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Study Title

Investigation of the metabolism of
NTN 33893 in potatoes
following granular application

Data Requirement

171-4 Nature of Residue (Metabolism) - Plants

Author

[redacted]

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Performing Laboratory

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T A B L E O F C O N T E N T S

I. Summary

The metabolism of the insecticide NTN 33893 was investigated in potatoes after application of [pyridinyl-14C-methyl] NTN 33893. An in-furrow application of 5% granules at a rate of 0.05 g active ingredient per running meter was made at the time of planting the potatoes. The vines and tubers were harvested 129 days after application. At the time of harvest the vines were withered and mostly dry as would be under practical conditions.

The total residues, expressed in a.i. equivalents, were 5.76 mg/kg in vines and 0.091 mg/kg in tubers. Of the radioactivity applied to the soil 2.2% was taken up by the vines and 0.3% by the tubers.

By chromatographic comparison with reference compounds and other physical methods the following could be identified (amounts given in per cent radioactivity and in mg/kg a.i. equivalents in vines and tubers respectively):

1. Vines

Unchanged parent compound	(I)	26.7%	(1.53 mg/kg)
5-Hydroxy compound	(IV)	4.6%	(0.26 mg/kg)
Dihydroxy compound	(VII)	0.3%	(0.02 mg/kg)
Olefine compound	(VI)	3.3%	(0.19 mg/kg)
Nitrosimine compound	(VIII)	2.6%	(0.15 mg/kg)
Guanidine compound	(II)	8.2%	(0.48 mg/kg)
6-Chloronicotinic acid	(XII)	8.3%	(0.48 mg/kg)
Glucoside of 6-chloropicolyl alcohol	(X)	1.4%	(0.08 mg/kg)

Another 14 unknown metabolites were detected in lower concentrations which in total amounted to 16.1%, 0.93 mg/kg. The non-extractable residue corresponded to 26.4%, 1.52 mg/kg.

2. Tubers

Unchanged parent compound	(I)	48.3%	(0.044 mg/kg)
5-Hydroxy compound	(IV)	8.0%	(0.007 mg/kg)
Olefine compound	(VI)	3.1%	(0.003 mg/kg)
Guanidine compound	(II)	11.3%	(0.010 mg/kg)
6-Chloronicotinic acid	(XII)	9.4%	(0.009 mg/kg)

Another 5 unknown metabolites occurred in very low concentrations and in total amounted to 13.1%, 0.012 mg/kg. The non-extractable residue was 6.4%, 0.006 mg/kg.

The identified compounds are shown in a proposed degradation pathway (Figure 15).

I. Zusammenfassung

Der Metabolismus des Insektizids NTN 33893 (I) wurde nach Applikation von [pyridinyl-14C-methyl] NTN 33893 an Kartoffeln untersucht. Es wurde ein 5 %iges Granulat bei der Aussaat der Kartoffeln in die Saatfurche in einer Aufwandmenge von 0,05 g Wirkstoff pro laufendem Meter appliziert. Kraut und Knollen wurden 129 Tage nach der Applikation geerntet. Zum Zeitpunkt der Ernte war das Kraut ähnlich wie in der Praxis welk und weitgehend trocken.

Die Gesamtrückstände, ausgedrückt in Wirkstoffäquivalenten, betrugen 5,76 mg/kg im Kraut und 0,091 mg/kg in den Knollen. Von der auf den Boden applizierten Radioaktivität wurden vom Kraut 2,2 % und von den Knollen 0,3 % aufgenommen.

Durch chromatographischen Vergleich mit Referenzsubstanzen und andere physikalische Methoden konnten identifiziert werden (Angaben in Prozent der Radioaktivität in Kraut bzw. Knolle und in mg/kg Wirkstoffäquivalenten):

1. Kraut:

Unveränderter Wirkstoff	(I)	26,7%	(1,53 mg/kg)
5-Hydroxy-Verbindung	(IV)	4,6%	(0,26 mg/kg)
Dihydroxy-Verbindung	(VII)	0,3%	(0,02 mg/kg)
Olefin-Verbindung	(VI)	3,3%	(0,19 mg/kg)
Nitrosimin-Verbindung	(VIII)	2,6%	(0,15 mg/kg)
Guanidin-Verbindung	(II)	8,2%	(0,48 mg/kg)
6-Chlornicotinsäure	(XII)	8,3%	(0,48 mg/kg)
Glucosid des 6-Chlorpicolylalkohol	(X)	1,4%	(0,08 mg/kg)

Weiterhin wurden 14 Metaboliten in geringer Konzentration nachgewiesen, die unbekannt waren, und deren Menge zusammen 16,1 % und 0,93 mg/kg betrug. Auf die nicht-extrahierbaren Rückstände entfielen 26,4 % und 1,52 mg/kg.

2. Knolle:

Unveränderter Wirkstoff	(I)	48,3%	(0,044 mg/kg)
5-Hydroxy-Verbindung	(IV)	8,0%	(0,007 mg/kg)
Olefin-Verbindung	(VI)	3,1%	(0,003 mg/kg)
Guanidin-Verbindung	(II)	11,3%	(0,010 mg/kg)
6-Chlornicotinsäure	(XII)	9,4%	(0,009 mg/kg)

Weiterhin traten 5 unbekannte Metaboliten in sehr geringer Konzentration auf, deren Menge zusammen 13,1 % und 0,012 mg/kg betrug. Die nicht-extrahierbaren Rückstände betrugen 6,4 % und 0,006 mg/kg.

Die identifizierten Verbindungen sind in einem Abbauschema (Abb. 15) dargestellt.

II. INTRODUCTION

The compound NTN 33893 (proposed common name imidacloprid, chemical designation 1-(6-chloro-3-pyridinyl)-methyl-4,5-dihydro-N-nitro-1H-imidazole-2-amine, CA 105 827-78-9, 1987) is an insecticide showing systemic activity. It was tested as a foliar and soil applied insecticide.

