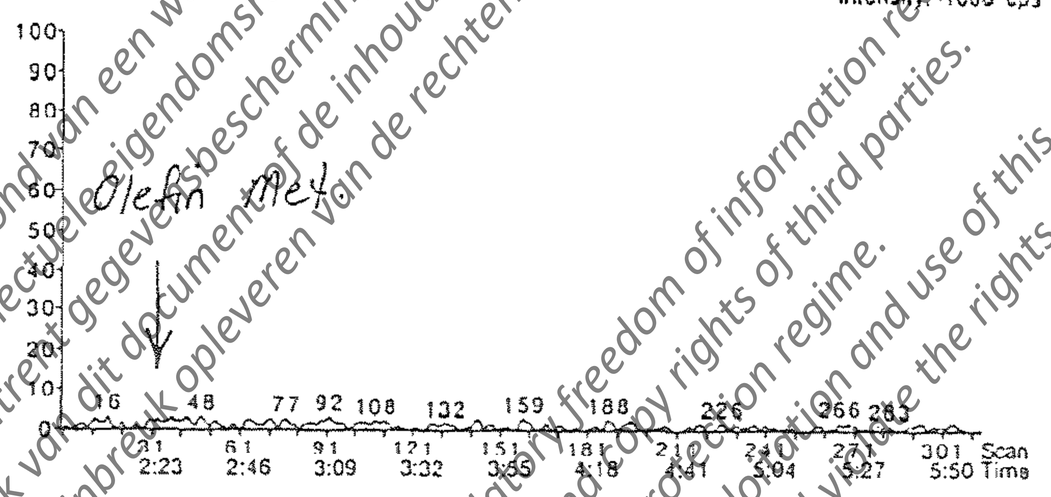
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A016 E0-08-003-06A, 1mL FV Thu, Dec 21, 2000 18:28
NECTAR GAUCHO ALL 12/07/00

5:59 In 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	M

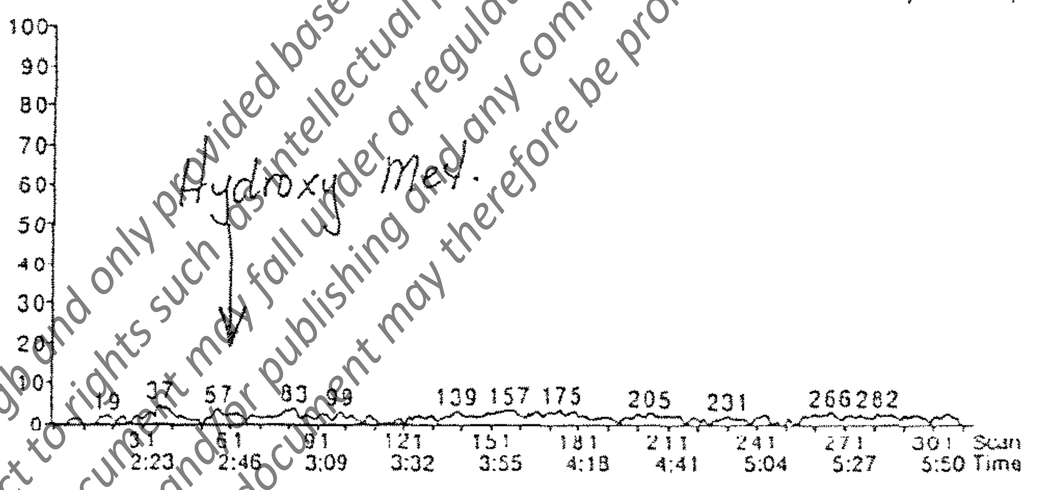


NM122100A016 E0-08-003-06A, 1mL FV Thu, Dec 21, 2000 18:28
NECTAR GAUCHO ALL 12/07/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->190.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

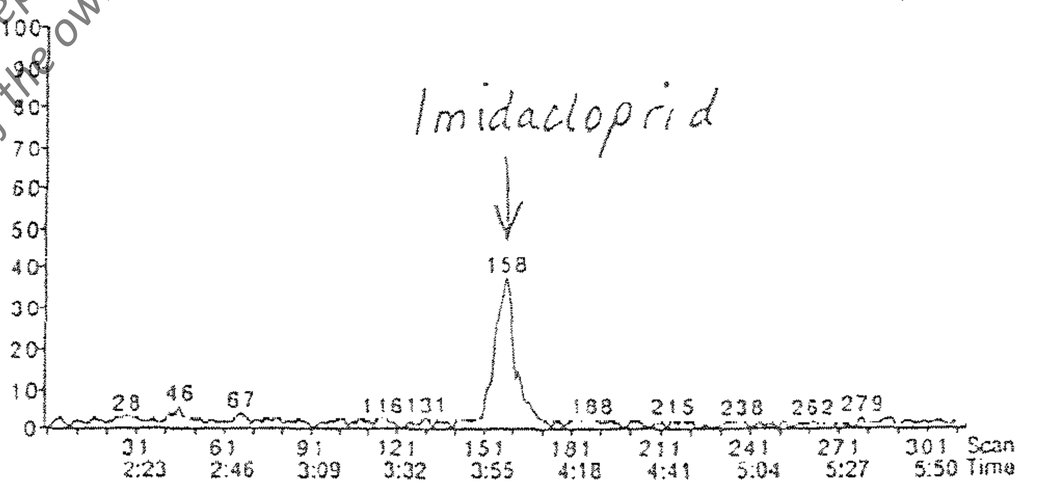


NM122100A016 E0-08-003-06A, 1mL FV Thu, Dec 21, 2000 18:28
NECTAR GAUCHO ALL 12/07/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	2442
Height	366
Start Time	3:51
End Time	4:11
Integration Width	0:19.5
Retention Time	4:00
Integration Type	M

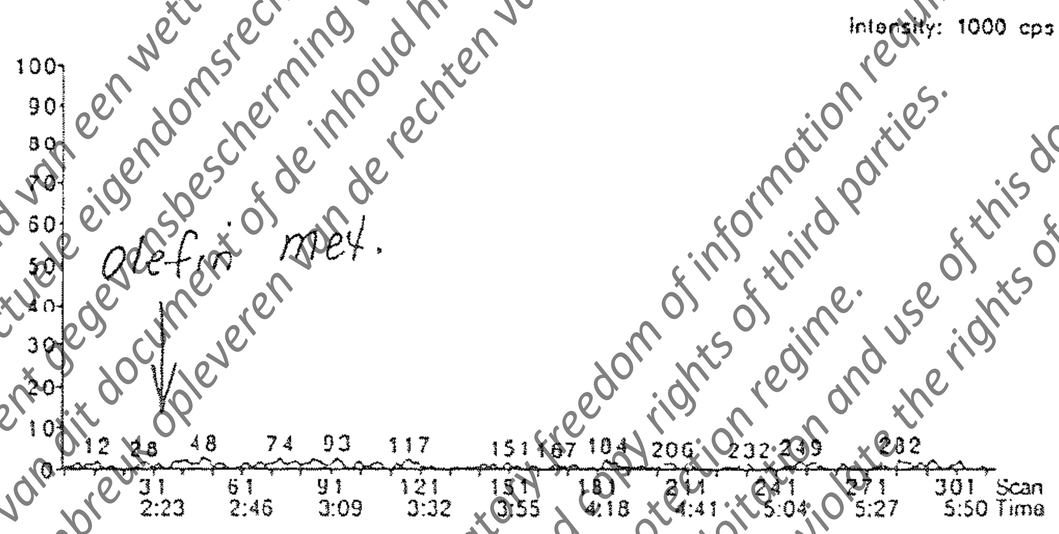


NM122100A017 E0-08-003-09AB, 1mL FV Thu, Dec 21, 2000 18:45
COMP-NECTAR CTL D8,D13 20/07/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

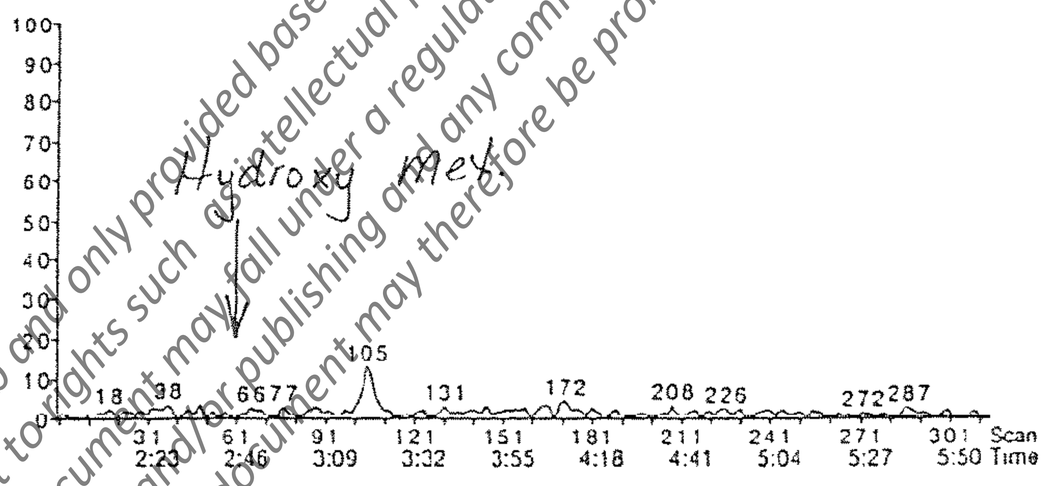


NM122100A017 E0-08-003-09AB, 1mL FV Thu, Dec 21, 2000 18:45
COMP-NECTAR CTL D8,D13 20/07/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

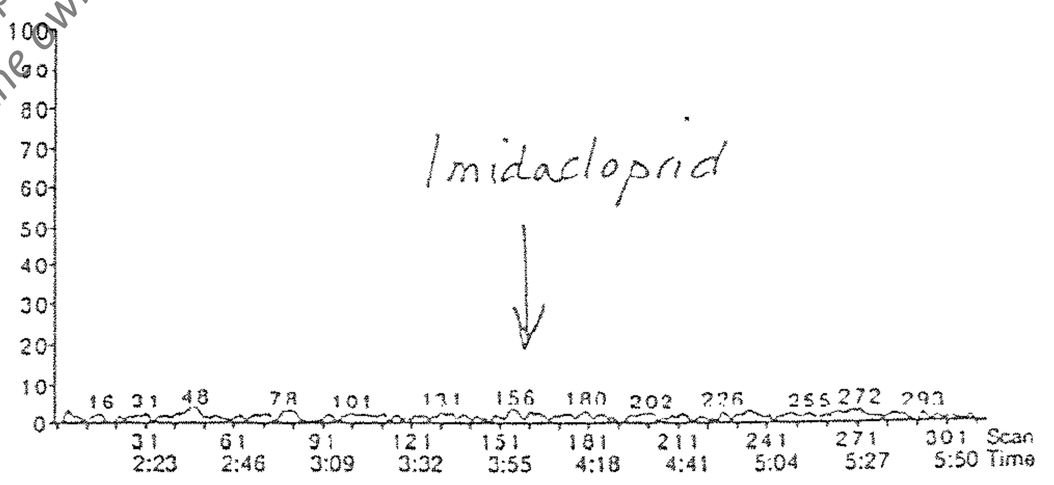


NM122100A017 E0-08-003-09AB, 1mL FV Thu, Dec 21, 2000 18:45
COMP-NECTAR CTL D8,D13 20/07/00

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	



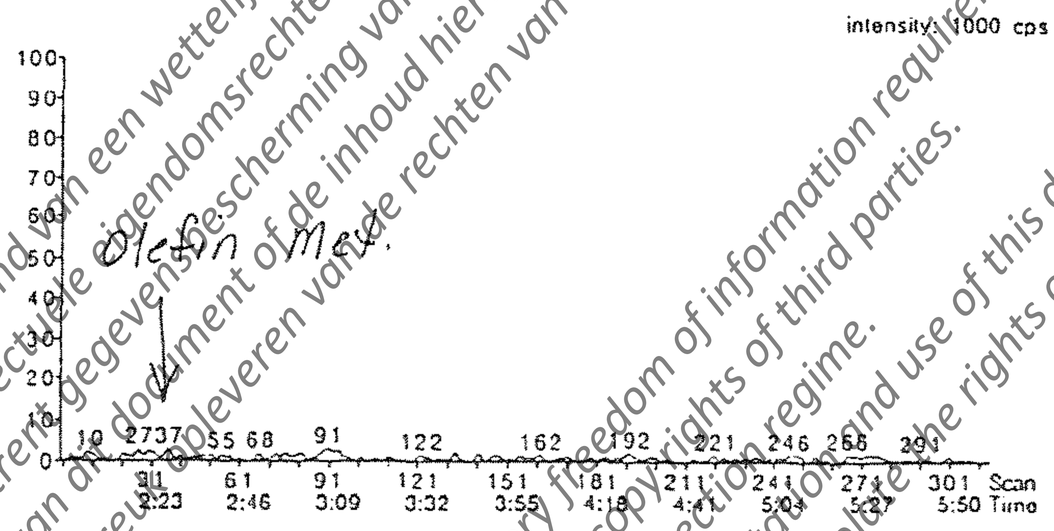
MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

NM122100A018 E0-08-003-10A, 1mL FV Thu, Dec 21, 2000 19:03
NECTAR GAUCHO ALL 20/07/00

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

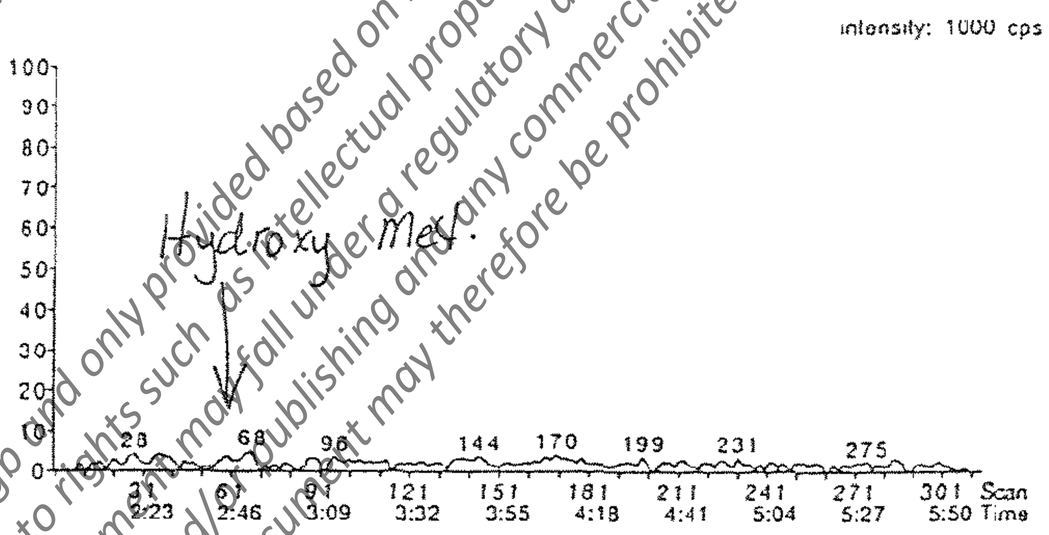


NM122100A018 E0-08-003-10A, 1mL FV Thu, Dec 21, 2000 19:03
NECTAR GAUCHO ALL 20/07/00

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	0
Height	0
Start Time	0:00.0
End Time	0:00.0
Integration Width	0:00.0
Retention Time	0:00.0
Integration Type	

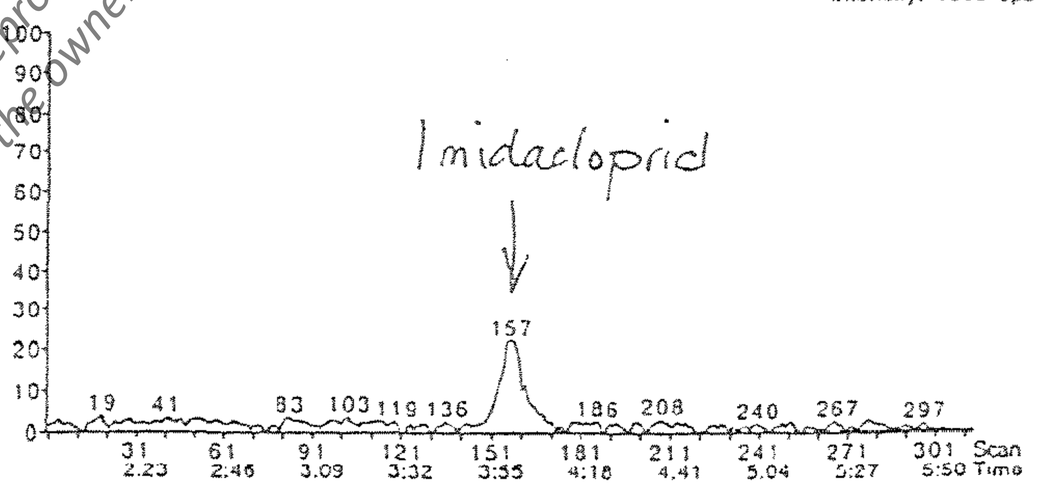


NM122100A018 E0-08-003-10A, 1mL FV Thu, Dec 21, 2000 19:03
NECTAR GAUCHO ALL 20/07/00

6:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	1561
Height	210
Start Time	3:51
End Time	4:10
Integration Width	0:19.1
Retention Time	3:60
Integration Type	M



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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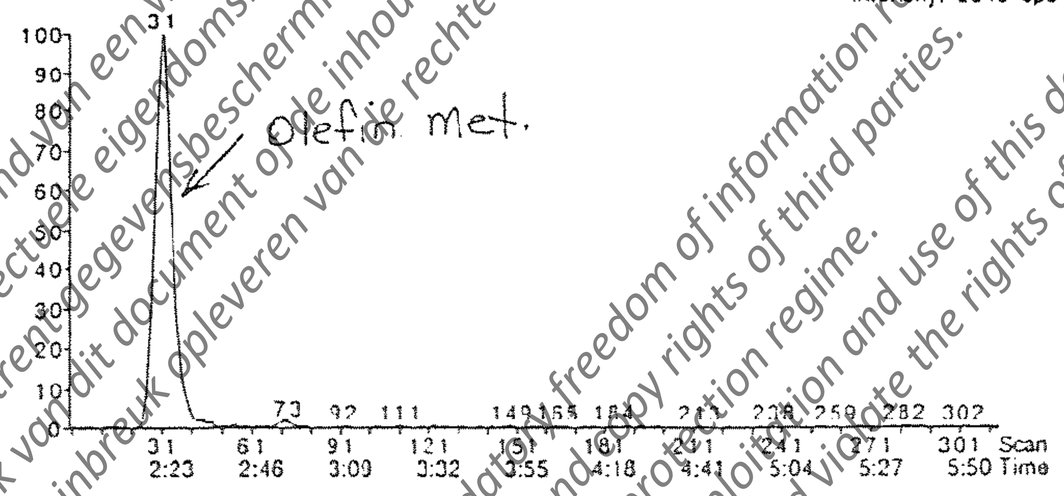
NM122100A019 standard — 50.0 ppb Imidicloprid and metabolites honey — Thu, Dec 21, 2000 19:21