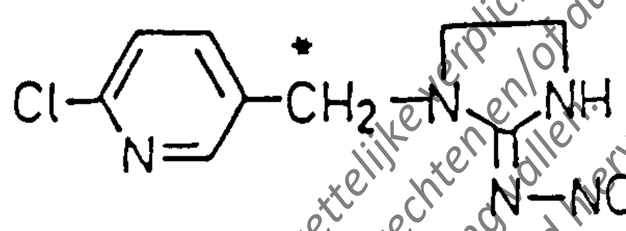
A great number of metabolites have been detected in plant metabolism studies reported so far: in cell cultures ([redacted] 1989), tomatoes ([redacted] 1989) and potatoes (after spray application; [redacted], 1990) as well as in studies on corn ([redacted] 1991), apples [redacted], 1991) and rice ([redacted], 1990) which have not yet been reported. The metabolites detected were the monohydroxy compound (IV) and its conjugate, the dihydroxy compound (VII), the keto compound (XVI), the olefine compound (VI), the guanidine compound (II), the urea compound (III), the nitrosimine compound (VIII), the ring-opened guanidine compound (XV), the 6-chloropicolyl alcohol (XIII) as well as its glucoside (X) and gentiobioside (XI) and the 6-chloronicotinic acid (XII). After spray application the parent compound was the main component of the residue. However, after uptake through the roots the proportion of parent compound was lower.

The objective of the present study was to investigate the metabolism of NTN 33893 in potatoes after granular application. The experimental part of the study was started on April 17, 1989 and completed on September 7, 1990.

III. MATERIALS

A. Test compound

Structural formula:



(The labelling position is marked with *)

Chemical designation: 1-(6-chloro-3-pyridinyl)methyl-4,5-dihydro-N-nitro-1H-imidazole-2-amine (C.A.)

CAS-No. : 105 827-78-9 (1987)

Molecular weight : 255.7

Solubility : 580 mg/l (water, 20°C)

46 g/l (acetone, 20°C)

Spec. Radioactivity : 0.944 MBq/mg (25.5 µCi/mg)

([redacted])

Radiochem. purity : 99.65% ([redacted])

A 5% GR-formulation was prepared from the pyridinyl-^{14}C-methyl-labelled active ingredient ([redacted] formulation ECW 6130). For this purpose the radiolabelled active ingredient together with the mixture of the formulation adjuvants was slowly concentrated by evaporation in a round-bottomed flask on a rotary evaporator. The conditions were tested in preliminary experiments in order to guarantee that a formulation corresponding to the commercial product was obtained. Content, purity and specific radioactivity of the active ingredient in the formulation were determined after extraction by means of HPLC and LS-measurement.

The active ingredient in the formulation contained such an amount of pyridinyl-^{13}C-methyl-labelled NTN 33893 to give a $^{12}C/^{13}C$-ratio of about 1:1. The identity of the active ingredient in the formulation was verified by MS and ^{1}H-NMR-spectrometry (for formulation details see Appendix I).

To obtain larger amounts of metabolites a mixture of non-radioactive and ^{13}C-labelled active ingredient, in a ratio of about 1:1, was used in a parallel experiment. However, the plant samples of this experiment were not needed and therefore this experiment is not reported.

B. Reference compounds

The structural formulae of the reference compounds for the identification of the metabolites and their chromatographic properties are summarized in Table I. The reference compounds were provided by [redacted] (6-chloronicotinic acid), [redacted] (all Bayer AG) and by [redacted]. In the course of the study the stability of the reference compounds was continuously checked by thin-layer chromatography.

C. Test facilities

The study was carried out in the vegetation area (Building 6682) of the Institute for Metabolism Research in the Pflanzenschutzzentrum Monheim of Bayer AG. The planting container (0.96 m²) was marked with the study number (M 173 0297-9). It was filled with a 10 cm layer of fine gravel for drainage as well as a 50 cm layer of a sandy loam soil (Monheim 1, for composition see Appendix II). The planting container was protected against rain and the plants were watered by drenching so that an optimal growth was achieved. All fertilization and crop protection measures which were carried out were recorded (see Appendix III).

IV. METHODS

A. Planting and application

The potatoes were planted on April 17, 1989. Six pre-germinated potatoes of the variety Clivia were planted into the planting box; two potatoes were planted per seed furrow, each 80 cm long. In each of the three seed furrows, 0.8 g of the radioactive granules (= 37.8 MBq) had been incorporated prior to planting. A total of 113.4 MBq were applied. The potatoes emerged on May 7, 1989.

The applied amount corresponds to that projected to be used in practice for seed furrow treatment which is 0.05 g a.i. or 1 g granules per running meter.

The stability of the active ingredient in the formulation was examined shortly before application. Based on thin-layer chromatography and LS measurement (after scraping off the respective zones), more than 98.6% of the radioactivity was accounted for by the active ingredient (Appendix IV).

B. Sampling

On August 24, 1989 (day 129) the potatoes were harvested after the vines had completely withered and dried as in practice. The tubers (3.833 kg) were carefully washed with cold water in order to remove adhering soil and any remaining formulation material. All of the tubers (except a 500 g portion) were cut into small pieces. The potato vines (462 g) were cut up by hand and subdivided into several portions. All samples were stored deep-frozen (- 20°C) until analysis.

C. Extraction of the samples

The potato tubers and vines were extracted by successive maceration with methanol/water (in case of tubers 2:1, in case of vines 1:1), methanol and dichloromethane using an Ultra-Turrax (see Fig. 1). The extracts were concentrated and the aqueous remainder was extracted with n-hexane followed by ethyl acetate (see Appendix V).

The three solvent phases - n-hexane, ethyl acetate, water - were concentrated under vacuum to a defined final volume. The solids were homogenized by maceration in liquid nitrogen. The ^{14}C-radioactivity of the solutions was determined by scintillation measurement and that of the solids by combustion and scintillation measurement.

The total residues in vines and tubers, expressed in mg active ingredient equivalents/kg, were determined from the sum of the radioactivity in the three solvent phases and in the solids (= 100%).

A radioactivity material balance is only given for the clean-up of the water phases since the ethyl acetate phases were directly analysed by TLC without any clean-up. After XAD 4 clean-up of the water phase of the potato vines 92.5% of the original radioactivity was recovered. After XAD 4 and SEP PAK clean-up of the water phase of the potato tubers 62.2% of the original radioactivity was recovered. These values were normalized to 100%.

D. Purification of the water phases for TLC analysis

The majority of polar plant constituents in the aqueous phases obtained according to IV.C were removed by column chromatography using an XAD resin (Type 4; 0.2-0.4 mm; Serva Co., Heidelberg, FRG) (see Fig. 1). For this purpose 20 g of resin was slurried in double distilled water into a column (2 cm diameter). The resin was washed with methanol and subsequently with water.

The aqueous phase (50 ml) was added to the column and the plant constituents eluted with 50 ml water (water eluate), this phase was discarded. The remaining matrix, containing the majority of the metabolites, was eluted with 100 ml methanol (methanol eluate). The radioactivity of both eluates was determined by scintillation measurement.

In the case of potato tubers the methanol eluate of the XAD 4 column was further cleaned up using SEP PAK cartridges (RP 18, Waters Co., Millipore GmbH, Eschborn, FRG). For this purpose the methanol eluate of the XAD 4 separation was concentrated and applied in 500 µl aliquots to a cartridge which had been pre-washed with water. Plant constituents were eluted with water and the metabolites with methanol.

E. Thin-layer chromatography

The radioactive solutions were analyzed by one- and two-dimensional TLC on commercial TLC plates (Silica gel 60 F_{254}, Merck Co., Darmstadt, FRG) using the following solvent systems:

SS I:	ethyl acetate/isopropanol/water	65:23:12
SS II:	ethyl acetate/toluene/methanol/acetic acid	80:20:20:1
SS III:	n-butanol/acetic acid/water	80:20:20
SS IV:	chloroform/methanol/acetic acid/water	65:25:3.5:3.5

The radioactive compounds were detected by means of a linear analyzer (linear analyzer TM 3000, Raytest Co.,) or by exposure on X-ray films (Curix RP-1, Agfa-Gevaert Co.). Co-chromatographed reference compounds were visualized by UV-light (254 nm).

F. Liquid chromatography

F.1 Low pressure chromatography

The metabolite mixture of the ethyl acetate phase of the potato vines was separated under the following conditions:

Chromatography system: Model 302 of Abimed Co.

Column: Commercial Lobar RP 8-column, size A (240x10 mm), 40-63 µm

Gradient:

min	H_2O	MeOH
0 - 10	100	0
11 - 25	90	10
26 - 40	85	15
41 - 55	80	20
56 - 70	70	30
71 - 85	40	60
86 - 100	0	100

Fraction collector: Model Foxy (Isco Co., Lincoln, Nebraska)

Flow rate: 2 ml/min

Detector: Radioactivity flow-through detector "Ramona D", Raytest Isotopenmeßgeräte GmbH

The ethyl acetate phase (50 ml) was concentrated to dryness and dissolved in 6 ml water/methanol (5:1). Two ml of this solution were injected.

F.2 High performance liquid chromatography

The fractions 1, 2 and 3 of the low-pressure chromatography of the ethyl acetate phase of potato vines were further cleaned up by HPLC and for identification were compared with reference compounds under the following conditions:

HPLC instrument: Model 5000 of Varian Co.

Column: RP 8 250 x 4 mm, 10 µm (Merck Co.)

Gradient:	Fractions 1 and 2: proportion of methanol in water from 0 to 45% in 60 min Fraction 3: proportion of methanol in water from 0 to 50% in 60 min
Fraction collector:	Model 202 of Gilson Co.
Flow rate:	1 ml/min
Detector:	Radioactivity flow-through detector "Ramona D", Raytest Isotopenmeßgeräte GmbH

G. NMR spectroscopy

The ^{1}H-NMR-spectra were recorded on a Bruker AC 300. Samples were dissolved in CD_3OD (EGA Co.) for analysis.

H. Reverse isotope dilution analysis of 6-chloronicotinic acid

For structure determination of the 6-chloronicotinic acid in the aqueous phase of the tuber extract, an aliquot of this with a defined ^{14}C-content was recrystallized from 20% aqueous ethanol together with non-labelled reference compound. The recrystallization was repeated until the specific ^{14}C-activity of the crystalline form reached a constant value. The selection of 6-chloronicotinic acid as reference compound was derived from thin-layer chromatographic comparison of this compound with the radioactive zones of the extract. The ^{14}C-activity of each crystalline form was determined by LS measurement after each recrystallization after dissolving an aliquot in methanol (Table X).

I. Radioactivity measurement

The ^{14}C-activities of liquid samples were measured in a liquid scintillation counter (LKB 1219 Rackbeta of LKB-Wallac Co.) using Instant-Scint-Gel (Packard Co.). Details concerning the scintillation measurement are given in Appendix IX.

Solid samples were combusted in an oxidizer OX 300 of Harvey Instrument Corp. (Zinsser Co.), the CO_2 being formed was absorbed in the scintillator solution (8 ml Carbosorb + 10 ml Permafluor V of Packard Co.) and the solution measured by liquid scintillation counting (PW 4700 of Philips Co.).

The radioactivity on TLC plates was determined either by scraping off the silica gel in the range of the radioactive zones followed by suspension in the scintillation cocktail and measurement or by quantifying the radioactive zones by means of a linear analyzer (TM 3000 of Raytest Co.).