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	13763
Height	2646
Start Time	2:10
End Time	2:34
Integration Width	0:18.4
Retention Time	2:23
Integration Type	A - BV

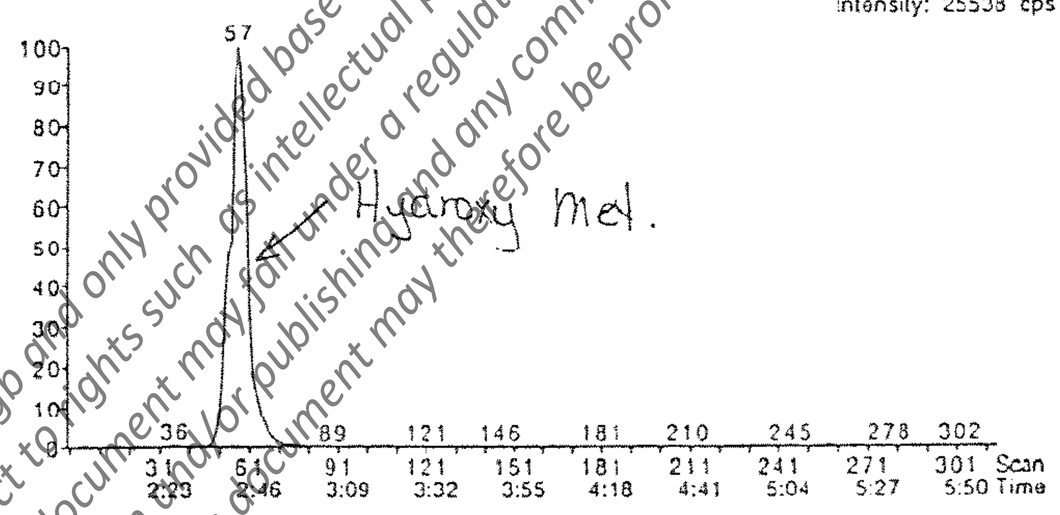


NM122100A019 standard — 50.0 ppb Imidicloprid and metabolites honey — Thu, Dec 21, 2000 19:21

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	138968
Height	25538
Start Time	2:32
End Time	3:03
Integration Width	0:30.6
Retention Time	2:43
Integration Type	A - VV

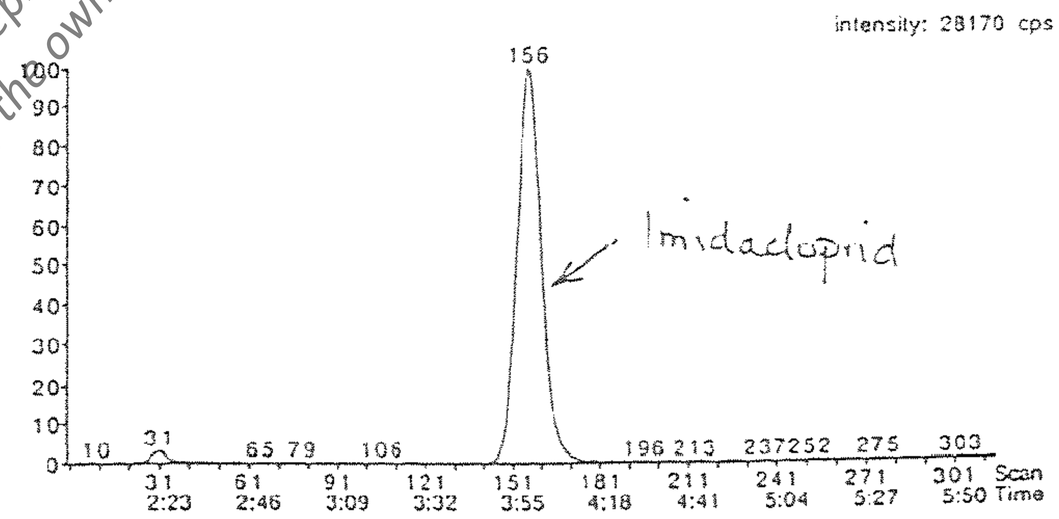


NM122100A019 standard — 50.0 ppb Imidicloprid and metabolites honey — Thu, Dec 21, 2000 19:21

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	211721
Height	28168
Start Time	3:46
End Time	4:23
Integration Width	0:36.7
Retention Time	3:59
Integration Type	A - BV

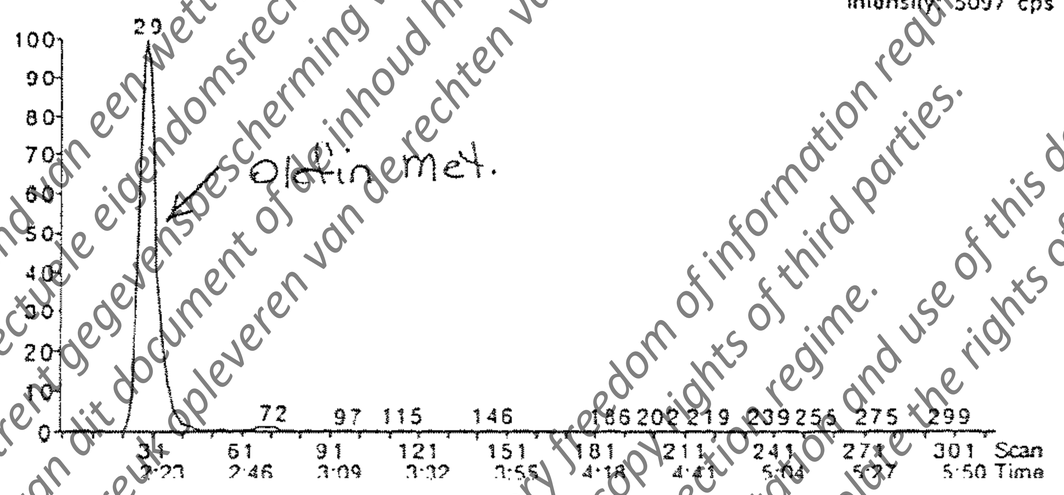


NM122100A020 100 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 19:39
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0
Noise Thres. 1.0
Quant Thres. 0.1
Min. Width 8
Mult. Width 2
Base. Width 100
RT Win. (secs) 10
Smooth 1
Expected RT 2:23

Area 26467
Height 5097
Start Time 2:13
End Time 2:36
Integration Width 0:22.2
Retention Time 2:22
Integration Type A - BV

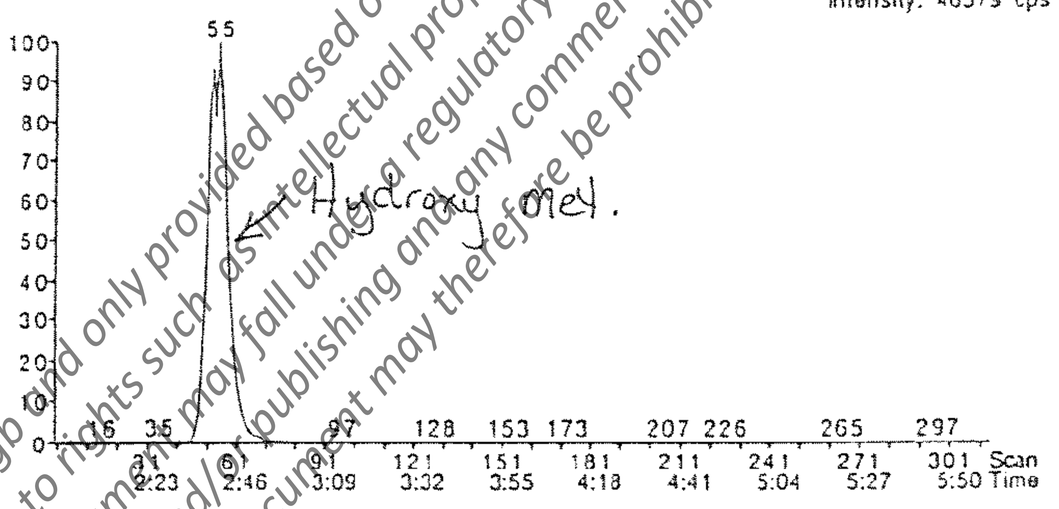


NM122100A020 100 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 19:39
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0
Noise Thres. 3.0
Quant Thres. 0.5
Min. Width 5
Mult. Width 4
Base. Width 100
RT Win. (secs) 5
Smooth 1
Expected RT 2:43

Area 283178
Height 46572
Start Time 2:31
End Time 3:01
Integration Width 0:29.8
Retention Time 2:42
Integration Type A - VV

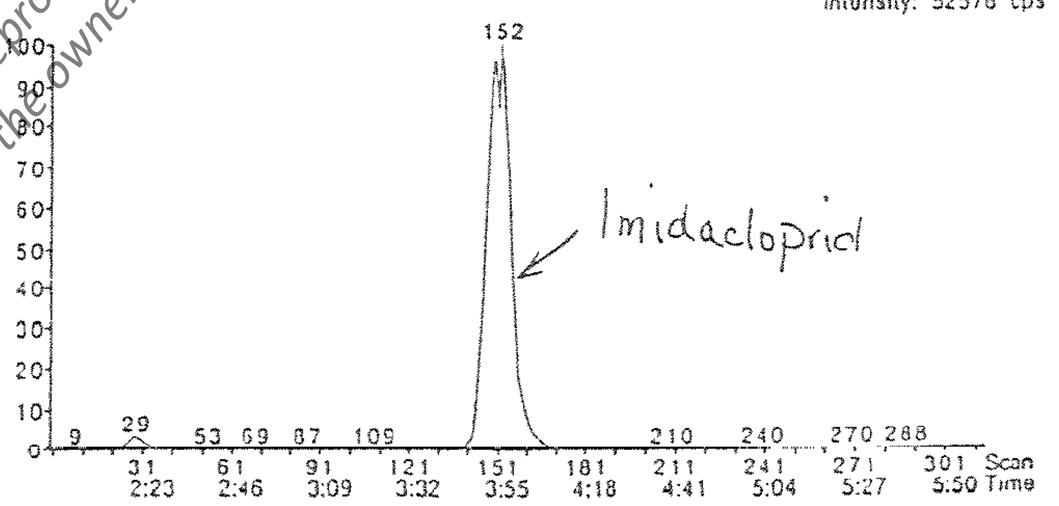


NM122100A020 100 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 19:39
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0
Noise Thres. 10.0
Quant Thres. 0.5
Min. Width 12
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 3:58

Area 401857
Height 52576
Start Time 3:41
End Time 4:09
Integration Width 0:36.0
Retention Time 3:55
Integration Type A - BV



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

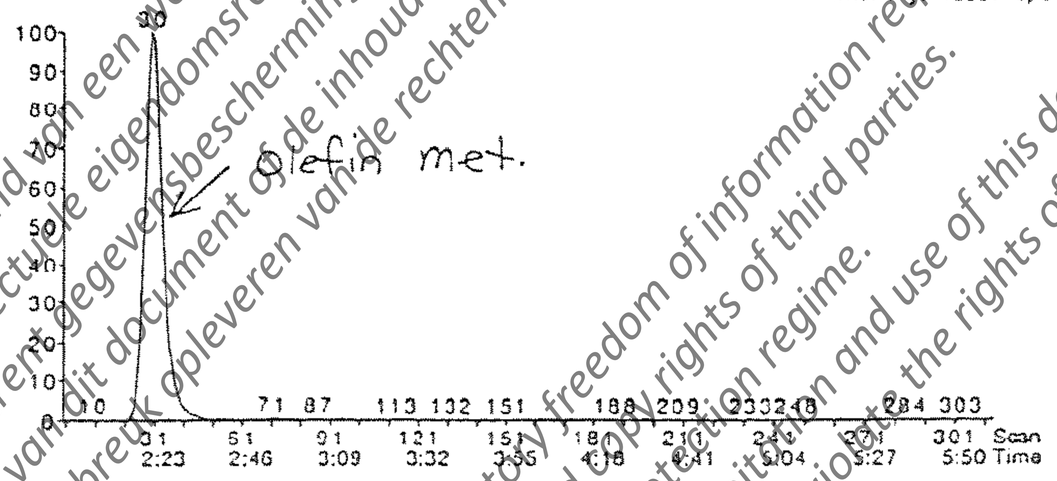
---

NM122100A021 250 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 19:57
standard

5:59 in 1 period
Imidacloprid Olefin metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
254.0->206.0

Noise Thres.	1.0
Quant Thres.	0.1
Min. Width	8
Mult. Width	2
Base. Width	100
RT Win. (secs)	10
Smooth	1
Expected RT	2:23
Area	66639
Height	12397
Start Time	2:15
End Time	2:38
Integration Width	0:23.0
Retention Time	2:23
Integration Type	A - BV

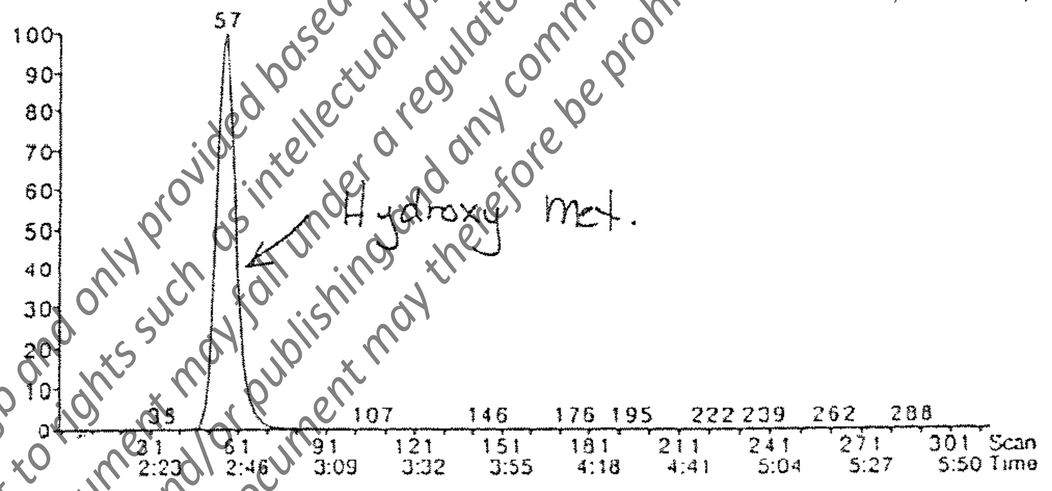


NM122100A021 250 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 19:57
standard

5:59 in 1 period
Imidacloprid Hydroxy metabolite
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
272.0->191.0

Noise Thres.	5.0
Quant Thres.	0.5
Min. Width	5
Mult. Width	4
Base. Width	100
RT Win. (secs)	5
Smooth	1
Expected RT	2:43
Area	753133
Height	124816
Start Time	2:33
End Time	3:02
Integration Width	0:29.1
Retention Time	2:43
Integration Type	A - VV

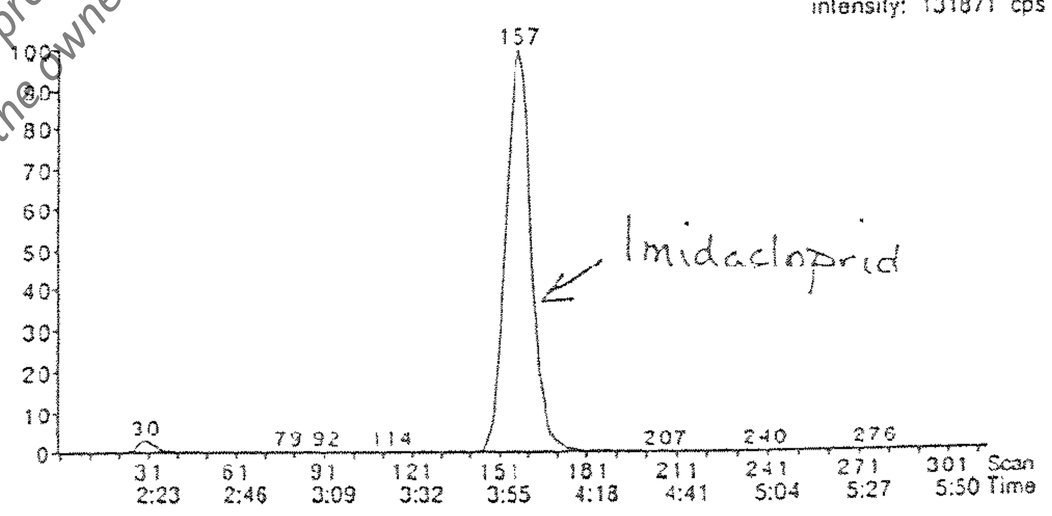


NM122100A021 250 ppb Imidicloprid and metabolites honey Thu, Dec 21, 2000 19:57
standard

5:59 in 1 period
Imidacloprid
No Internal Standard
Use Area

1: 3:59 MRM, 313 scans
256.0->209.0

Noise Thres.	10.0
Quant Thres.	0.5
Min. Width	12
Mult. Width	5
Base. Width	100
RT Win. (secs)	20
Smooth	1
Expected RT	3:58
Area	1031795
Height	131869
Start Time	3:47
End Time	4:28
Integration Width	0:40.5
Retention Time	3:58
Integration Type	A - BV