Where quantitation was achieved by scraping off the silica gel, only the visible darkened zones were determined. In cases of poor peak resolution with the linear analyzer, an autoradiogram was prepared. The positions of the peaks were determined in the autoradiogram and their location was transferred to the respective linear analyzer chromatogram.

J. Storage stability investigation

Samples of potato vines and tubers harvested at maturity were extracted and investigated quantitatively for the main metabolites after a storage period of 5, 203 and 342 days (vines) and 169 and 379 days (tubers), respectively. The samples were cut into smaller pieces and stored deep-frozen (- 20°C) until extraction.

The conditions of processing (weight of the samples, amounts of solvents for extraction) were the same as for the metabolism study (see Appendix V). Exceptions were the potato vines from day 5 and day 342. In the case of the potato vines from day 5 the water eluate of the XAD 4 column (see section IV. D.) was chromatographed a second time on the XAD 4 column after concentration to 30 ml as it still contained a considerable amount of radioactivity. The methanol eluates of both separation processes were combined and concentrated. In the case of the potato vines from day 342 only 67 g were used for extraction instead of 100 g (for details see Appendices VII and VIII).

In contrast to the metabolism experiment the determination of the main metabolites for all samples in the storage stability investigation was done using a linear analyser after separation of the main metabolites by TLC (solvent

system SS II for the ethyl acetate phases and solvent system SS III for the water phases). The identity of parent compound and metabolites was confirmed by co-chromatography with the respective reference compounds. Some peaks in the water phase contained more than one metabolite.

V. Results and discussion

A. Balance and distribution of the ^{14}C-radioactivity

At the time of harvest of the potatoes on day 129 the applied ^{14}C-radioactivity (113.4 MBq = 100%) was recovered quantitatively from the plant/soil system of the planting container (114.47 MBq = 100.9%). Of this total amount 2.51 MBq (= 2.2%) was found in the potato vines, 0.33 MBq (= 0.3%) in the tubers and 111.63 MBq (= 98.4%) in the soil (Table VII).

The raw data of weights, volumes and radioactivity measurements are given in Appendices V and VI and the total residues calculated from these are given in Table II.

Potato vines

The ^{14}C-radioactivity in the potato vines corresponded to 5.76 mg/kg total residue. During liquid/liquid partition of the extracted ^{14}C-radioactivity, 2.1% of this residue was found in the n-hexane phase. The n-hexane phase contained a significant amount of lipophilic plant constituents. The ethyl acetate phase and the water phase contained about equal proportions of the ^{14}C-radioactivity (38.2% lipophilic and 33.3% hydrophilic portions, resp.). The non-extractable residues amounted to 26.4%.

Potato tuber

The potato tubers contained low amounts of total residues at a level of 0.091 mg/kg. Of this 61.3% were lipophilic and 31.9% hydrophilic components. The non-extractable residues amounted to 6.4%.

Soil

A total of 98.1% of the ^{14}C-radioactivity of the soil was located in the top 20 cm. The remaining 1.9% was almost uniformly distributed in a soil depth between 20 and 50 cm (Table VII).

B. Quantitative determination and identification of the metabolites

The quantitative determination of the individual metabolites was made via evaluation of the thin-layer chromatograms by scraping off the radioactive TLC zones and subsequent scintillation measurement (Figures 2 - 5, Tables III - VI).

Synthesized compounds which had been applied in earlier investigations (metabolism of NTN 33893 on tomatoes; Dräger, Brauner and Bornatsch, 1989), were used for identification of metabolites by various chromatographic methods. The metabolites are indicated with Arabic and the reference compounds with Roman numerals.

B.1 Metabolites in potato vines

Components 1 and 17 = NTN 33893 (I)

These two components which occurred in the ethyl acetate phase (25.4% of the radioactivity in the potato vines) and in the water phase (1.3%) were identified as unchanged parent compound (I) by two-dimensional TLC and HPLC (Figs. 2, 3 and 13). The unchanged parent compound constituted the main proportion of the residue in the potato vines with a total content of 26.7%, 1.53 mg/kg (Table IV).

The structure of component 1 was further confirmed by ^{1}H-NMR spectroscopy after clean-up of the ethyl acetate phase by low-pressure chromatography and HPLC. Despite numerous impurities the following signals could be assigned in the ^{1}H-NMR-spectrum: H2 of the aromatic ring at 8.35 ppm (doublet), H4 at 7.82 ppm (double-doublet) and H5 at 7.46 ppm (doublet) with coupling constants $J_{(2,4)}$ of 2.6 Hz and $J_{(4,5)}$ of 8.5 Hz. The methylene group appeared at 4.54 ppm as a singlet, the two methylene groups of the five-membered ring

appeared at 3.73 and 3.55 ppm as a multiplet (Fig. 14). A mass spectroscopic investigation was unsuccessful due to large amounts of impurities.

Metabolite 2 = 5-hydroxy compound (IV)

The content of this metabolite was 4.6%, 0.26 mg/kg and was identified as WAK 4103 (IV) by two-dimensional TLC and HPLC (Figures 2 and 11).

The somewhat asymmetric shape of the radioactive zone in the TLC chromatogram indicated an additional compound with similar chromatographic behaviour as IV. This was probably the isomeric 4-hydroxy form which had been detected spectroscopically during structure elucidation of NTN 33893-metabolites from tomato plants ([redacted], 1989).

Metabolite 3 = dihydroxy compound (VII)

The metabolite 3 was identified by thin-layer chromatographic comparison with the reference compound WAK 3772 (VII) (Fig. 2). It accounted for 0.3% of the total residue and an amount of 0.02 mg/kg.

Metabolite 4 and metabolite 19 = olefine compound (VI)

These metabolites were detected in the ethyl acetate phase and the water phase (a total of 3.3% or 0.19 mg/kg). They were identified as NTN 35884 (VI) by two-dimensional TLC (Figs. 2 and 3) and by HPLC comparison (only metabolite 4) by co-chromatography with the standard (Fig. 12). As HPLC separation of NTN 35884 (VI) from WAK 3839 (VIII) was not possible due to the presence of a high proportion of plant matrix, the metabolites were partially purified by HPLC. After this, almost baseline separation of the metabolites was obtained and they had the same retention times as the corresponding standards.

Metabolite 5 and metabolite 18 = nitrosimine compound (VIII)

These metabolites occurred also in the ethyl acetate phase and in the water phase (a total of 2.6% or 0.15 mg/kg). They were identified as WAK 3839 (VIII) by two-dimensional TLC (Figs. 2 and 3) as well as by HPLC (only metabolite 5) (Fig. 12).

Metabolite 6 and metabolite 22 = guanidine compound (II)

The two metabolites identified as NTN 33823 (II) constituted the main metabolites besides the 6-chloronicotinic acid. This guanidine metabolite occurred predominantly in the water phase of the potato vines. It amounted in total to 8.2%, 0.48 mg/kg in both phases. It was identified by two-dimensional TLC (Figs. 2 and 3).

Metabolite 20 = 6-chloronicotinic acid (XII)

Metabolite 20 was identified as 6-chloronicotinic acid by two-dimensional TLC (Fig. 3) and was one of the main metabolites (8.3%, 0.48 mg/kg).

Metabolite 21 = glucoside of 6-chloropicolyl alcohol (X)

Metabolite 21 was detected by two-dimensional TLC as RBN 1114 (X) in an amount of 1.4%, 0.08 mg/kg (Fig. 3).

B.2 Metabolites in the potato tubers

The metabolites in the potato tuber (0.091 mg/kg total residue) were identified and quantified by one-dimensional TLC (Figs. 4 and 5, Tables V and VI). Some of the metabolites detected in the vines also occurred in the tubers. These were the unchanged parent compound I (48.3% of the radioactivity in the tuber, 0.044 mg/kg), the 5-hydroxy compound IV (8.0%, 0.007 mg/kg), the ole-

fine compound VI (3.1%, 0.003 mg/kg), the 6-chloronicotinic acid XII (9.4%, 0.009 mg/kg) and the guanidine compound II which was the main metabolite (11.3%, 0.010 mg/kg).

The structure of metabolite 8 (6-chloronicotinic acid) was further verified by reverse isotope dilution analysis (Table X). For this purpose non-labelled reference compound was added to the ^{14}C-containing aqueous phase and the mixture recrystallized several times. The constant value for the specific ^{14}C-activity (dpm/g) indicated the identity of 6-chloronicotinic acid and metabolite 8.

C. Storage stability of parent compound and metabolites

The results of the storage stability studies showed that no significant changes in the metabolite pattern (Figs. 6 to 9) and in the quantitative composition of parent compound and main metabolites (Tables VIII and IX) resulted during repeated analysis of potato vines and tubers.

VI. CONCLUSIONS

After application of a granular formulation containing 5% NTN 33893 at a rate of 1 g granules per running meter as projected to be used in practice, the potato tubers contained only low amounts of residues. At the time of harvest on day 129 a total residue of 0.091 mg/kg was found. The potato vines contained 5.76 mg/kg total residues. The unchanged parent compound (48.3%, 0.044 mg/kg in the tubers and 26.7%, 1.53 mg/kg in the vines) constituted the main proportion of the total residue.

Similarly to earlier plant metabolism studies, 3 basic metabolic pathways could be ascertained:

a) Reduction and loss of the nitro group
b) Hydroxylation of the dihydroimidazole ring followed by loss of water
c) Metabolism to 6-chloropicolyl alcohol, glucoside formation and oxidation to 6-chloronicotinic acid.

The main metabolites were the guanidine compound II (in the tuber 11.3%, 0.01 mg/kg, in the vines 8.2%, 0.48 mg/kg), the 6-chloronicotinic acid XII (in the tubers 9.4%, 0.009 mg/kg, in the vines 8.3%, 0.48 mg/kg) and the 5-hydroxy compound IV (in the tubers 8.0%, 0.007 mg/kg, in the vines 4.6%, 0.26 mg/kg).

A total of 80.1% of the radioactivity in the tubers and 55.4% of the radioactivity in the vines could be identified. Another 5 metabolites (together with the n-hexane phase = 13.5%) occurred in the tubers and another 14 metabolites (together with the n-hexane phase = 18.2%) occurred in the vines each in very low concentrations and these could not be identified.

The amount of non-extractable residues in the tubers were very low (6.4%, 0.006 mg/kg). In the vines they amounted to 26.4%, 1.52 mg/kg; they were not further investigated since potato vines are not used as foodstuff or as animal feed.

The total toxic residues of NTN 33893 and its metabolites containing the 6-chloropicolyl structure can be determined by means of a method based on 6-chloronicotinic acid ([redacted], 1990a). The 6-chloronicotinic acid method was checked with various plant materials originating from metabolism studies ([redacted], 1990b). The comparison of the residues determined according to the 6-chloronicotinic acid method with those of the metabolism study consisting of parent compound and identified metabolites showed the residue levels in the investigated plant materials to be of the same order of magnitude. Thus, for instance, the total residue determined in the potato vines from the metabolism study after spray application ([redacted], 1990) averaged 61.5% (58% and 65%) of the radioactivity in the plant and the residue totalling parent compound and identified metabolites amounted to 67%. Consequently, the 6-chloronicotinic acid method and the degree of identifiation of the metabolism study support one another.

A degradational pathway for NTN 33893 in potatoes after granular application is given in Figure 15.