CB Jan. 3, 2001

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APPENDIX II

EXAMPLES OF SPREADSHEETS AND LINEARITY CURVES

TI 435 Validation		Determination of TI-435 in Nectar and Pollen										
Project No.:					Matrix: Honey							
Site:					Analytical Method: MS224.00							
ETL Sample/Standard I.D.	Client Sample/Standard I.D.	Analyte	Analyte Peak Area	ISTD Peak Area	Weight (g)	Final Volume (mL)	Y Intercept	Slope	Results Dry weight (ug/g)	Spike Level (ug/g)	Recovery (%)	LCMS Chro Filename
blank	solvent	TI 435	n / a	n / a	-	-	0.1068	0.921	-	-	-	NM120900A001
0.250 ppb TI-435 honey	standard	TI 435	1963	6551	-	-	0.1068	0.921	-	-	-	NM120900A002
0.500 ppb TI-435 honey	standard	TI 435	3437	6384	-	-	0.1068	0.921	-	-	-	NM120900A003
1.00 ppb TI-435 honey	standard	TI 435	7150	6067	-	-	0.1068	0.921	-	-	-	NM120900A004
2.50 ppb TI-435 honey	standard	TI 435	16658	6845	-	-	0.1068	0.921	-	-	-	NM120900A005
E0-08-010-02A-3, 1mL FV	HONEY UTC	TI 435	n / a	6270	1.00	1.0	0.1068	0.921	<0.000500	-	-	NM120900A006
E0-08-010-02A-4, 1mL FV	HONEY UTC	TI 435	n / a	6144	1.00	1.0	0.1068	0.921	<0.000500	-	-	NM120900A007
E0-08-010-02A+5, 1mL FV	HONEY UTC	TI 435	3179	6556	1.00	1.0	0.1068	0.921	0.000411	0.000505	81	NM120900A008
5.00 ppb TI-435 honey	standard	TI 435	31388	6481	-	-	0.1068	0.921	-	-	-	NM120900A009
E0-08-010-02A+6, 1mL FV	HONEY UTC	TI 435	3080	6404	1.00	1.0	0.1068	0.921	0.000406	0.000505	80	NM120900A010
E0-08-010-02A+7, 1mL FV	HONEY UTC	TI 435	3099	6801	1.00	1.0	0.1068	0.921	0.000379	0.000505	75	NM120900A011
E0-08-010-02A+8, 1mL FV	HONEY UTC	TI 435	14696	6749	1.00	1.0	0.1068	0.921	0.00275	0.00250	90	NM120900A012
10.0 ppb TI-435 honey	standard	TI 435	72883	7411	-	-	0.1068	0.921	-	-	-	NM120900A013
E0-08-010-02A+9, 1mL FV	HONEY UTC	TI 435	14582	7063	1.00	1.0	0.1068	0.921	0.00213	0.00250	85	NM120900A014
E0-08-010-02A+10, 1mL FV	HONEY UTC	TI 435	29195	6321	1.00	1.0	0.1068	0.921	0.00490	0.00500	98	NM120900A015
E0-08-010-02A+11, 1mL FV	HONEY UTC	TI 435	27491	6356	1.00	1.0	0.1068	0.921	0.00460	0.00500	92	NM120900A016
25.0 ppb TI-435 honey	standard	TI 435	160188	7168	-	-	0.1068	0.921	-	-	-	NM120900A017

Comments:

Calibration Filename: NM120900ACal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppb)=[(Analyte Peak area / ISTD Peak area) - y-intercept]/slope X final volume / sample weight / 1000)) ~~/ 1000~~

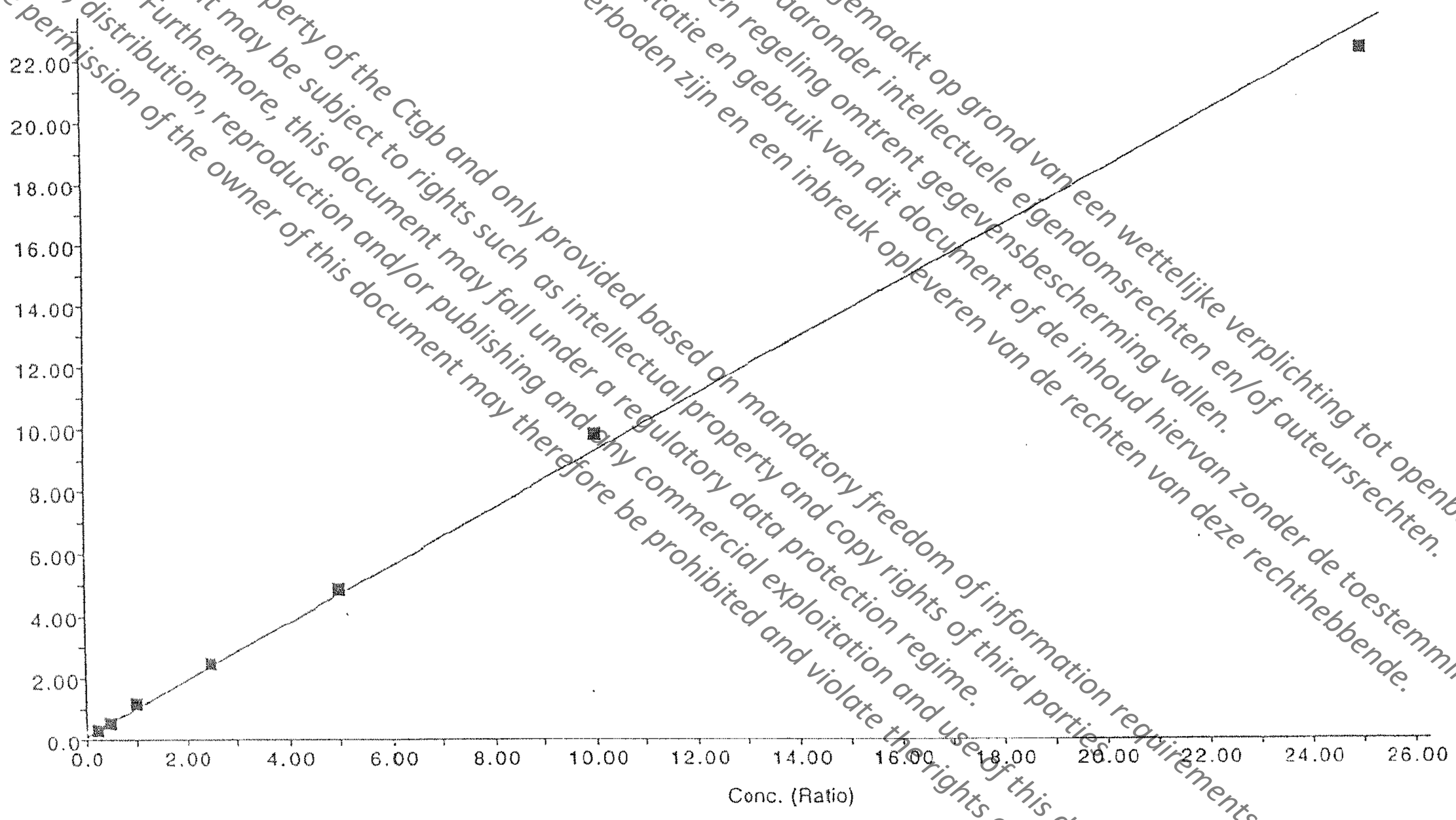
Data Entered By: [redacted] Date: Jan. 9, 2001 CB (EE) Feb. 5, 2001 updated DFS AB Jan 9, 2001

Protocol No.: EVL00BAY02
ETL Report No.: 00BAY09.REP

MacQuan, version 1.6
Printed: Mon, Dec 11, 2000 07:41
Calibration File: NM120900ACal, Path:Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM120900A:
Comments: Protocol ETL00BAY02.PRO - TI 485 Honey (Validation)

TI 435 250.0->169.0 Internal Standard: TI 435 d3
Weighted (1/x)

Intercept = 0.1068
Slope = 0.921
Correlation Coeff. = 0.9987



Set: Honey #1 — Determination of TI-435 in Nectar and Pollen

Project No.: ETL00BAY02.PRO — Matrix: Honey — Analysis Date: December 20, 2000

Site: — Analytical Method: MS225.00

ETL Sample/Standard I.D.	Client Sample/Standard I.D.	Analyte	Analyte Peak Area	ISTD Peak Area	Weight (g)	Aliquot (mL/mL)	Final Volume (mL)	Y Intercept	Slope	Results Dry weight (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS Chro Filename
blank	solvent	TI 435	0	0	-	-	-	0.2220	0.772	-	-	-	NM122000A001
0.250 ppb TI-435 honey	standard	TI 435	1011	2900	-	-	-	0.2220	0.772	-	-	-	NM122000A002
0.500 ppb TI-435 honey	standard	TI 435	1802	2800	-	-	-	0.2220	0.772	-	-	-	NM122000A003
1.00 ppb TI-435 honey	standard	TI 435	3346	3235	-	-	-	0.2220	0.772	-	-	-	NM122000A004
2.50 ppb TI-435 honey	standard	TI 435	7313	3497	-	-	-	0.2220	0.772	-	-	-	NM122000A005
E0-08-001-09A-1, 1mL FV	GVS ROAD NECTAR 07/10/00	TI 435	0	3546	1.00	1.00	1.0	0.2220	0.772	<0.000500	-	-	NM122000A006
E0-08-001-09A+1, 1mL FV	GVS ROAD NECTAR 07/10/00 0.000500ppm spike	TI 435	1744	3306	1.00	1.00	1.0	0.2220	0.772	0.000396	0.000505	78	NM122000A007
E0-08-001-09A+2, 1mL FV	GVS ROAD NECTAR 07/10/00 0.00250ppm spike	TI 435	7427	3233	1.01	1.00	1.0	0.2220	0.772	0.00272	0.00250	109	NM122000A008
5.00 ppb TI-435 honey	standard	TI 435	14083	3280	-	-	-	0.2220	0.772	-	-	-	NM122000A009
E0-08-001-01A, 1mL FV	ELORA NECTAR 07/03/00	TI 435	7831	2540	1.01	1.00	1.0	0.2220	0.772	0.0037	-	-	NM122000A010
E0-08-001-03A, 1mL FV	ELORA NECTAR 07/10/00	TI 435	3051	3215	1.00	1.00	1.0	0.2220	0.772	0.00094	-	-	NM122000A011
E0-08-001-11A, 1mL FV	GVS ROAD NECTAR 07/19/00	TI 435	0	3386	1.00	1.00	1.0	0.2220	0.772	<0.000500	-	-	NM122000A012
10.0 ppb TI-435 honey	standard	TI 435	30040	3448	-	-	-	0.2220	0.772	-	-	-	NM122000A013
E0-08-003-01A, 1mL FV	NECTAR TI-435 ALL 06/07/00	TI 435	3221	3056	1.00	1.00	1.0	0.2220	0.772	0.0011	-	-	NM122000A014
E0-08-003-02A, 1mL FV	NECTAR CONTROL ALL 06/07/00	TI 435	0	3169	1.01	1.00	1.0	0.2220	0.772	<0.000500	-	-	NM122000A015
E0-08-003-04A, 1mL FV	NECTAR TI-435 ALL 12/07/00	TI 435	2733	2721	1.00	1.00	1.0	0.2220	0.772	0.0010	-	-	NM122000A016
25.0 ppb TI-435 honey	standard	TI 435	67480	3321	-	-	-	0.2220	0.772	-	-	-	NM122000A017
E0-08-003-05A, 1mL FV	NECTAR CONTROL ALL 12/07/00	TI 435	0	2970	1.00	1.00	1.0	0.2220	0.772	<0.000500	-	-	NM122000A018
E0-08-003-09A, 1mL FV	COMP NECTAR CONTROL D13-D8	TI 435	0	2579	1.00	1.00	1.0	0.2220	0.772	<0.000500	-	-	NM122000A019
50.0 ppb TI-435 honey	standard	TI 435	136671	3691	-	-	-	0.2220	0.772	-	-	-	NM122000A020
100 ppb TI-435 honey	standard	TI 435	291462	4862	-	-	-	0.2220	0.772	-	-	-	NM122000A021
2.50 ppb TI-435 honey	standard	TI 435	7204	3109	-	-	-	0.2220	0.772	-	-	-	NM122000A022

Comments:

Calibration Filename: NM122000ACal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppb)=[(Analyte Peak area / ISTD Peak area) - y-intercept]/slope X final volume / sample weight / 1000))

Data Entered By: [redacted] Date: Jan. 9, 2001 Validated by AB Jan. 9, 2001

66

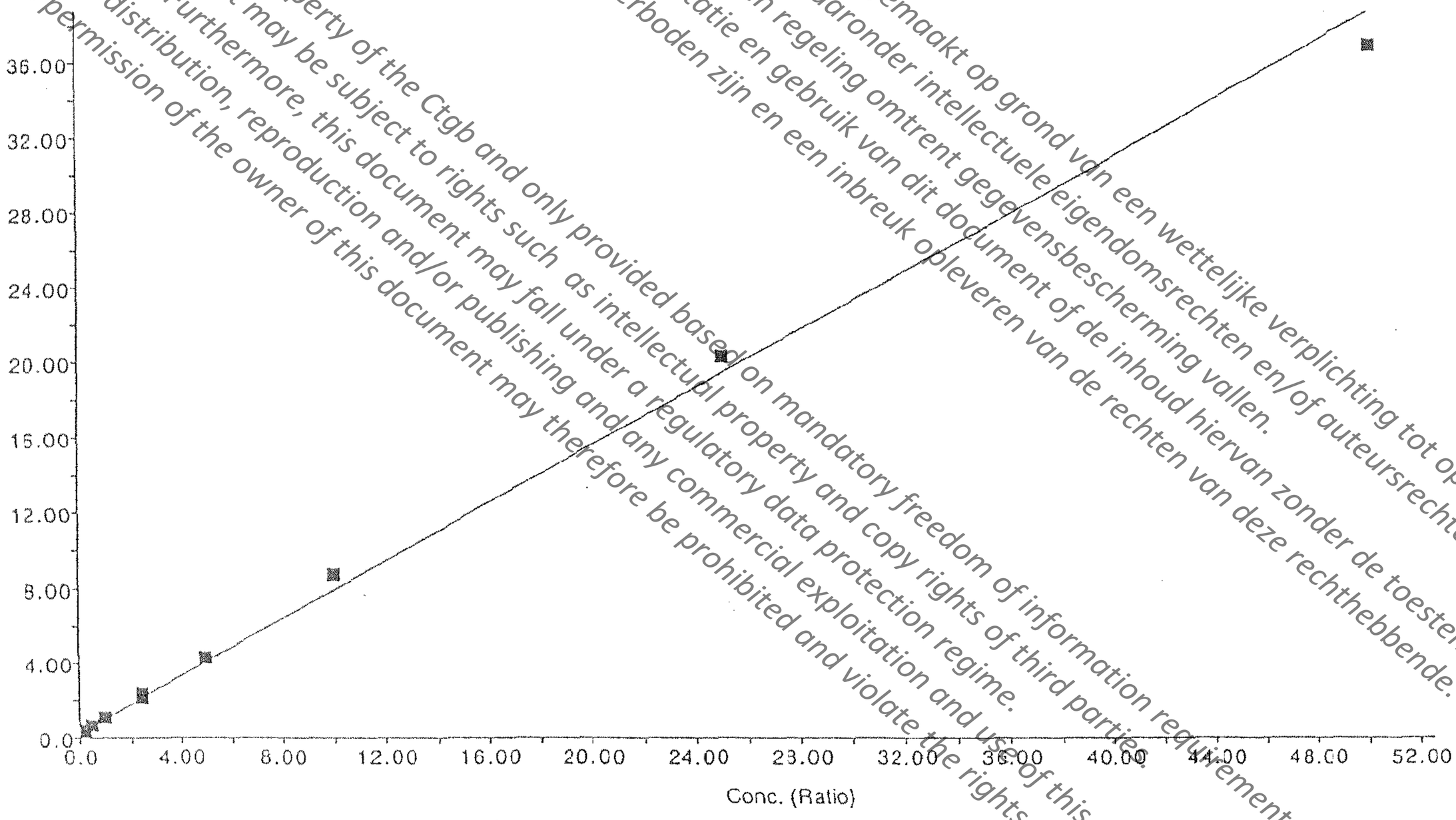
Protocol No.: ETL00BAY02
Report No.: 01BAY09.REP

110403

88

TI 435 250.0>>169.0 Internal Standard: TI 435 d3
Weighted (1/x)

Intercept = 0.222
Slope = 0.772
Correlation Coeff. = 0.9981



Protocol No.: ETL00BAY02
ETL Report No.: 00BAY09.REP

110403

Set: Validation		Determination of Imidicloprid and Metabolites in Pollen and Nectar										
Protocol No.: ETL00BAY02.PRO				Matrix: Honey					Weight = 1.0g			
SITE:				Analytical Method: MS224.00								
ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
blank	solvent	Olefin metabolite	0	-	-	-	15	782	-	-	-	NM121300A001
Wed, Dec 13, 2000 14:49		Hydroxy metabolite	0	-	-	-	-411	7268	-	-	-	NM121300A001
		Imidacloprid	0	-	-	-	-523	10113	-	-	-	NM121300A001
1.00 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	811	-	-	-	15	782	-	-	-	NM121300A002
Wed, Dec 13, 2000 15:07		Hydroxy metabolite	7603	-	-	-	-411	7268	-	-	-	NM121300A002
		Imidacloprid	10506	-	-	-	-524	10113	-	-	-	NM121300A002
2.50 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	1949	-	-	-	15	782	-	-	-	NM121300A003
Wed, Dec 13, 2000 15:25		Hydroxy metabolite	17084	-	-	-	-411	7268	-	-	-	NM121300A003
		Imidacloprid	23980	-	-	-	-523	10113	-	-	-	NM121300A003
E0-08-010-02A-1, 1mL FV	HONEY UTC	Olefin metabolite	0	1.00	1.0	1.0	15	782	<0.00100	-	-	NM121300A004
Wed, Dec 13, 2000 15:42		Hydroxy metabolite	0	1.00	1.0	1.0	-411	7268	<0.00100	-	-	NM121300A004
		Imidacloprid	0	1.00	1.0	1.0	-523	10113	<0.00100	-	-	NM121300A004
E0-08-010-02A-2, 1mL FV	HONEY UTC	Olefin metabolite	0	1.00	1.0	1.0	15	782	<0.00100	-	-	NM121300A005
Wed, Dec 13, 2000 16:00		Hydroxy metabolite	0	1.00	1.0	1.0	-411	7268	<0.00100	-	-	NM121300A005
		Imidacloprid	0	1.00	1.0	1.0	-523	10113	<0.00100	-	-	NM121300A005
E0-08-010-02A+1, 1mL FV	HONEY UTC 0.001 ppm spike	Olefin metabolite	908	1.00	1.0	1.0	15	782	0.00114	0.00100	114	NM121300A006
Wed, Dec 13, 2000 16:18		Hydroxy metabolite	4919	1.00	1.0	1.0	-411	7268	0.000733	0.00100	73	NM121300A006
		Imidacloprid	16306	1.00	1.0	1.0	-523	10113	0.00107	0.00101	106	NM121300A006
5.00 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	3925	-	-	-	15	782	-	-	-	NM121300A007
Wed, Dec 13, 2000 16:36		Hydroxy metabolite	34032	-	-	-	-411	7268	-	-	-	NM121300A007
		Imidacloprid	46731	-	-	-	-523	10113	-	-	-	NM121300A007
E0-08-010-02A+2, 1mL FV	HONEY UTC 0.001 ppm spike	Olefin metabolite	883	1.00	1.0	1.0	15	782	0.00111	0.00100	111	NM121300A008
Wed, Dec 13, 2000 16:54		Hydroxy metabolite	7257	1.00	1.0	1.0	-411	7268	0.00106	0.00100	106	NM121300A008
		Imidacloprid	9846	1.00	1.0	1.0	-523	10113	0.00103	0.00101	102	NM121300A008