VII. SIGNATURES

[redacted]

December 11, 1991

Date

December 11, 1991

Date

December 11, 1991

Date

Jan. 30, 1992

Date

We thank [redacted] for the good performance of the experimental work.

All raw data and documentation and the original report are stored in the archive of the Institute for Metabolism Research, building 6660.

VIII. QUALITY ASSURANCE STATEMENT

[redacted]

IX. LITERATURE

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Bayer AG, Internal PF-Report No. 3311, 2/2/1990

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Investigation on the Metabolism of NTN 33893 after Application to Tomatoes.
Bayer AG, Internal PF-Report No. 3257, 12/10/1989

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Metabolism of [pyridinyl-^{14}C-methyl]NTN 33893 in Potato, Wheat and Corn Cell Suspension Cultures.
Bayer AG, Internal PF-Report No. 3179, 16/5/1989

[redacted] (1990)
Metabolism of [pyridinyl-^{14}C-methyl]NTN 33893 in Rice Plants (Nursery Box Application).
Nihon Tokushu Noyaku Seizo, K.K., Yuki Research Center, Japan, Report No. 1284 (ESR/ENG) in preparation

[redacted] (1991)
Metabolism of NTN 33893 in Corn after Seed Dressing.
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Report in Preparation.

[redacted] (1991)
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Bayer AG, Crop Protection Research, Institute for Metabolism Research
Report in preparation

[redacted] (1990a)
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Bayer AG, Crop Protection Research, Institute for Residue Analysis, Internal Report RA 418/90 of 31/5/1990

[redacted] (1990b)
Validation of the Residue Method for the Total Imidacloprid Residue in Vegetable Samples using Radioactive Residues.
Bayer AG, Crop Protection Research, Institute for Residue Analysis, Internal Report RA-419/90 of 31/5/1990

X. TABLES AND FIGURES

A. Tables

Table I: R_f-values of reference compounds

Solvent Systems
SS I : ethyl acetate/i-propanol/water 65:23:12
SS II: ethyl acetate/toluene/methanol/acetic acid 80:20:20:1
SS III: n-butanol/acetic acid/water 80:20:20
SS IV: chloroform/methanol/acetic acid/water 65:25:3.5:3.5

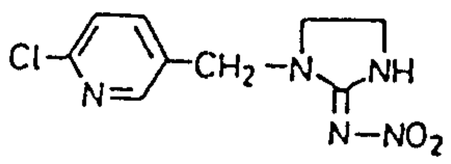
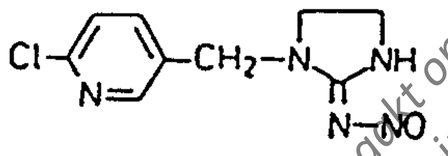
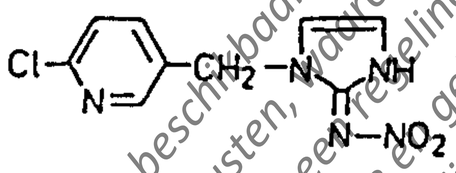
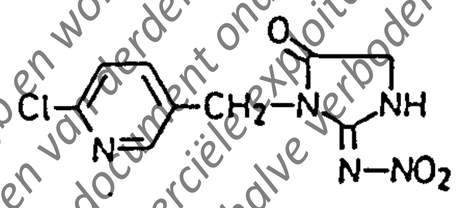
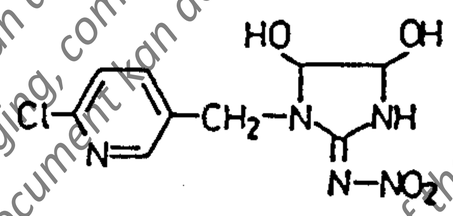
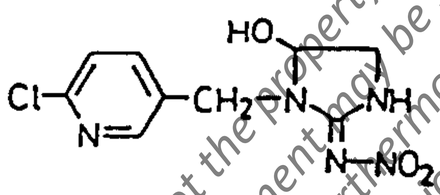
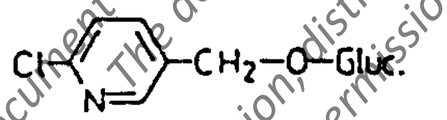
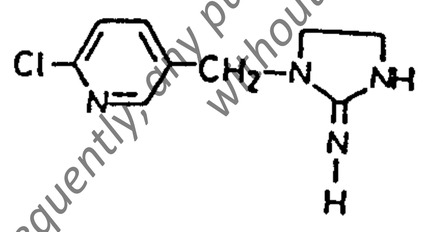
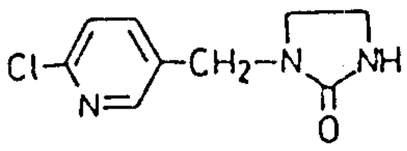
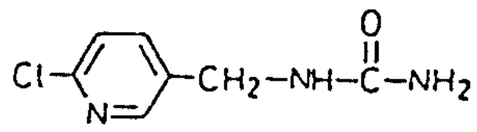
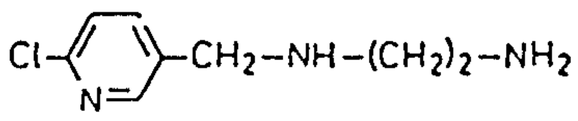
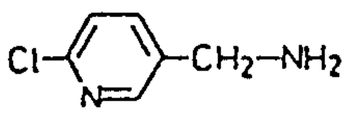
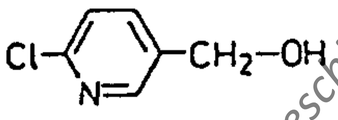
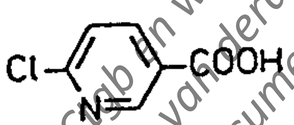
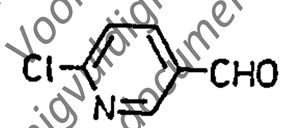
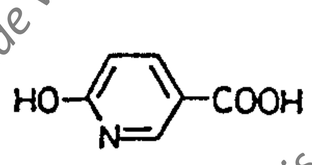
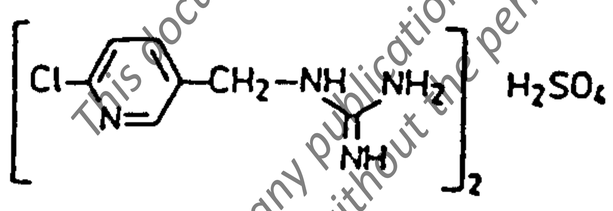
Compound		SS I	SS II	SS III	SS IV
	NTN 33393 I	0.83	0.63	0.68	0.91
	WAK 3839 VIII	0.62	0.36	0.60	0.82
	NTN 35884 VI	0.78	0.52	0.67	0.82
	WAK 3738 XVI	0.95	0.85	0.82	0.91
	WAK 3772 VII	0.95	0.74	0.90	0.80
	WAK 4103 IV	0.81	0.74	0.78	0.97
	RBN 1114 X	0.56	0.32	0.60	0.75
 ×HBr	NTN 33823 II	0.06	Origin	0.40	0.47