Comments:

Calibration Filename: NM121300ACal

Regression type: Weighted (1/x)

Quantitation formula (ppm)=(Peak area - y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.) ÷ 1000

Data Entered By: [redacted] Date: Jan. 9, 2001

QC'ed by audited by AB Jan. 9, 2001

Set: Validation		Determination of Imidicloprid and Metabolites in Pollen and Nectar										
Protocol No.: ETL00BAY02.PRO				Matrix: Honey					Weight = 1.0g			
SITE:				Analytical Method: MS224.00								
ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-010-02A+3, 1mL FV	HONEY UTC 0.001 ppm spike	Olefin metabolite	891	1.00	1.0	1.0	15	782	0.00112	0.00100	112	NM121300A009
Wed, Dec 13, 2000 17:11		Hydroxy metabolite	7363	1.00	1.0	1.0	-411	7268	0.00107	0.00100	107	NM121300A009
		Imidacloprid	10480	1.00	1.0	1.0	-523	10113	0.00109	0.00101	108	NM121300A009
E0-08-010-02A+4, 1mL FV	HONEY UTC 0.005 ppm spike	Olefin metabolite	3524	1.00	1.0	1.0	15	782	0.00449	0.00500	90	NM121300A010
Wed, Dec 13, 2000 5:29 PM		Hydroxy metabolite	27420	1.00	1.0	1.0	-411	7268	0.00383	0.00500	77	NM121300A010
		Imidacloprid	44847	1.00	1.0	1.0	-523	10113	0.00449	0.00505	89	NM121300A010
10.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	7657				15	782	-	-	-	NM121300A011
Wed, Dec 13, 2000 5:47 PM		Hydroxy metabolite	68622	-	-	-	-411	7268	-	-	-	NM121300A011
		Imidacloprid	98257	-	-	-	-523	10113	-	-	-	NM121300A011
E0-08-010-02A+5, 1mL FV	HONEY UTC 0.005 ppm spike	Olefin metabolite	3331	1.00	1.0	1.0	15	782	0.00473	0.00500	85	NM121300A012
Wed, Dec 13, 2000 6:05 PM		Hydroxy metabolite	30080	1.00	1.0	1.0	-411	7268	0.00420	0.00500	84	NM121300A012
		Imidacloprid	42947	1.00	1.0	1.0	-523	10113	0.00439	0.00505	85	NM121300A012
E0-08-010-02A+6, 1mL FV	HONEY UTC 0.01 ppm spike	Olefin metabolite	7860	1.00	1.0	1.0	15	782	0.0100	0.0100	100	NM121300A013
Wed, Dec 13, 2000 6:23 PM		Hydroxy metabolite	67513	1.00	1.0	1.0	-411	7268	0.00935	0.0100	94	NM121300A013
		Imidacloprid	96216	1.00	1.0	1.0	-523	10113	0.00957	0.0101	95	NM121300A013
E0-08-010-02A+7, 1mL FV	HONEY UTC 0.01 ppm spike	Olefin metabolite	7816	1.00	1.0	1.0	15	782	0.00993	0.0100	100	NM121300A014
Wed, Dec 13, 2000 6:41 PM		Hydroxy metabolite	72219	1.00	1.0	1.0	-411	7268	0.00995	0.0100	100	NM121300A014
		Imidacloprid	105521	1.00	1.0	1.0	-523	10113	0.0105	0.0101	104	NM121300A014
25.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	19992				15	782	-	-	-	NM121300A015
Wed, Dec 13, 2000 6:58 PM		Hydroxy metabolite	185905	-	-	-	-411	7268	-	-	-	NM121300A015
		Imidacloprid	257787				-523	10113	-	-	-	NM121300A015
50.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	39008	-		15	782	-	-	-	NM121300A016	
Wed, Dec 13, 2000 7:15 PM		Hydroxy metabolite	370365	-	-	-411	7268	-	-	-	NM121300A016	
		Imidacloprid	512529	-	-	-523	10113	-	-	-	NM121300A016	

Comments:

Calibration Filename: NM121300ACal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppm)=(Peak area - y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt)

Data Entered By: [redacted] Date: Jan. 9, 2001

QC'ed by audited by [redacted] Jan. 9, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

Set: Validation — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Honey

SITE: — Analytical Method: MS224.00 — Weight = 1.0g

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
100 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	78128	-	-	-	15	782	-	-	-	NM121300A017
Wed, Dec 13, 2000 7:34 PM		Hydroxy metabolite	213815	-	-	-	-411	7268	-	-	-	NM121300A017
		Imidacloprid	1003426			-	-523	10113	-	-	-	NM121300A017

Comments:

Calibration Filename: NM121300ACal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppm)=(Peak area - y-Intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.)

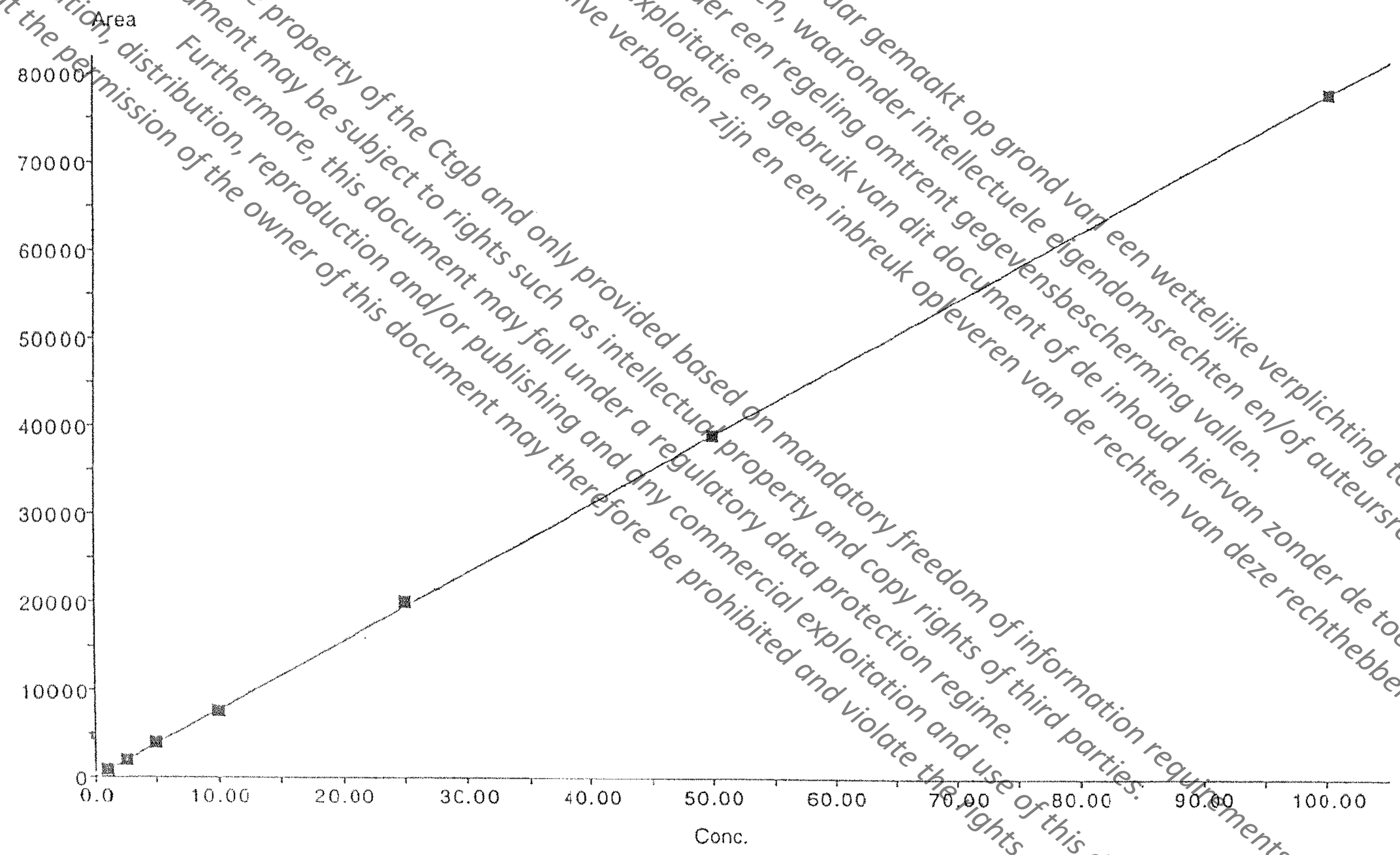
Data Entered By: [redacted] Date: Jan. 9, 2001

QC'ed by Audited by KS Jan. 9, 2001

MacQuan, version 1.6
Printed: Thu, Dec 14, 2000 08:37
Calibration File: NM121300ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM121300A:
Comments: No Comments

Imidacloprid Olefin metabolite 254.0->206.0 No Internal Standard
Weighted (1/x)

Intercept = 15
Slope = 5782
Correlation Coeff. = 0.9999

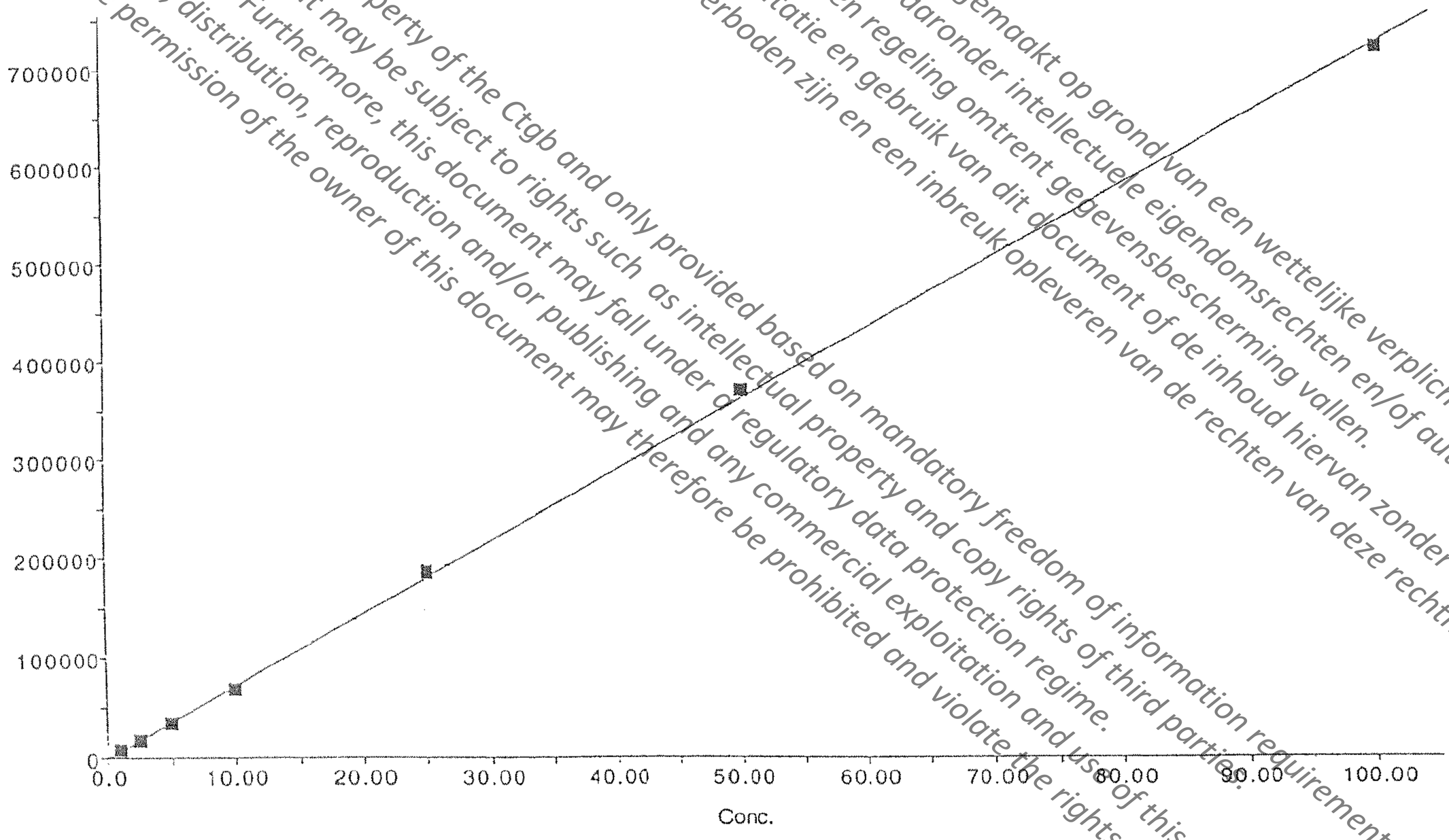


Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

110403

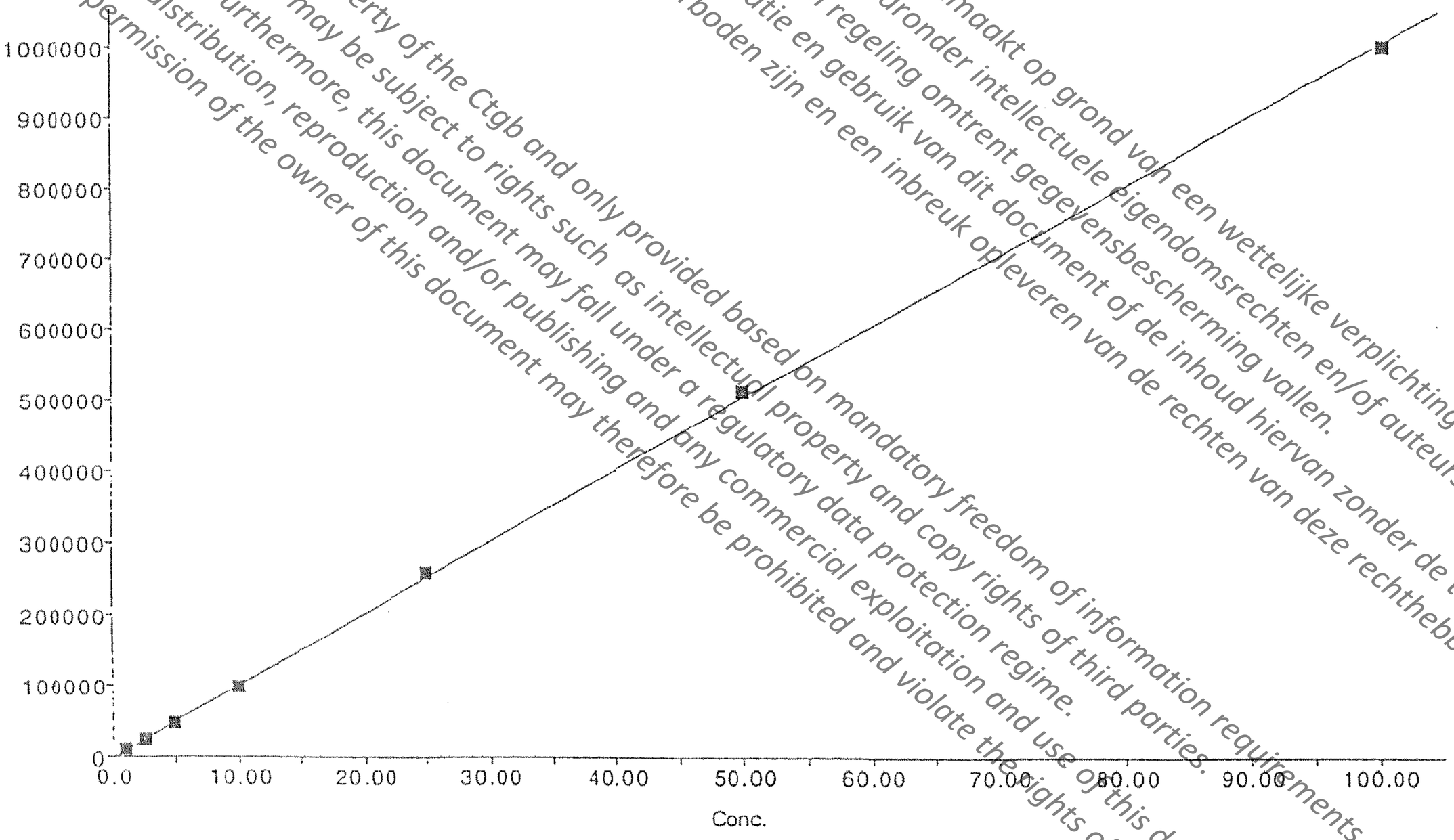
MacQuan, version 1.6
Printed: Thu, Dec 14, 2000 08:37
Calibration File: NM121300ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM121300A:
Comments: No Comments

Imidacloprid Hydroxy metabolite 272.0->191.0 No Internal Standard
Weighted (1/x)
Intercept = -411
Slope = 7268
Correlation Coeff. = 0.9997



MacQuan, version 1.6
Printed: Thu, Dec 14, 2000 08:57
Calibration File: NM121300ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM121300A:
Comments: No Comments

Imidacloprid 256.0->209.0 No Internal Standard
Weighted (1/x)
Intercept = -523
Slope = 10113
Correlation Coeff. = 0.9998



Protocol No.: ETL00BAY02
ETL Report No.: 01BAY09.REP

110403

Set: Honey#1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Honey — Analysis Date: December 21, 2000

SITE: — Analytical Method: MS224.00 — Weight = 1g

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
blank	solvent	Olefin metabolite	0	-	-	-	28	267	-	-	-	NM122100A001
Thu, Dec 21, 2000 14:01		Hydroxy metabolite	0	-	-	-	-720	2946	-	-	-	NM122100A001
		Imidacloprid	0	-	-	-	-898	4118	-	-	-	NM122100A001
1.00 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	323	-	-	-	28	267	-	-	-	NM122100A002
Thu, Dec 21, 2000 14:18		Hydroxy metabolite	2668	-	-	-	-720	2946	-	-	-	NM122100A002
		Imidacloprid	3713	-	-	-	-898	4118	-	-	-	NM122100A002
2.50 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	700	-	-	-	28	267	-	-	-	NM122100A003
Thu, Dec 21, 2000 14:36		Hydroxy metabolite	5795	-	-	-	-720	2946	-	-	-	NM122100A003
		Imidacloprid	8458	-	-	-	-898	4118	-	-	-	NM122100A003
E0-08-001-11A-2, 1mL FV	GVS ROAD NECTAR 07/19/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A004
Thu, Dec 21, 2000 14:54		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A004
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A004
E0-08-001-11A+1, 1mL FV	GVS ROAD NECTAR 07/19/00 0.001ppm spike	Olefin metabolite	398	1.00	1.0	1.0	28	267	0.00105	0.00100	105	NM122100A005
Thu, Dec 21, 2000 15:12		Hydroxy metabolite	2508	1.00	1.0	1.0	-720	2946	0.00110	0.00100	110	NM122100A005
		Imidacloprid	3949	1.00	1.0	1.0	-898	4118	0.00118	0.00101	117	NM122100A005
E0-08-001-11A+2, 1mL FV	GVS ROAD NECTAR 07/19/00 0.005ppm spike	Olefin metabolite	1093	1.00	1.0	1.0	28	267	0.00399	0.00500	80	NM122100A006
Thu, Dec 21, 2000 15:30		Hydroxy metabolite	12687	1.00	1.0	1.0	-720	2946	0.00435	0.00500	91	NM122100A006
		Imidacloprid	18036	1.00	1.0	1.0	-898	4118	0.00460	0.00505	91	NM122100A006
5.00 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	1263	-	-	-	28	267	-	-	-	NM122100A007
Thu, Dec 21, 2000 15:47		Hydroxy metabolite	14015	-	-	-	-720	2946	-	-	-	NM122100A007
		Imidacloprid	18585	-	-	-	-898	4118	-	-	-	NM122100A007
E0-08-001-05A, 1mL FV	GVH FARM NECTAR 07/10/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A008
Thu, Dec 21, 2000 16:05		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A008
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A008

Comments:

Calibration Filename: NM122100ACal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppm)=(Peak area - y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt) ÷1000 *

Data Entered By: [redacted] Date: Jan. 9, 2001

QC'ed by audited by AB Jan. 9, 2001

Protocol No.: ETL00BAY02
ETL Report No.: 01BAY09.REP

110403

Set: Honey#1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Honey — Weight = 1g

SITE: — Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-001-08A, 1mL FV	GVH FARM NECTAR 07/19/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A009
Thu Dec 21, 2000 16:23		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A009
		Imidacloprid	802	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A009
E0-08-001-09AB, 1mL FV	GVS FARM NECTAR 07/10/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A010
Thu Dec 21, 2000 16:41		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A010
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A010
10.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	2486	-	-	-	28	267	-	-	-	NM122100A011
Thu Dec 21, 2000 16:59		Hydroxy metabolite	29345				-720	2946	-	-	-	NM122100A011
		Imidacloprid	40554	-	-	-	-898	4118	-	-	-	NM122100A011
E0-08-003-02A, 1mL FV	NECTAR CONTROL ALL 06/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A012
Thu Dec 21, 2000 17:16		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A012
		Imidacloprid	0	1.00	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A012
E0-08-003-03A, 1mL FV	NECTAR GAUCHO ALL 06/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A013
Thu Dec 21, 2000 17:34		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A013
		Imidacloprid	2783	1.00	1.0	1.0	-898	4118	0.00075	-	-	NM122100A013
E0-08-003-05AB, 1mL FV	NECTAR CONTROL ALL 12/07/00	Olefin metabolite	0	1.01	1.0	1.0	28	267	<0.00100	-	-	NM122100A014
Thu Dec 21, 2000 17:52		Hydroxy metabolite	0	1.01	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A014
		Imidacloprid	0	1.01	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A014
25.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	6810	-	-	-	28	267	-	-	-	NM122100A015
Thu Dec 21, 2000 18:10		Hydroxy metabolite	73515	-	-	-	-720	2946	-	-	-	NM122100A015
		Imidacloprid	102590	-	-	-	-898	4118	-	-	-	NM122100A015
E0-08-003-06A, 1mL FV	NECTAR GAUCHO ALL 12/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A016
Thu Dec 21, 2000 18:28		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A016
		Imidacloprid	2442	1.00	1.0	1.0	-898	4118	0.00061	-	-	NM122100A016

Comments:

Calibration Filename: NM122100ACal

Regression type: Weighted (1/x)

Quantitation formula (from the linearity curve (ppm)=(Peak area - y-Intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.) ÷1000

Data Entered By: [redacted] Date: Jan. 5, 2001

QC'ed by audited by AB Jan. 5, 2001

Protocol No.: ETL00BAY02
ETL Report No.: 016BAY09.REP

Set: Honey#1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.FRO — Matrix: Honey — Analytical Method: MS224.00 — Weight = 1g

SITE:

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-003-09AB_1mL FV	COMP-NECTAR CTL D8 D13 20/07/00	Olefin metabolite	0	1.01	1.0	1.0	28	267	<0.00100	-	-	NM122100A017
Thu, Dec 21, 2000 16:45		Hydroxy metabolite	0	1.01	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A017
		Imidacloprid	0	1.01	1.0	1.0	-898	4118	<0.00100	-	-	NM122100A017
E0-08-003-10A_1mL FV	NECTAR GAUCHO ALL 20/07/00	Olefin metabolite	0	1.00	1.0	1.0	28	267	<0.00100	-	-	NM122100A018
Thu, Dec 21, 2000 19:03		Hydroxy metabolite	0	1.00	1.0	1.0	-720	2946	<0.00100	-	-	NM122100A018
		Imidacloprid	1561	1.00	1.0	1.0	-898	4118	0.00060	-	-	NM122100A018
50.0 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	13763	-	-	-	28	267	-	-	-	NM122100A019
Thu, Dec 21, 2000 19:21		Hydroxy metabolite	138968	-	-	-	-720	2946	-	-	-	NM122100A019
		Imidacloprid	211721	-	-	-	-898	4118	-	-	-	NM122100A019
100 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	26467	-	-	-	28	267	-	-	-	NM122100A020
Thu, Dec 21, 2000 19:39		Hydroxy metabolite	283177	-	-	-	-720	2946	-	-	-	NM122100A020
		Imidacloprid	401857	-	-	-	-898	4118	-	-	-	NM122100A020
250 ppb Imidicloprid and metabolites honey	standard	Olefin metabolite	66639	-	-	-	28	267	-	-	-	NM122100A021
Thu, Dec 21, 2000 19:57		Hydroxy metabolite	753183	-	-	-	-720	2946	-	-	-	NM122100A021
		Imidacloprid	1031795	-	-	-	-898	4118	-	-	-	NM122100A021

Comments:

Calbration Filename: NM122100AC3I

Regression type: Weighted (1/x)

Quantitation formula: from the linearity curve (area/Peak area-y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.) ÷1000

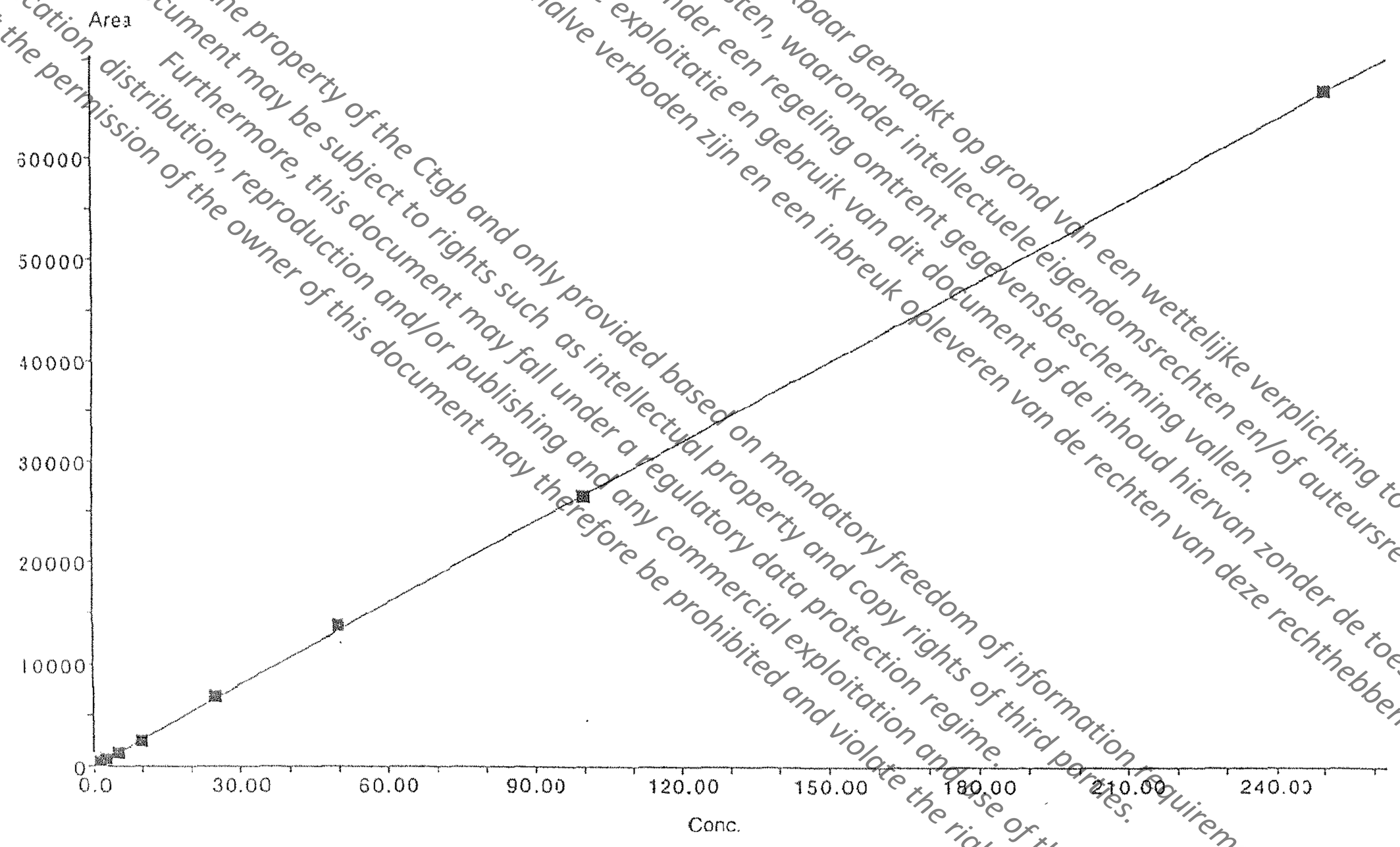
Data Entered By: [redacted] Date: Jan-5, 2001

QC'ed by: audited by AB, Jan-5, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

Imidaclopid Olefin metabolite 254.0->206.0 No Internal Standard
Weighted (1/x)

Intercept = 28
Slope = 267
Correlation Coeff. = 0.9998



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:04
Calibration File: NM122100ACal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100A:
Comments: Protocol ETL00BAY02.PRO Imidicloprid and metabolites / Honey

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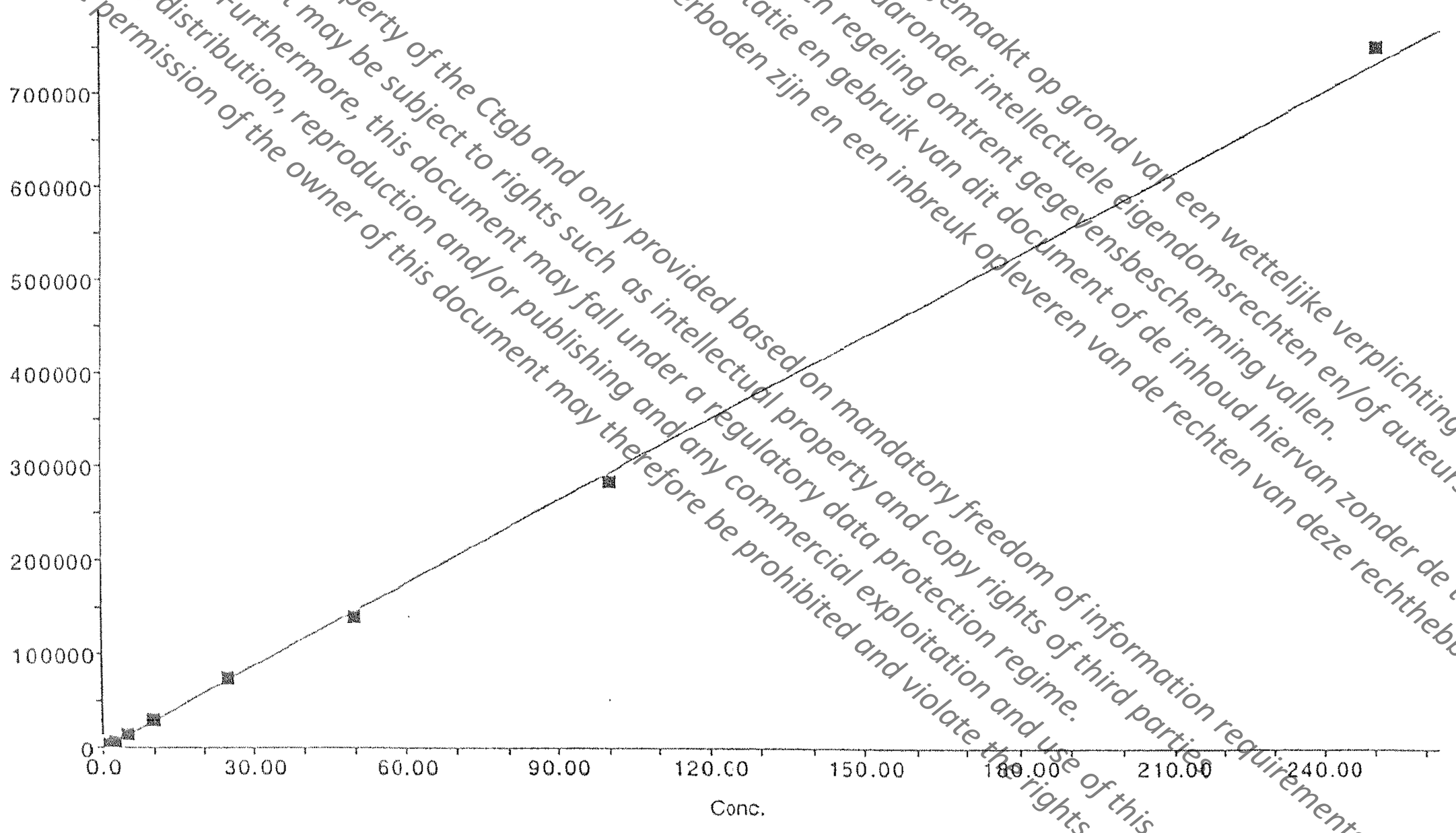
Imidacloprid Hydroxy metabolite 272.0->191.0 No Internal Standard
Weighted (1/x)
Intercept = -720
Slope = 2946
Correlation Coeff. = 0.9994



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Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Honey

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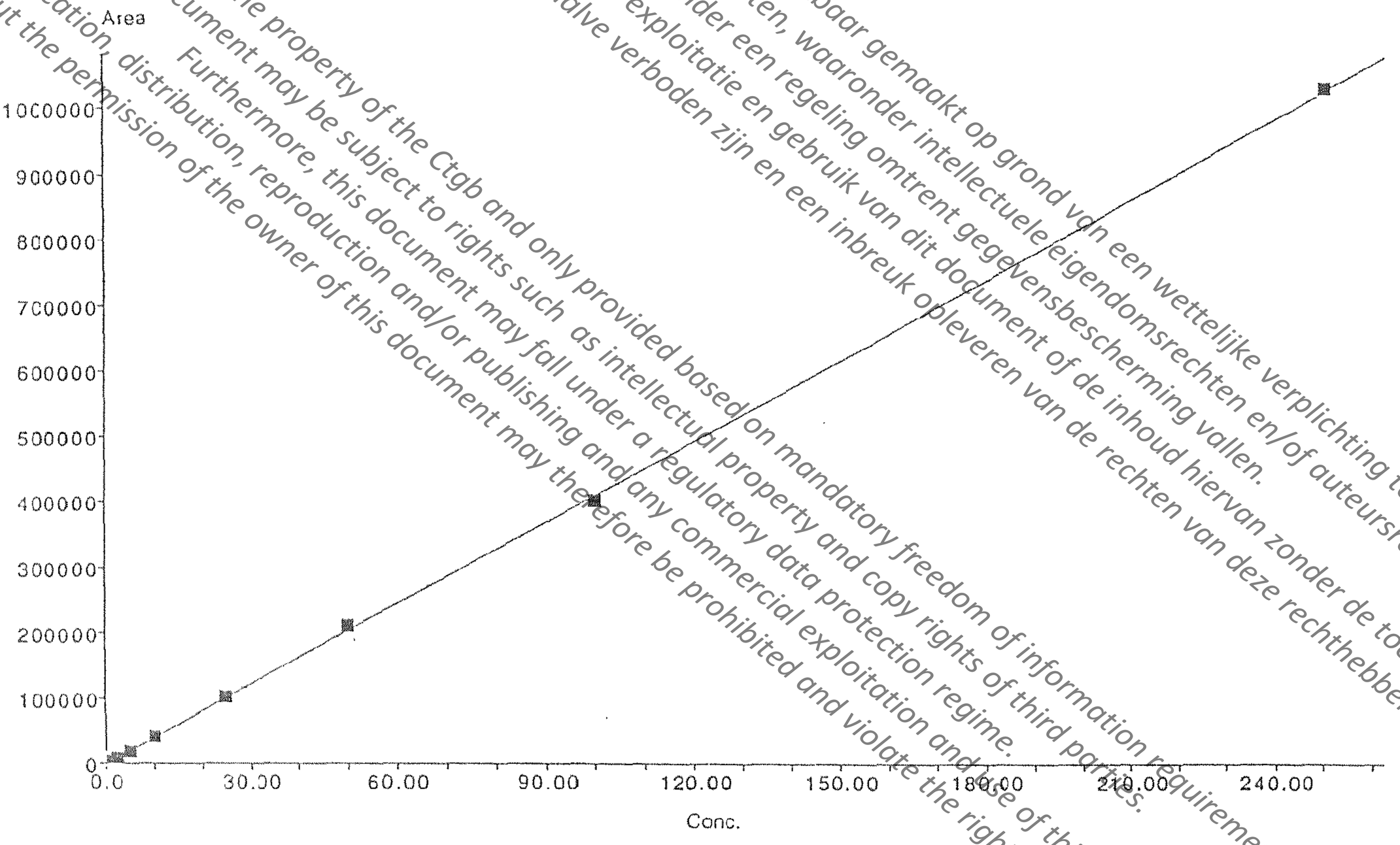
Imidacloprid 256.0->209.0 No Internal Standard
Weighted (1/x)