Table I continued: R_f-values of reference compounds

Compound		SS I	SS II	SS III	SS IV
	DIJ 9817 III	0.70	0.50	0.70	0.91
	NTN 36749 XIII	0.10	Origin	0.50	0.39
	DIJ 9646-2 XII	Origin	Origin	0.2	0.15
	GSE 1478 XII	0.11	Origin	0.5	0.39
	DIJ 9805 XIII	0.93	0.79	0.87	0.91
	6-Chloro-nicotinic-acid XII	0.39	0.49	0.86	0.81
	MAT 10249-D XII	0.96	0.91	0.94	0.91
	GBH 4315 XII	0.14	0.14	0.75	0.61
	WAK 4126 XV	nd[1]	nd[1]	nd[1]	nd[1]

[1] nd = not determined

Table II:
^{14}C-Residues in fractions after extraction of potatoes following granular application of NTN 33893

Radioactivity in the plant part = 100%
mg/kg expressed as NTN 33893 equivalents

Fraction	Vines		Tubers	
	%	mg/kg	%	mg/kg
n-Hexane phase	2.1	0.12	0.4	< 0.0004
Ethyl acetate phase	38.2	2.20	61.3	0.056
Aqueous phase	33.3	1.92	31.9	0.029
Non-extractable residue	26.4	1.52	6.4	0.006
Total residue	100	5.76	100	0.091

Table III:

Distribution of the metabolites in the extracts of potato vines following granular application of NTN 33893
Radioactivity in the vines = 100%
mg/kg values expressed as NTN 33893 equivalents

Metabolite/Fraction	%	mg/kg
n-Hexane phase	2.1	0.12
Ethyl acetate phase	(38.2)	(2.20)
component 1 = NTN 33893 (I)	25.4	1.46
metabolite 2 = 5-hydroxy compound, WAK 4103 (IV)	4.6	0.26
metabolite 3 = dihydroxy compound, WAK 3772 (VII)	0.3	0.02
metabolite 4 = olefine compound, NTN 35884 (VI)	1.0	0.06
metaboilte 5 = nitrosimine compound, WAK 3839 (VIII)	1.1	0.06
metabolite 6 = guanidine compound, NTN 33823 (II)	1.5	0.09
10 unknown metabolites No. 7-16 between 0.1 and 0.9% and ≤ 0.01 and 0.05 mg/kg each	4.3	0.25
Aqueous phase	(33.3)	(1.92)
component 17 = NTN 33893 (I)	1.3	0.07
metabolite 18 = nitrosimine compound, WAK 3839 (VIII)	1.5	0.09
metabolite 19 = olefine compound, NTN 35884 (VI)	2.3	0.13
metabolite 20 = 6-chloronicotinic acid (XII)	8.3	0.48
metabolite 21 = glucoside RBN 1114 (X)	1.4	0.08
metabolite 22 = guanidine compound, NTN 33823 (II)	6.7	0.39
4 unknown metabolites No. 23-26 between 1.0 and 5.4% and 0.07 and 0.3 mg/kg each	11.8	0.68
Non-extractable residue	26.4	1.52
Total	100.0	5.76

Table IV:

Metabolites in potato vines following granular application of NTN 33893*

Radioactivity in the vines = 100%

mg/kg values expressed as NTN 33893 equivalents

Metabolite/compound	%	mg/kg
components 1 + 17 = NTN 33893 (I)	26.7	1.53
metabolite 2 = 5-hydroxy compound, WAK 4103 (IV)	4.6	0.26
metabolite 3 = dihydroxy compound, WAK 3772 (VII)	0.3	0.02
metabolites 4 + 19 = olefine compound, NTN 35884 (VI)	3.3	0.19
metabolites 5 + 18 = nitrosimine compound, WAK 3839 (VIII)	2.6	0.15
metabolites 6 + 22 = guanidine compound, NTN 33823 (II)	8.2	0.48
metabolite 20 = 6-chloronicotinic acid (XII)	8.3	0.48
metabolite 21 = glucoside RBN 1114 (X)	1.4	0.08
total identified	55.4	3.19
14 unknown metabolites	16.1	0.93
n-hexane phase	2.1	0.12
non-extractable residue	26.4	1.52
Total	100.0	5.76

* summation of the quantities of the individual metabolites from the different phases (see Table III)

Table V:

Distribution of the metabolites in the extracts of potato tubers following granular application of NTN 33893
Radioactivity in the tubers = 100%
mg/kg values expressed as NTN 33893 equivalents