Intercept = -898
Slope = 4118
Correlation Coeff. = 0.9998



Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

TI 435 Validation		Determination of TI-435 in Nectar and Pollen											
Project No.:					Matrix: Pollen								
Site:					Analytical Method: MS225.00								
ETL Sample/Standard I.D.	Client Sample/Standard I.D.	Analyte	Analyte Peak Area	ISTD Peak Area	Weight (g)	Aliquot (mL/mL)	Final Volume (mL)	Y Intercept	Slope	Results Dry weight (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS Chro Filename
blank	solvent	TI 435	n/a	n/a	-		-	0.0956	0.991	-	-	-	NM120900B001
0.250 ppb TI-435 honey	standard	TI 435	2008	6258	-	-	-	0.0956	0.991	-	-	-	NM120900B002
0.500 ppb TI-435 honey	standard	TI 435	3665	5890	-	-	-	0.0956	0.991	-	-	-	NM120900B003
1.00 ppb TI-435 honey	standard	TI 435	7096	6370	-	-	-	0.0956	0.991	-	-	-	NM120900B004
2.50 ppb TI-435 honey	standard	TI 435	16825	6704	-	-	-	0.0956	0.991	-	-	-	NM120900B005
E0-08-010-01A-3, 1mL FV	POLLEN UTC	TI 435	0	6580	2.00	2.22	1.0	0.0956	0.991	<0.000500	-	-	NM120900B006
E0-08-010-01A-4, 1mL FV	POLLEN UTC	TI 435	0	6200	2.00	2.22	1.0	0.0956	0.991	<0.000500	-	-	NM120900B007
E0-08-010-01A+5, 1mL FV	POLLEN UTC	TI 435	2971	6358	2.00	2.22	1.0	0.0956	0.991	0.000415	0.000505	82	NM120900B008
5.00 ppb TI-435 honey	standard	TI 435	31434	6063	-	-		0.0956	0.991	-	-	-	NM120900B009
E0-08-010-01A+6, 1mL FV	POLLEN UTC	TI 435	3150	6301	2.00	2.22	1.0	0.0956	0.991	0.000453	0.000505	90	NM120900B010
E0-08-010-01A+7, 1mL FV	POLLEN UTC	TI 435	2744	6143	2.00	2.22	1.0	0.0956	0.991	0.000393	0.000505	78	NM120900B011
E0-08-010-01A+8, 1mL FV	POLLEN UTC	TI 435	13800	6015	2.00	2.22	1.0	0.0956	0.991	0.00246	0.00250	98	NM120900B012
10.0 ppb TI-435 honey	standard	TI 435	61945	6135	-	-		0.0956	0.991	-	-	-	NM120900B013
E0-08-010-01A+9, 1mL FV	POLLEN UTC	TI 435	13728	5973	2.00	2.22	1.0	0.0956	0.991	0.00247	0.00250	99	NM120900B014
E0-08-010-01A+10, 1mL FV	POLLEN UTC	TI 435	25993	6013	2.00	2.22	1.0	0.0956	0.991	0.00473	0.00500	95	NM120900B015
E0-08-010-01A+11, 1mL FV	POLLEN UTC	TI 435	28811	6063	2.00	2.22	1.0	0.0956	0.991	0.00522	0.00500	104	NM120900B016
25.0 ppb TI-435 honey	standard	TI 435	147766	5985	-	-	-	0.0956	0.991	-	-	-	NM120900B017

Comments:

Calibration Filename: NM120900BCAL

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppb)=[(Analyte Peak area / ISTD Peak area) - y-intercept] / slope X final volume X aliquot / sample weight / 1000)

Data Entered By: [redacted] Date: Jan. 9, 2001 Verified By: Jan 9, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

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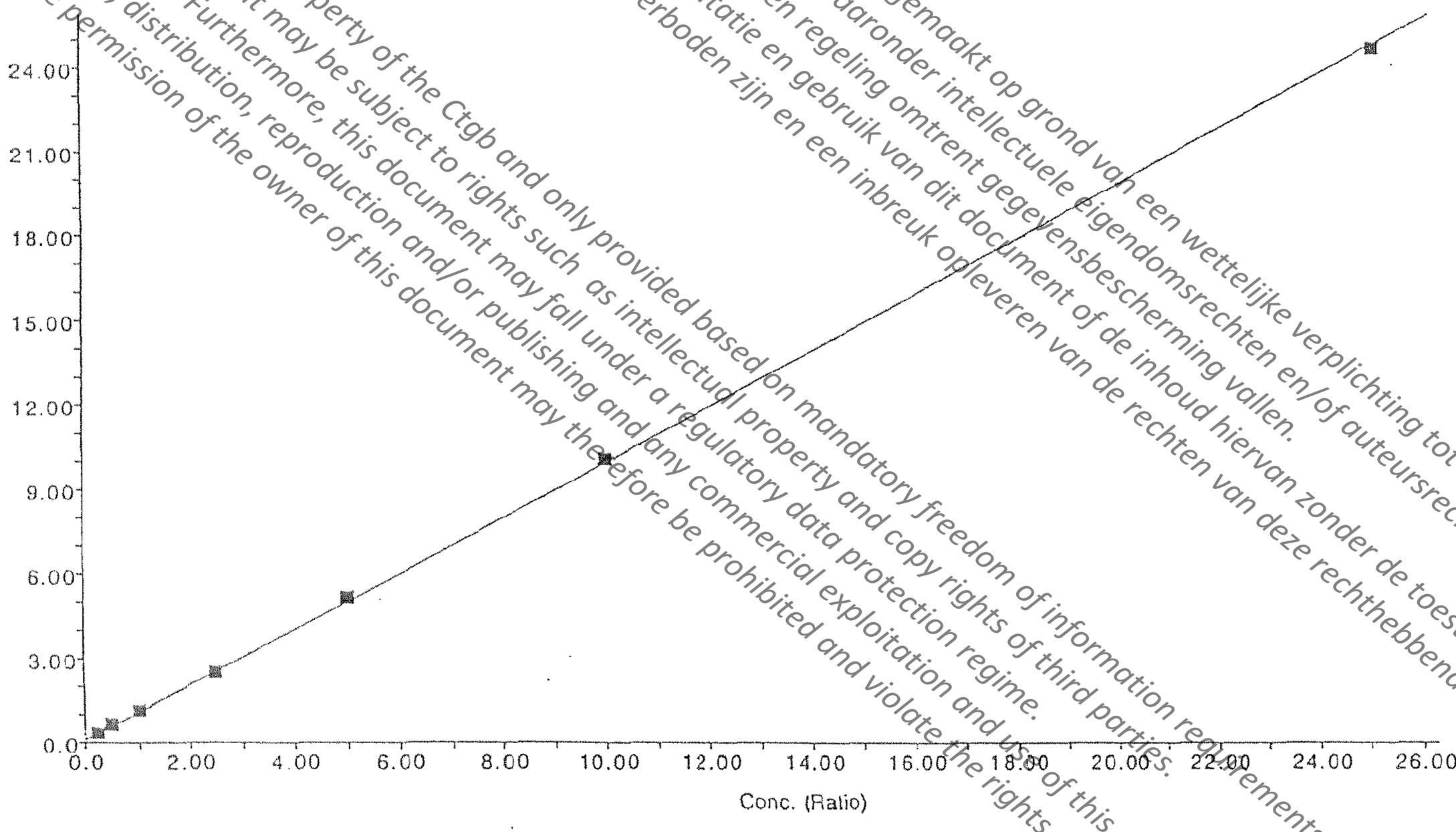
MacQuan, version 1.6
Printed: Mon, Dec 11, 2000 07:46
Calibration File: NM120900BCal Path: Macintosh HD:AP/3000:DATA:BAYER:IMIDACLOPRID:NM120900B:
Comments: Protocol ETL00BAY02.PRO - TI 435 Pollen (Validation)

TI 435 250.0->169.0 Internal Standard: TI 435 d3
Weighted (1/x)
Intercept = 0.0956
Slope = 0.991
Correlation Coeff. = 0.9998



Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

Set: Pollen #1 — Determination of TI-435 in Nectar and Pollen

Project No.: ETL00BAY02.PRO — Matrix: Pollen — Analysis Date: December 20, 2000

Site: — Analytical Method: MS225.00

ETL Sample/Standard I.D.	Client Sample/Standard I.D.	Analyte	Analyte Peak Area	ISTD Peak Area	Weight (g)	Aliquot (mL/mL)	Final Volume (mL)	Y Intercept	Slope	Results Dry weight (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS Chro Filename
blank	solvent	TI 435	0	3	-	-	-	0.2550	0.831	-	-	-	NM122000B001
0.250 ppb TI-435 pollen	standard	TI 435	1163	2792	-	-	-	0.2550	0.831	-	-	-	NM122000B002
0.500 ppb TI-435 pollen	standard	TI 435	1566	2578	-	-	-	0.2550	0.831	-	-	-	NM122000B003
1.00 ppb TI-435 pollen	standard	TI 435	3084	2864	-	-	-	0.2550	0.831	-	-	-	NM122000B004
2.50 ppb TI-435 pollen	standard	TI 435	7023	2945	-	-	-	0.2550	0.831	-	-	-	NM122000B005
E0-08-001-10A-1, 1mL FV	GVS ROAD POLLEN 07/11/00	TI 435	0	2918	2.00	2.22	1.0	0.2550	0.831	<0.000500	-	-	NM122000B006
E0-08-001-10A+1, 1mL FV	GVS ROAD POLLEN 07/11/00 0.000500ppm spike	TI 435	1690	2920	2.00	2.22	1.0	0.2550	0.831	0.000432	0.000505	86	NM122000B007
E0-08-001-10A+2, 1mL FV	GVS ROAD POLLEN 07/11/00 0.00250ppm spike	TI 435	7094	2824	2.00	2.14	1.0	0.2550	0.831	0.00289	0.00250	116	NM122000B008
5.00 ppb TI-435 pollen	standard	TI 435	13535	2459	-	-	-	0.2550	0.831	-	-	-	NM122000B009
E0-08-001-02A, 1mL FV	ELORA POLLEN 07/05/00	TI 435	7379	2908	2.00	2.22	1.0	0.2550	0.831	0.0030	-	-	NM122000B010
E0-08-001-04A, 1mL FV	ELORA POLLEN 07/11/00	TI 435	3901	2611	2.00	2.14	1.0	0.2550	0.831	0.0016	-	-	NM122000B011
E0-08-001-12A, 1mL FV	GVS ROAD POLLEN 07/21/00	TI 435	0	2983	2.00	2.22	1.0	0.2550	0.831	<0.000500	-	-	NM122000B012
10.0 ppb TI-435 pollen	standard	TI 435	27164	2749	-	-	-	0.2550	0.831	-	-	-	NM122000B013
E0-08-003-11A, 1mL FV	POLLEN TI-435 ALL 06/07/00	TI 435	5460	2794	2.00	2.22	1.0	0.2550	0.831	0.0023	-	-	NM122000B014
E0-08-003-13A, 1mL FV	POLLEN CONTROL ALL 06/07/00	TI 435	0	2765	0.34	1.05	1.0	0.2550	0.831	<0.000500	-	-	NM122000B015
E0-08-003-17A, 1mL FV	COMP POLLEN D10-12 12/07/00	TI 435	7099	2998	2.00	2.22	1.0	0.2550	0.831	0.0026	-	-	NM122000B016
25.0 ppb TI-435 pollen	standard	TI 435	66042	3081	-	-	-	0.2550	0.831	-	-	-	NM122000B017
E0-08-003-25A, 1mL FV	COMP POLLEN CTL D8,D13,D12 12/07/00	TI 435	0	2991	2.00	2.14	1.0	0.2550	0.831	<0.000500	-	-	NM122000B018
E0-08-003-35A, 1mL FV	COMP POLLEN CTL D8,D13,D14,O12 20/07/00	TI 435	0	3106	2.00	2.22	1.0	0.2550	0.831	<0.000500	-	-	NM122000B019
50.0 ppb TI-435 pollen	standard	TI 435	127028	3099	-	-	-	0.2550	0.831	-	-	-	NM122000B020
100 ppb TI-435 pollen	standard	TI 435	272764	3346	-	-	-	0.2550	0.831	-	-	-	NM122000B021
2.50 ppb TI-435 pollen	standard	TI 435	7149	3179	-	-	-	0.2550	0.831	-	-	-	NM122000B022

Comments:

Calibration Filename: NM122000BCal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppb)=[(Analyte Peak area / ISTD Peak area) - y-intercept] / slope X final volume X aliquot / sample weight / 1000

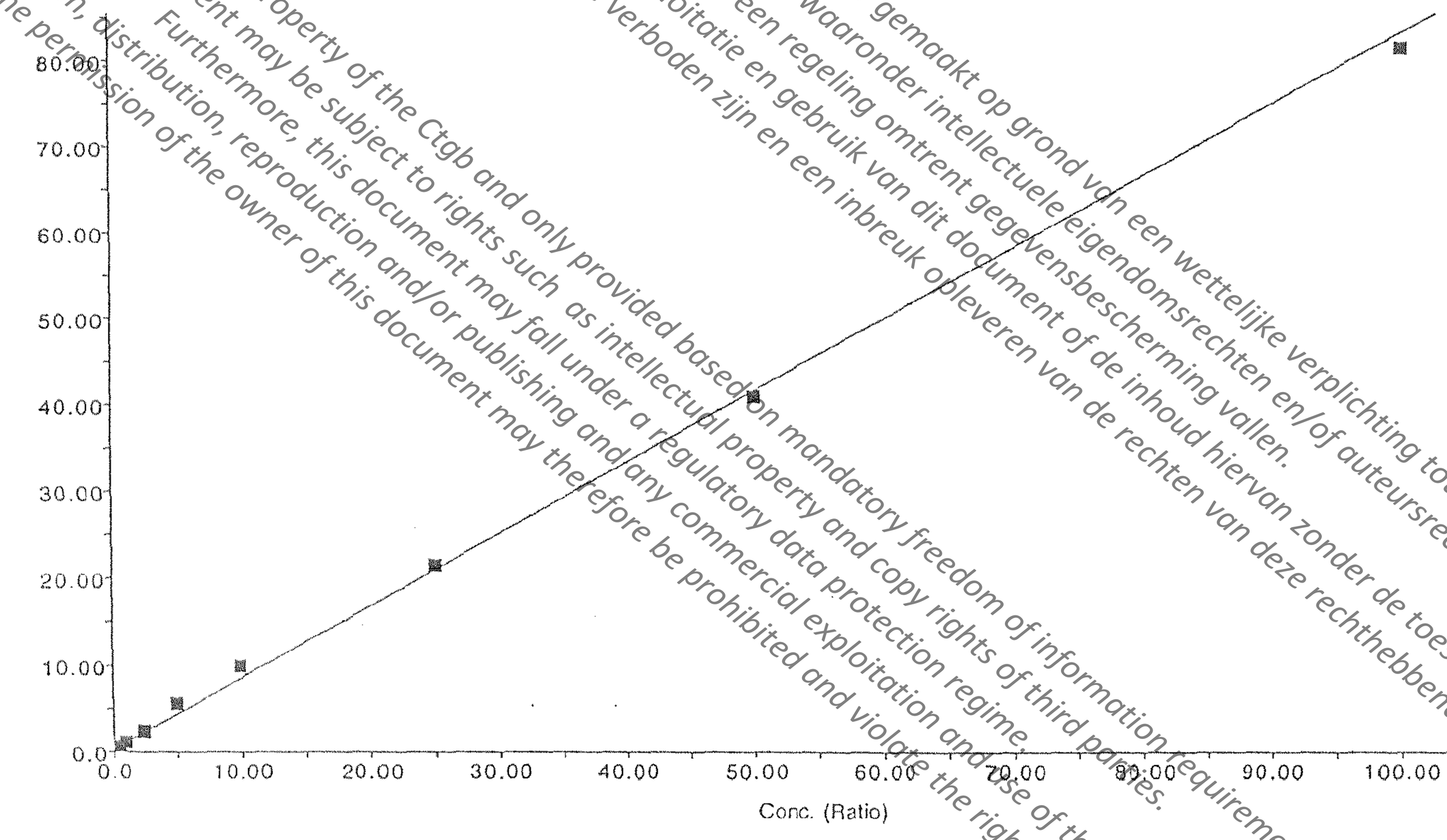
Data Entered By: [redacted] Date: Jan. 9. 2001 Audited By [redacted] Jan. 9, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

MacQuan, version 1.6
Printed: Thu, Dec 21, 2000 08:22
Calibration File: NM122000B.Cal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122000B:
Comments: Protocol ETL008AY02.PRO; TI 435 Pollen

TI 435 250.0->169.0 Internal Standard: TI 435 d3
Weighted (1/x)

Intercept = 0.255
Slope = 0.831
Correlation Coeff. = 0.9981



Protocol No.: ETL008AY02
ETL Report No.:01BAY09.REP

Set: Pollen Validation | Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAYC2.PRO | Matrix: Pollen | Weight = 2g

SITE: | Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
blank	solvent	Olefin metabolite	0	-	-	-	187	492	-	-	-	NM12:900E001
Wed, Dec 20, 2000 05:05		Hydroxy metabolite	0	-	-	-	430	4533	-	-	-	NM12:900E001
		Imidacloprid	0	-	-	-	-300	6827	-	-	-	NM12:900E001
1.00 ppb Imidacloprid and metabolites pollen	standard	Olefin metabolite	712	-	-	-	187	492	-	-	-	NM12:900E002
Wed, Dec 20, 2000 05:23		Hydroxy metabolite	5624	-	-	-	430	4533	-	-	-	NM12:900E002
		Imidacloprid	6745	-	-	-	-300	6827	-	-	-	NM12:900E002
2.50 ppb Imidacloprid and metabolites pollen	standard	Olefin metabolite	1466	-	-	-	187	492	-	-	-	NM12:900E003
Wed, Dec 20, 2000 05:41		Hydroxy metabolite	11917	-	-	-	430	4533	-	-	-	NM121900E003
		Imidacloprid	16958	-	-	-	-300	6827	-	-	-	NM121900E003
E0-08-010-01A-1, 1mL FV	POLLEN UTC	Olefin metabolite	0	2.00	1.0	2.14	187	492	<0.00100	-	-	NM121900E004
Wed, Dec 20, 2000 05:59		Hydroxy metabolite	0	2.00	1.0	2.14	430	4633	<0.00100	-	-	NM121900E004
		Imidacloprid	0	2.00	1.0	2.14	-300	6827	<0.00100	-	-	NM121900E004
E0-08-010-01A-2, 1mL FV	POLLEN UTC	Olefin metabolite	0	2.00	1.0	2.14	187	492	<0.00100	-	-	NM121900E005
Wed, Dec 20, 2000 06:16		Hydroxy metabolite	0	2.00	1.0	2.14	430	4633	<0.00100	-	-	NM121900E005
		Imidacloprid	0	2.00	1.0	2.14	-300	6827	<0.00100	-	-	NM121900E005
E0-08-010-01A+1, 1mL FV	POLLEN UTC 0.001 ppm spike	Olefin metabolite	634	2.00	1.0	2.14	187	492	0.000972	0.00100	97	NM121900E006
Wed, Dec 20, 2000 06:34		Hydroxy metabolite	3869	2.00	1.0	2.14	430	4633	0.000793	0.00100	79	NM121900E006
		Imidacloprid	7317	2.00	1.0	2.14	-300	6827	0.00119	0.00101	118	NM121900E006
5.00 ppb Imidacloprid and metabolites pollen	standard	Olefin metabolite	2355	-	-	-	187	492	-	-	-	NM121900E007
Wed, Dec 20, 2000 06:52		Hydroxy metabolite	22467	-	-	-	430	4633	-	-	-	NM121900E007
		Imidacloprid	33728	-	-	-	-300	6827	-	-	-	NM121900E007
E0-08-010-01A+2, 1mL FV	POLLEN UTC 0.001 ppm spike	Olefin metabolite	563	2.00	1.0	2.14	187	492	0.000818	0.00100	82	NM121900E008
Wed, Dec 20, 2000 07:10		Hydroxy metabolite	4547	2.00	1.0	2.14	430	4633	0.000951	0.00100	95	NM121900E008
		Imidacloprid	6937	2.00	1.0	2.14	-300	6827	0.00113	0.00101	112	NM121900E008

Comments:

Calibration Filename: NM121900ECal

Regression type: Weighted (1/x)

Quantitation formu[redacted]ercptVslope x Final Volume x Aliquot factor / (Sample Wt.) ÷1000

Data Entered By: [redacted] Date: Jan. 9, 2001 Jan. 9, 2001

QC'ed by

Set: Pollen Validation — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02PRO — Matrix: Pollen — Weight = 2g

SITE: — Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-010-01A+3, 1mL FV	POLLEN UTC 0.001 ppm spike	Olefin metabolite	849	2.00	1.0	2.14	187	492	0.00100	0.00100	100	NM121900E009
Wed, Dec 20, 2000 07:28		Hydroxy metabolite	5279	2.00	1.0	2.14	430	4633	0.00112	0.00100	112	NM121900E009
		Imidacloprid	7678	2.00	1.0	2.14	-300	6827	0.00123	0.00101	122	NM121900E009
E0-08-010-01A+4, 1mL FV	POLLEN UTC 0.005 ppm spike	Olefin metabolite	2339	2.00	1.0	2.14	187	492	0.00468	0.00500	94	NM121900E010
Wed, Dec 20, 2000 07:45		Hydroxy metabolite	18782	2.00	1.0	2.14	430	4633	0.00424	0.00500	85	NM121900E010
		Imidacloprid	30903	2.00	1.0	2.14	-300	6827	0.00489	0.00505	97	NM121900E010
10.0 ppb Imidacloprid and metabolites pollen	standard	Olefin metabolite	5056	-	-	-	187	492	-	-	-	NM121900E011
Wed, Dec 20, 2000 08:03		Hydroxy metabolite	41969	-	-	-	430	4633	-	-	-	NM121900E011
		Imidacloprid	67107	-	-	-	-300	6827	-	-	-	NM121900E011
E0-08-010-01A+5, 1mL FV	POLLEN UTC 0.005 ppm spike	Olefin metabolite	2164	2.00	1.0	2.14	187	492	0.00430	0.00500	86	NM121900E012
Wed, Dec 20, 2000 08:21		Hydroxy metabolite	20040	2.00	1.0	2.14	430	4633	0.00453	0.00500	91	NM121900E012
		Imidacloprid	29483	2.00	1.0	2.14	-300	6827	0.00457	0.00505	92	NM121900E012
E0-08-010-01A+6, 1mL FV	POLLEN UTC 0.01 ppm spike	Olefin metabolite	4556	2.00	1.0	2.14	187	492	0.00950	0.0100	95	NM121900E013
Wed, Dec 20, 2000 08:39		Hydroxy metabolite	44614	2.00	1.0	2.14	430	4633	0.0102	0.0100	102	NM121900E013
		Imidacloprid	65521	2.00	1.0	2.14	-300	6827	0.0105	0.0101	104	NM121900E013
E0-08-010-01A+7, 1mL FV	POLLEN UTC 0.01 ppm spike	Olefin metabolite	5487	2.00	1.0	2.14	187	492	0.0115	0.0100	115	NM121900E014
Wed, Dec 20, 2000 08:57		Hydroxy metabolite	47343	2.00	1.0	2.14	430	4633	0.0108	0.0100	108	NM121900E014
		Imidacloprid	71328	2.00	1.0	2.14	-300	6827	0.0112	0.0101	111	NM121900E014
25.0 ppb Imidacloprid and metabolites pollen	standard	Olefin metabolite	12776	-	-	-	187	492	-	-	-	NM121900E015
Wed, Dec 20, 2000 09:15		Hydroxy metabolite	121048	-	-	-	430	4633	-	-	-	NM121900E015
		Imidacloprid	167472	-	-	-	-300	6827	-	-	-	NM121900E015
50.0 ppb Imidacloprid and metabolites pollen	standard	Olefin metabolite	25764	-	-	-	187	492	-	-	-	NM121900E016
Wed, Dec 20, 2000 09:32		Hydroxy metabolite	233018	-	-	-	430	4633	-	-	-	NM121900E016
		Imidacloprid	322813	-	-	-	-300	6827	-	-	-	NM121900E016

Comments:

Calibration Filename: NM121900ECal

Regression type: Weighted (1/x)

Quantitation formula (from the linearity curve): (Peak area - y-Intercept)/slope x Final Volume x Aliquot factor / (Sample Wt)

Data Entered By: [redacted] Date: Jan. 9, 2001

QC'ed by: Jan. 9, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

Set: Pollen Validation — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Pollen — Weight = 2g

SITE: — Analytical Method: MS224.00

ETL Sample/Standard ID	Client Sample ID	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
100 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	50249	-	-	-	187	492	-	-	-	NM121900E017
Wed Dec 20, 2000 09:50		Hydroxy metabolite	475242			-	430	4633	-	-	-	NM121900E017
		Imidacloprid	7715886			-	-300	6827	-	-	-	NM121900E017
250 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	121585			-	187	492	-	-	-	NM121900E018
Wed Dec 20, 2000 10:08		Hydroxy metabolite	1147065			-	430	4633	-	-	-	NM121900E018
		Imidacloprid	1694545			-	-300	6827	-	-	-	NM121900E018

Comments:

Calibration Filename: NM121500ECal

Regression type: Weighted (1/x)

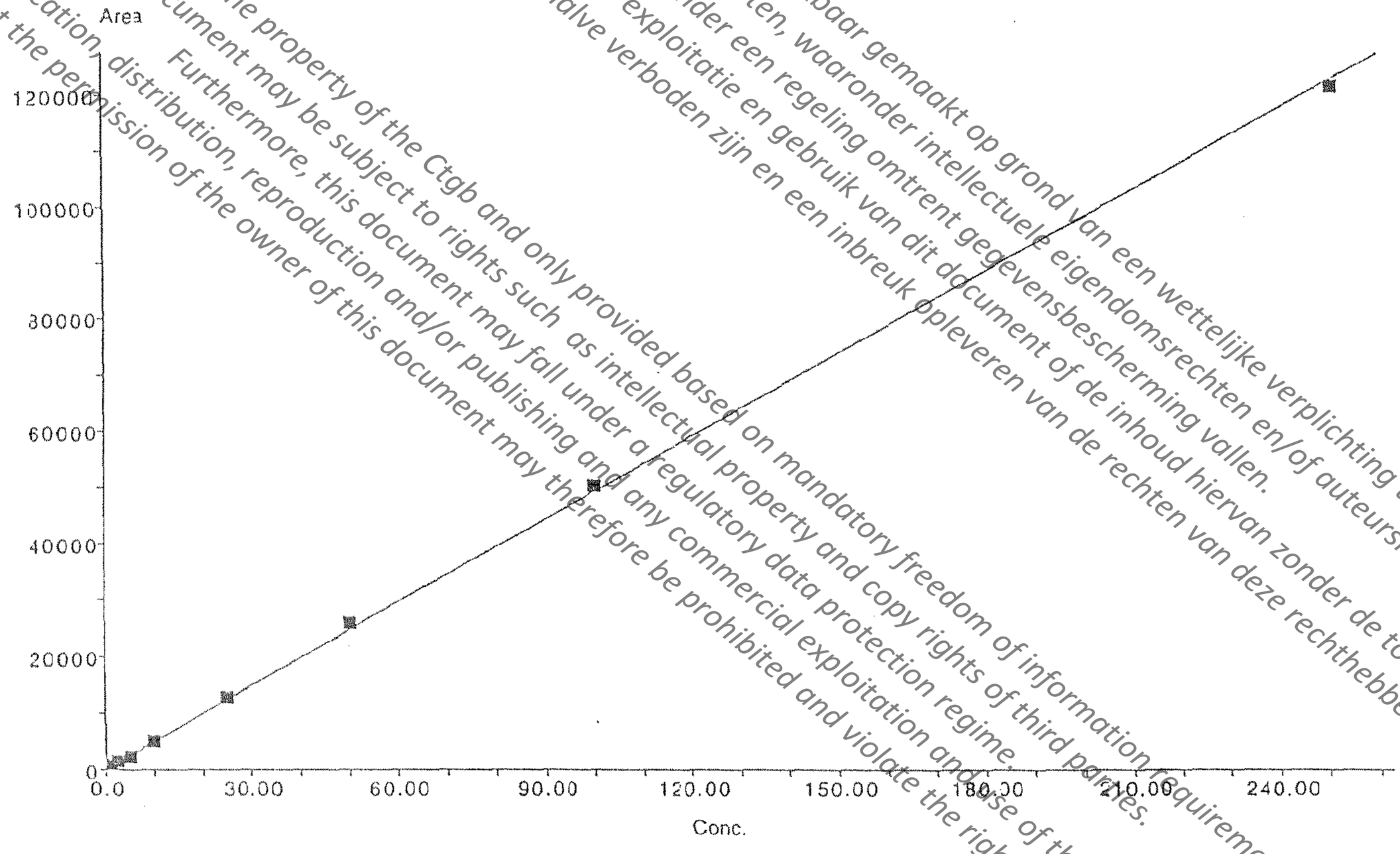
Quantitation formula: [illegible] x Intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.)

Data Entered By: [redacted] Date: Jan. 9, 2001

QC'ed by

Imidacloprid Olefin metabolite 254.0->208.0 No Internal Standard
Weighted (1/x)

Intercept = 187
Slope = 492
Correlation Coeff. = 0.9996



MacQuan, Version 1.6
Printed: Thu, Dec 21, 2000 08:24
Calibration File: NM121900ECal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM121900E:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Pollen Validation

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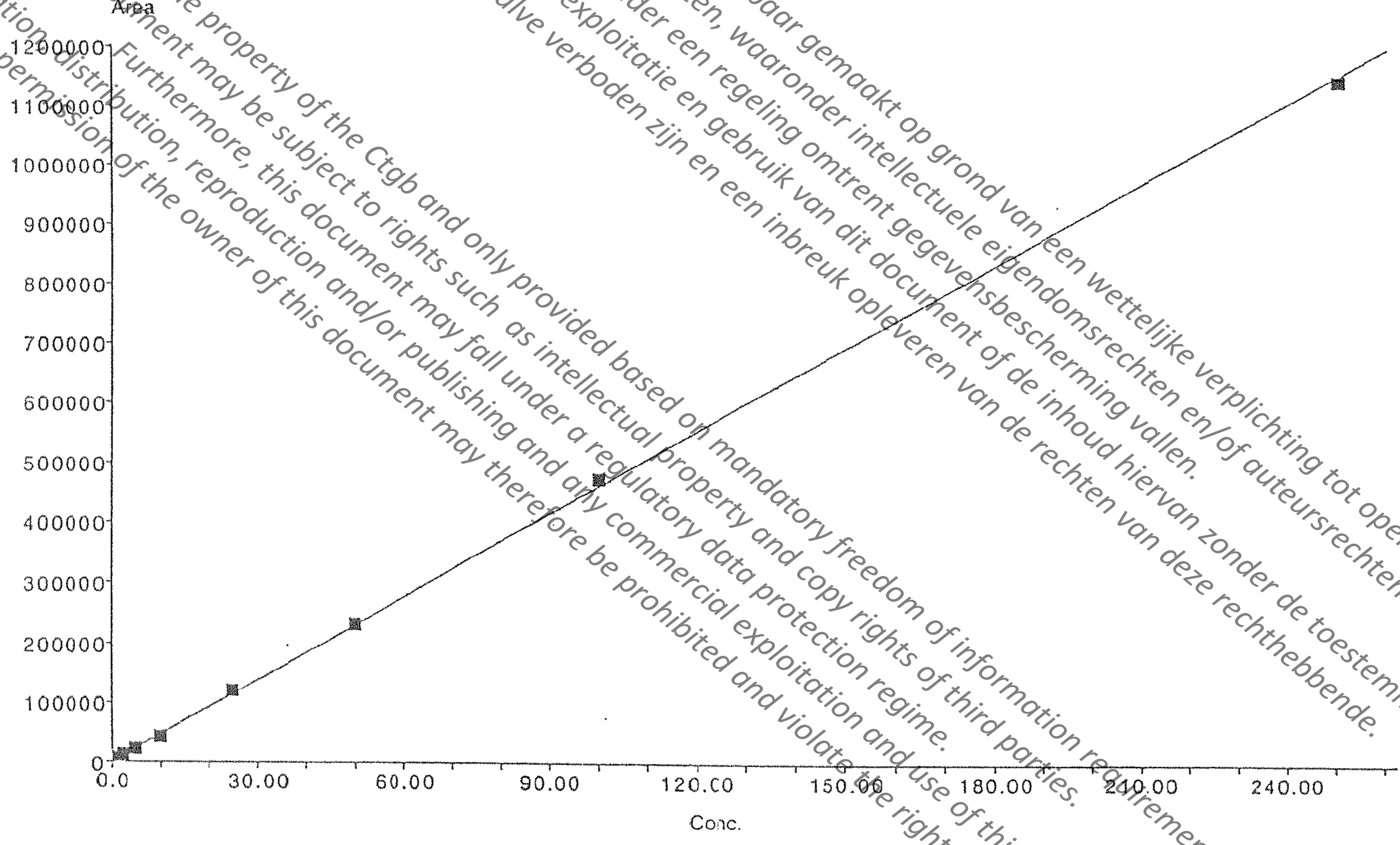
Imidacloprid Hydroxy metabolite 272.0->191.0 No Internal Standard
Weighted (1/x)
Intercept = 430
Slope = 4633
Correlation Coeff. = 0.9997



MacQuan, version 1.6
Printed: Thu, Dec 21, 2000 08:24
Calibration File: NM121900ECal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM121900E:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Pollen Validation

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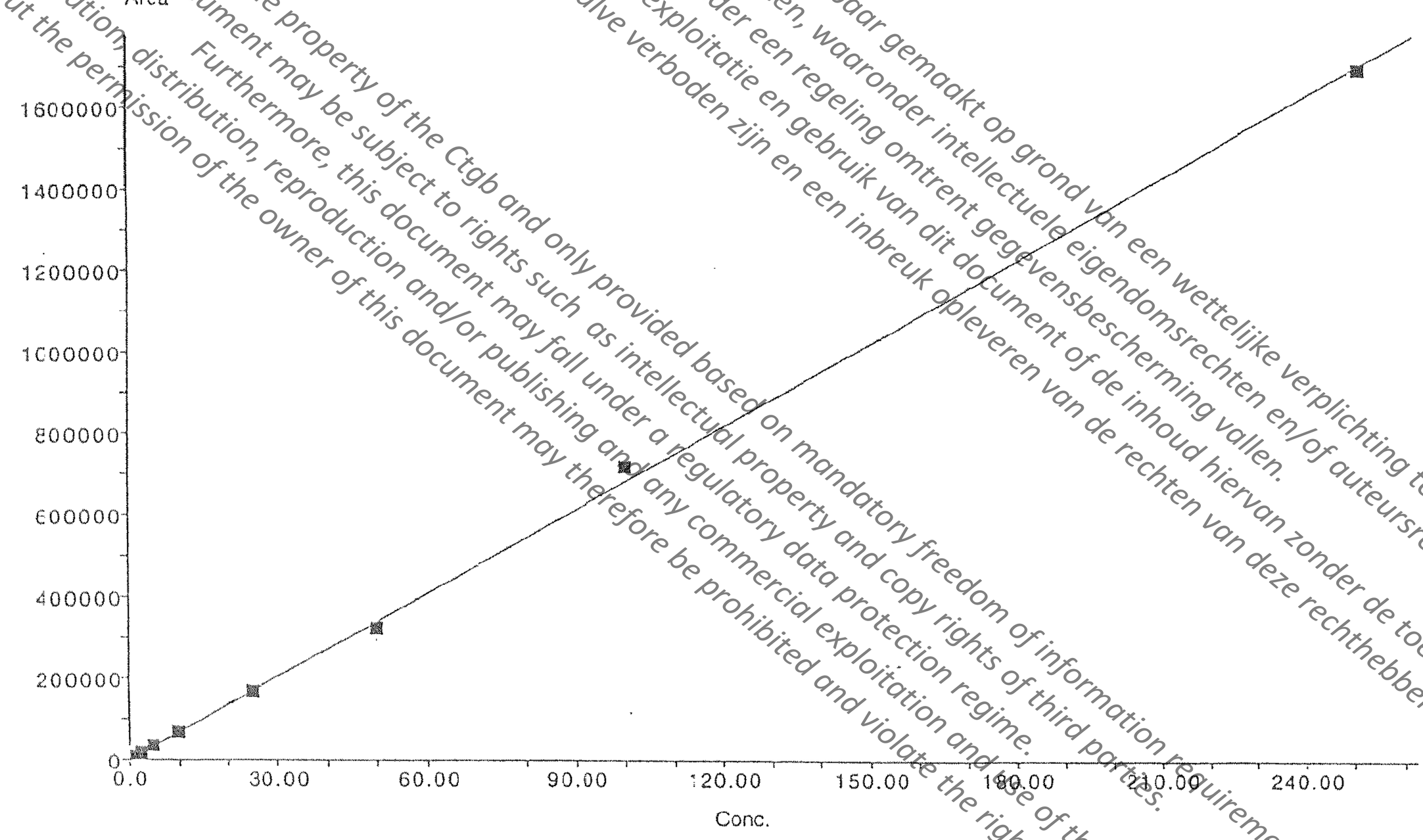
Imidaclopprid 256.0->209.0 No Internal Standard
Weighted (1/x)
Intercept = -300
Slope = 6827
Correlation Coeff. = 0.9995



Set: Pollen #1		Determination of Imidicloprid and Metabolites in Pollen and Necta										
Protocol No.: ETL00BAY02.PRO				Matrix: Pollen					Analysis Date: December 21, 2000			
SITE:				Analytical Method: MS224.00					Weight = 2g			
ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
blank	solvent	Olefin metabolite	0	-	-	-	91	239	-	-	-	NM122100B001
Thu, Dec 21, 2000 20:15		Hydroxy metabolite	0	-	-	-	514	2719	-	-	-	NM122100B001
		Imidacloprid	0	-	-	-	-232	4285	-	-	-	NM122100B001
1.00 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	362	-	-	-	91	239	-	-	-	NM122100B002
Thu, Dec 21, 2000 20:32		Hydroxy metabolite	2942	-	-	-	514	2719	-	-	-	NM122100B002
		Imidacloprid	4404	-	-	-	-232	4285	-	-	-	NM122100B002
2.50 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	745	-	-	-	91	239	-	-	-	NM122100B003
Thu, Dec 21, 2000 20:50		Hydroxy metabolite	7502	-	-	-	514	2719	-	-	-	NM122100B003
		Imidacloprid	10817	-	-	-	-232	4285	-	-	-	NM122100B003
E0-08-001-12A-2, 1mL FV	GVS ROAD POLLEN 07/21/00	Olefin metabolite	0	2.00	1.0	2.14	91	239	<0.00400	-	-	NM122100B004
Thu, Dec 21, 2000 21:08		Hydroxy metabolite	0	2.00	1.0	2.14	514	2719	<0.00100	-	-	NM122100B004
		Imidacloprid	0	2.00	1.0	2.14	-232	4285	<0.00100	-	-	NM122100B004
E0-08-001-12A+2, 1mL FV	GVS ROAD POLLEN 07/21/00 0.005ppm spike	Olefin metabolite	1061	2.00	1.0	2.14	91	239	0.00437	0.00500	87	NM122100B005
Thu, Dec 21, 2000 21:26		Hydroxy metabolite	13157	2.00	1.0	2.14	514	2719	0.00496	0.00500	100	NM122100B005
		Imidacloprid	18918	2.00	1.0	2.14	-232	4285	0.00478	0.00505	95	NM122100B005
E0-08-001-12A+1, 1mL FV	GVS ROAD POLLEN 07/21/00 0.001ppm spike	Olefin metabolite	316	2.00	1.0	2.14	91	239	0.00101	0.00100	101	NM122100B006
Thu, Dec 21, 2000 21:44		Hydroxy metabolite	2926	2.00	1.0	2.14	514	2719	0.000948	0.00100	95	NM122100B006
		Imidacloprid	4567	2.00	1.0	2.14	-232	4285	0.00120	0.00101	119	NM122100B006
5.00 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	1171	-	-	-	91	239	-	-	-	NM122100B007
Thu, Dec 21, 2000 22:01		Hydroxy metabolite	13648	-	-	-	514	2719	-	-	-	NM122100B007
		Imidacloprid	20099	-	-	-	-232	4285	-	-	-	NM122100B007
E0-08-001-06A, 1mL FV	GVH FARM POLLEN 07/11/00	Olefin metabolite	0	2.00	1.0	2.14	91	239	<0.00100	-	-	NM122100B008
Thu, Dec 21, 2000 22:19		Hydroxy metabolite	0	2.00	1.0	2.14	514	2719	<0.00100	-	-	NM122100B008
		Imidacloprid	0	2.00	1.0	2.14	-232	4285	<0.00100	-	-	NM122100B008

Comments:

Calibration Filename: NM122100BCal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve: (ppm)=(Peak area - y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.) ÷1000

Data Entered By: [redacted] Date: Jan-9-2001

QC'ed by: audited by AB Jan 5, 2001

Protocol No.: ETL00BAY02
ETL Report No.: 01BAY09.REP

Set: Pollen #1 | Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO | Matrix: Pollen | Weight = 2g

SITE: | Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-001-07A, 1mL FV	GVH FARM POLLEN 07/17/00	Olefin metabolite	0	2.00	1.0	2.22	91	239	<0.00100	-	-	NM122100B009
Thu, Dec 21, 2000 22:37		Hydroxy metabolite	0	2.00	1.0	2.22	514	2719	<0.00100	-	-	NM122100B009
		Imidacloprid	0	2.00	1.0	2.22	-232	4285	<0.00100	-	-	NM122100B009
E0-08-001-10AB, 1mL FV	GVH FARM POLLEN 07/17/00	Olefin metabolite	0	2.00	1.0	2.14	91	239	<0.00100	-	-	NM122100B010
Thu, Dec 21, 2000 22:55		Hydroxy metabolite	0	2.00	1.0	2.14	514	2719	<0.00100	-	-	NM122100B010
		Imidacloprid	0	2.00	1.0	2.14	-232	4285	<0.00100	-	-	NM122100B010
10.0 ppb Imidicloprid and metabolites pollen	Standard	Olefin metabolite	2204	-	-	-	91	239	-	-	-	NM122100B011
Thu, Dec 21, 2000 23:13		Hydroxy metabolite	29088	-	-	-	514	2719	-	-	-	NM122100B011
		Imidacloprid	42311	-	-	-	-232	4285	-	-	-	NM122100B011
E0-08-003-12A, 1mL FV	POLLEN GAUCHO ALL 06/07/00	Olefin metabolite	0	0.52	0.50	1.09	91	239	<0.00100	-	-	NM122100B012
Thu, Dec 21, 2000 23:31		Hydroxy metabolite	0	0.52	0.50	1.09	514	2719	<0.00100	-	-	NM122100B012
		Imidacloprid	5728	0.52	0.50	1.09	-232	4285	0.0015	-	-	NM122100B012
E0-08-003-13AB, 1mL FV	POLLEN CONTROL ALL 06/07/00	Olefin metabolite	0	0.34	0.50	1.09	91	239	<0.00100	-	-	NM122100B013
Thu, Dec 21, 2000 23:48		Hydroxy metabolite	0	0.34	0.50	1.09	514	2719	<0.00100	-	-	NM122100B013
		Imidacloprid	0	0.34	0.50	1.09	-232	4285	<0.00100	-	-	NM122100B013
E0-08-003-21A, 1mL FV	COMP-POLLEN GAUCHO D1,D3,O22 12/07/00	Olefin metabolite	0	2.00	1.0	2.22	91	239	<0.00100	-	-	NM122100B014
Fri, Dec 22, 2000 00:06		Hydroxy metabolite	0	2.00	1.0	2.22	514	2719	<0.00100	-	-	NM122100B014
		Imidacloprid	29183	2.00	1.0	2.22	-232	4285	0.0076	-	-	NM122100B014
25.0 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	5891	-	-	-	91	239	-	-	-	NM122100B015
Fri, Dec 22, 2000 00:24		Hydroxy metabolite	65788	-	-	-	514	2719	-	-	-	NM122100B015
		Imidacloprid	103159	-	-	-	-232	4285	-	-	-	NM122100B015
E0-08-003-25AB, 1mL FV	COMP-POLLEN CTL D8,D13,O12 12/07/00	Olefin metabolite	0	2.00	1.0	2.22	91	239	<0.00100	-	-	NM122100B016
Fri, Dec 22, 2000 00:42		Hydroxy metabolite	0	2.00	1.0	2.22	514	2719	<0.00100	-	-	NM122100B016
		Imidacloprid	749	2.00	1.0	2.22	-232	4285	<0.00100	-	-	NM122100B016

Comments:

Calibration Filename: NM122100BCal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppm)=(Peak area - y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.)

Data Entered By: [redacted] Date: Jan. 5. 2001

QC'ed by [illegible] Jan. 5, 2001

Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

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Protocol No.: ETL00BAY02
ETL Report No.:01BAY09.REP

Set: Pollen #1 — Determination of Imidicloprid and Metabolites in Pollen and Nectar

Protocol No.: ETL00BAY02.PRO — Matrix: Pollen — Weigh: = 2g

SITE: — Analytical Method: MS224.00

ETL Sample/Standard I.D.	Client Sample I.D.	Analyte	Peak Area	Weight (g)	Final Volume (mL)	Aliquot Factor (mL/mL)	Y Intercept	Slope	Results (ug/g)	Spike Level (ug/g)	Recovery (%)	LC/MS/MS Chrom. Filename
E0-08-003-30A, 1mL FV	COMP-POLLEN GAUCHO D1,D3,D5,O22 20/07/	Olefin metabolite	0	2.00	1.0	2.14	91	239	<0.00100	-	-	NM122100B017
Fri, Dec 22, 2000 01:00		Hydroxy metabolite	1246	2.00	1.0	2.14	514	2719	<0.00100	-	-	NM122100B017
		Imidacloprid	17557	2.00	1.0	2.14	-232	4285	0.0044	-	-	NM122100B017
E0-08-003-35AB, 1mL FV	COMP-POLLEN CTL D8,D13,D14,O12 20/07/00	Olefin metabolite	0	2.00	1.0	2.22	91	239	<0.00100	-	-	NM122100B018
Fri, Dec 22, 2000 01:17		Hydroxy metabolite	0	2.00	1.0	2.22	514	2719	<0.00100	-	-	NM122100B018
		Imidacloprid	825	2.00	1.0	2.22	-232	4285	<0.00100	-	-	NM122100B018
50.0 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	12282	-	-	-	91	239	-	-	-	NM122100B019
Fri, Dec 22, 2000 01:35		Hydroxy metabolite	148962	-	-	-	514	2719	-	-	-	NM122100B019
		Imidacloprid	206865	-	-	-	-232	4285	-	-	-	NM122100B019
100 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	23576	-	-	-	91	239	-	-	-	NM122100B020
Fri, Dec 22, 2000 01:53		Hydroxy metabolite	283322	-	-	-	514	2719	-	-	-	NM122100B020
		Imidacloprid	429450	-	-	-	-232	4285	-	-	-	NM122100B020
250 ppb Imidicloprid and metabolites pollen	standard	Olefin metabolite	60554	-	-	-	91	239	-	-	-	NM122100B021
Fri, Dec 22, 2000 02:11		Hydroxy metabolite	698523	-	-	-	514	2719	-	-	-	NM122100B021
		Imidacloprid	1051256	-	-	-	-232	4285	-	-	-	NM122100B021

Comments:

Calibration Filename: NM122100BCal

Regression type: Weighted (1/x)

Quantitation formula from the linearity curve (ppm)=(Peak area - y-intercept)/slope x Final Volume x Aliquot factor / (Sample Wt.)

Data Entered By: [redacted] Date: Jan 5, 2001

QC'ed by: audited by AB Jan. 5, 2001

MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:11
Calibration File: NM122100BCal Path: Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100B:
Comments: Protocol ETL00BAY02.PRO - Imidicloprid and metabolites - Pollen

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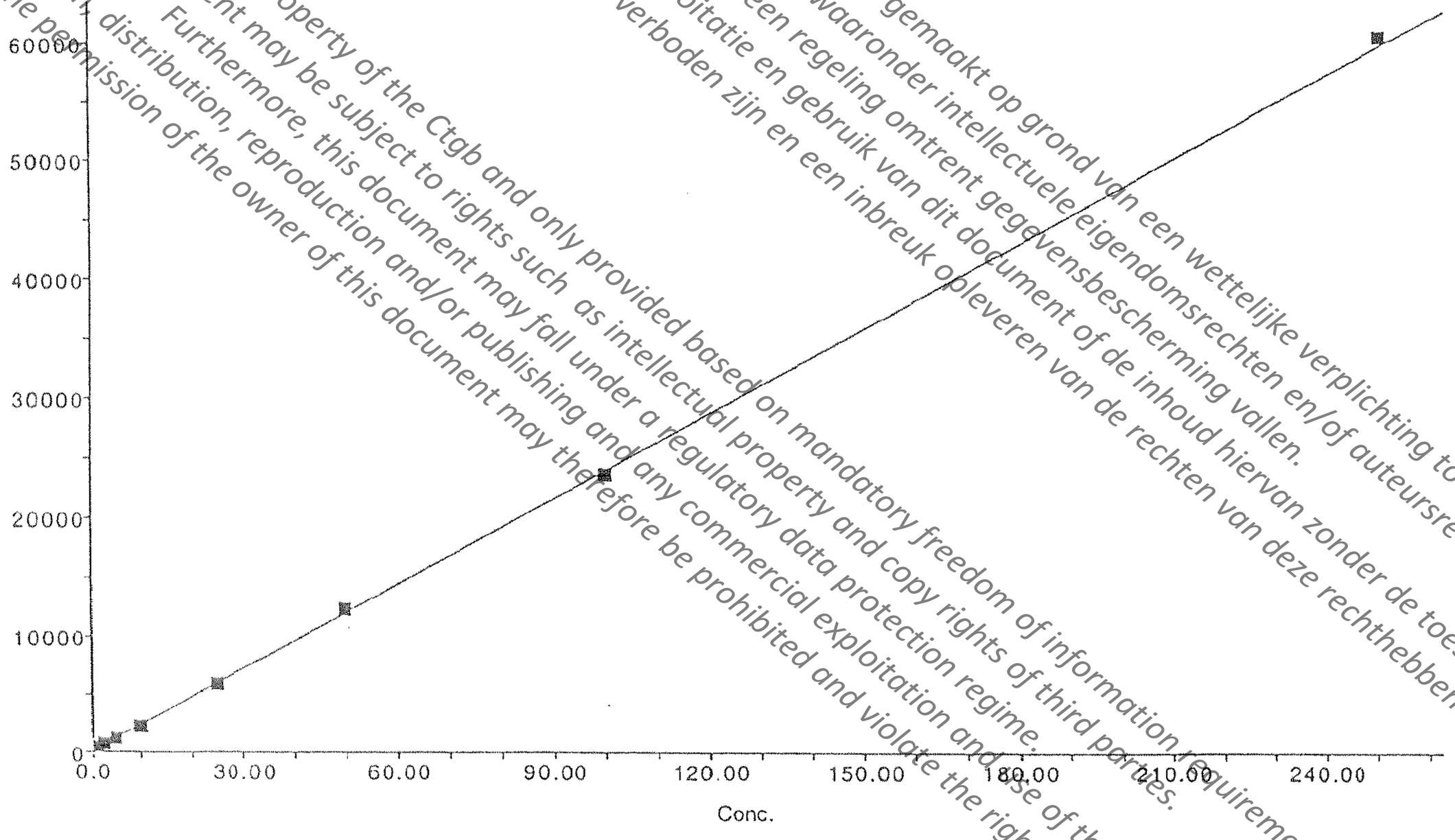
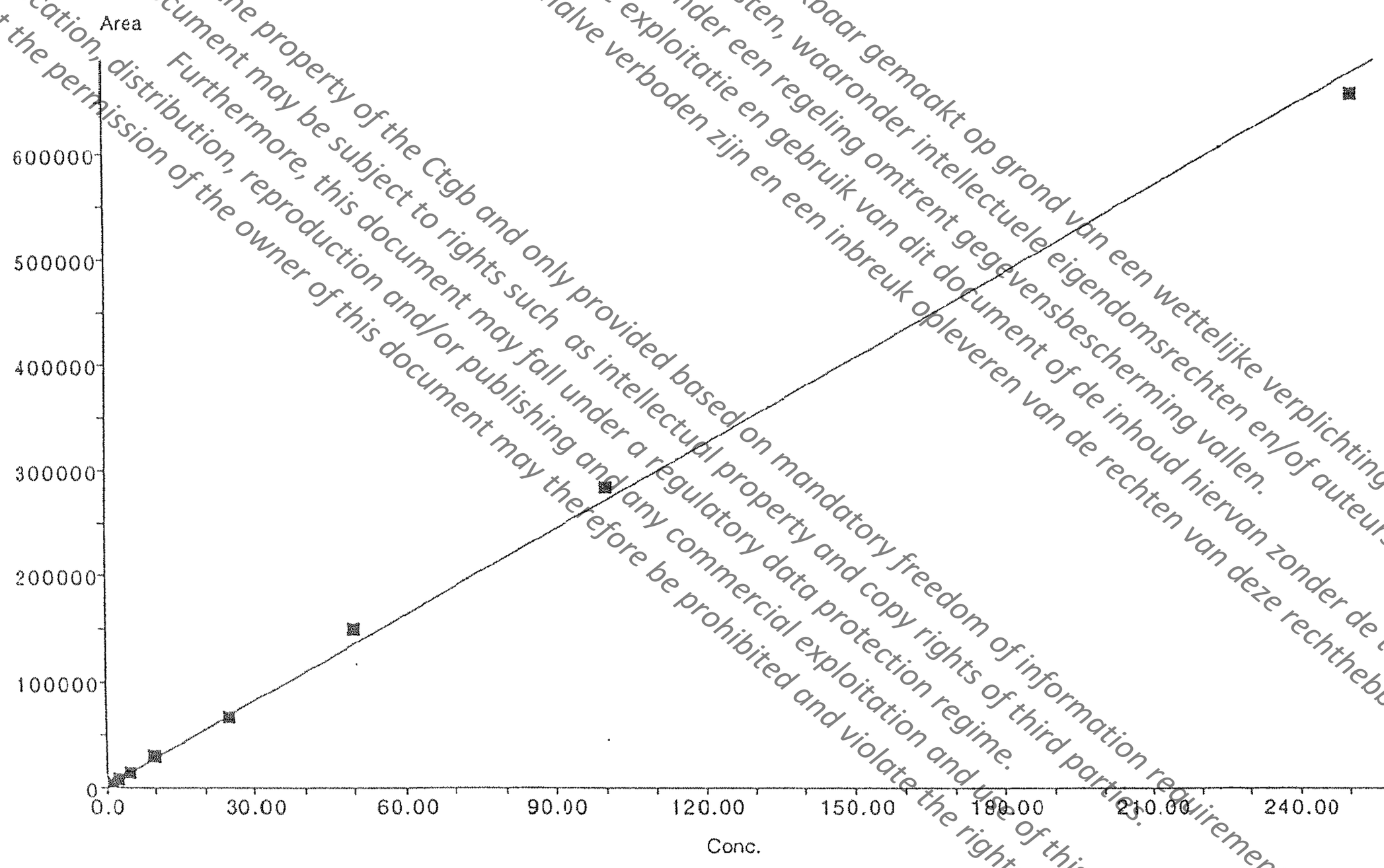
Imidacloprid Olefin metabolite 254.0->208.0 No Internal Standard
Weighted (1/x)
Intercept = 91
Slope = 239
Correlation Coeff. = 0.9996



Imidacloprid Hydroxy metabolite 272.0->191.0 No Internal Standard
Weighted (1/x)
Intercept = 514
Slope = 2719
Correlation Coeff. = 0.9989



MacQuan, version 1.6
Printed: Fri, Dec 22, 2000 08:13
Calibration File: NM122100BCal Path:Macintosh HD:API3000:DATA:BAYER:IMIDACLOPRID:NM122100B:
Comments: Protocol ETL00BAY02-PRO - Imidicloprid and metabolites - Pollen

Imidacloprid 256.0->209.0 No Internal Standard
Weighted (1/x)

Intercept = -232
Slope = 4285
Correlation Coeff. = 0.9998