Metabolite/compound	%	mg/kg
n-Hexane phase	0.4	< 0.001
Ethyl acetate phase	(61.3)	(0.056)
component 1 = NTN 33893 (I)	45.3	0.041
metabolite 2 = 5-hydroxy compound, WAK 4103 (IV)	8.0	0.007
metabolite 3 = olefine compound, NTN 35884 (VI)	3.1	0.003
metabolite 4 = 6-chloronicotinic acid (XII)	2.1	0.002
metabolite 5 = unknown	2.8	0.003
Aqueous phase	(31.9)	(0.029)
component 6 = NTN 33893 (I)	3.0	0.003
metabolite 7 = guanidine compound, NTN 33823 (II)	11.3	0.010
metabolite 8 = 6-chloronicotinic acid (XII)	7.3	0.007
4 unknown metabolites No. 9-12 between 1.6 and 4.2% and 0.001 and 0.004 mg/kg each	10.3	0.009
Non-extractable residue	6.4	0.006
Total	100.0	0.091

Table VI:
Metabolites in the potato tubers following granular application of NTN 33893*

Radioactivity in the tubers = 100%
mg/kg values expressed as NTN 33893 equivalents

Metabolite/compound	%	mg/kg
metabolites 1 + 6 = NTN 33893 (I)	48.3	0.044
metabolite 2 = 5-hydroxy compound, WAK 4103 (IV)	8.0	0.007
metabolite 3 = olefine compound, NTN 35884 (VI)	3.1	0.003
metabolites 4 + 8 = 6-chloronicotinic acid (XII)	9.4	0.009
metabolite 7 = guanidine compound, NTN 33823 (II)	11.3	0.010
total identified	80.1	0.073
5 unknown metabolites	13.1	0.012
n-hexane phase	0.4	< 0.001
non-extractable residue	6.4	0.006
Total	100.0	0.091

* summation of the quantities of the individual metabolites from the different phases (see Table V)

Table VII:

^{14}C-Radioactivity in soil following granular application of NTN 33893 to potatoes

Radioactivity in the soil = 100%

mg/kg expressed as NTN 33893 equivalents

Soil Depth (cm)	Radioactivity %	MBq	Soil residues Bq/g	mg/kg
0 - 10	66.4	74.08	926	0.98
10 - 20	31.7	35.44	443	0.47
20 - 30	0.6	0.70	6.97	0.006
30 - 40	0.7	0.78	7.83	0.007
40 - 50	0.6	0.63	4.17	0.002
total	100.0	111.63		

Table VIII:
Results of the storage stability study of parent compound and main metabolites in potato vines following granular application of NTN 33893.

Radioactivity in the vines = 100%
mg/kg values expressed as NTN 33893 equivalents

	1. Analysis Extraction date: 29.8.89 (day 5)		2. Analysis Extraction date: 15.3.90 (day 203)		3. Analysis Extraction date: 1.8.90 (day 342)	
Compound/metabolite	%	mg/kg	%	mg/kg	%	mg/kg
n-Hexane phase	1.42	0.092	2.14	0.12	1.47	0.11
Ethyl acetate phase	36.2	2.34	38.2	2.20	35.2	2.51
component 1 = NTN 33893 (I)	21.6	1.39	21.9	1.26	20.3	1.45
metabolite 2 = 5-hydroxy compound (IV)	4.87	0.31	4.10	0.24	3.00	0.21
metabolite 4 = olefine compound (VI)	2.42	0.16	2.13	0.12	2.13	0.15
metabolite 7 = unknown	1.78	0.12	2.58	0.15	2.02	0.14
Aqueous phase	31.0	2.00	33.3	1.92	34.4	2.46
component 17 = mostly NTN 33893 (I)	3.58	0.23	3.43	0.20	4.20	0.30
metabolite 20 = 6-chloronicotinic acid (XII)	4.73	0.31	5.69	0.33	5.42	0.39
metabolite 22 = guanidine compound (II)	5.45	0.35	6.03	0.35	6.65	0.48
Non-extractable residue	31.4	2.03	26.4	1.52	28.9	2.06

Table IX:

Results of the storage stability study of parent compound and main metabolites in potato tubers following granular application of NTN 33893.

Radioactivity in the tubers = 100%
mg/kg values expressed as NTN 33893 equivalents

	1. Analysis Extraction date: 9.2.1990 (day 169)		2. Analysis Extraction date: 7.9.1990 (day 379)	
Compound/metabolite	%	mg/kg	%	mg/kg
n-Hexane phase	0.37	0.0003	0.79	0.0008
Ethyl acetate phase	61.3	0.056	62.0	0.065
component 1 = NTN 33893 (I)	49.4	0.045	49.4	0.052
metabolite 2 = 5-hydroxy compound (IV)	4.41	0.0040	3.29	0.0034
metabolite 3 = olefine compound (VI)	1.95	0.0018	1.74	0.0018
Aqueous phase	31.9	0.029	27.5	0.029
metabolite group 6 = mostly NTN 33893 (I)	4.83	0.0044	5.31	0.0056
metabolite 7 = guanidine compound (II) and metabolite 10	12.7	0.012	9.97	0.010
metabolite 8 = 6-chloronicotinic acid (XII)	5.73	0.0052	4.37	0.0047
Non-extractable residue	6.43	0.0058	9.71	0.010

Table X: Identification of 6-Chloronicotinic acid in the Potato Tuber Extract by Reverse Isotope Dilution Analysis

Number of crystallization	Initial quantity	Solvent used Water	Ethanol	Initial Radioactivity	Crystallized compound Recovery	Specific ^{14}C-radioactivity
	g	ml	ml	dpm	g	dpm/g
1	5.0	150	50	224000	4.5	9490
2	4.0	120	30	37960	3.8	7770
3	3.5	100	20	27195	3.2	7420
4	3.0	80	20	22260	2.7	7550

X. Tables and Figures

B. Figures

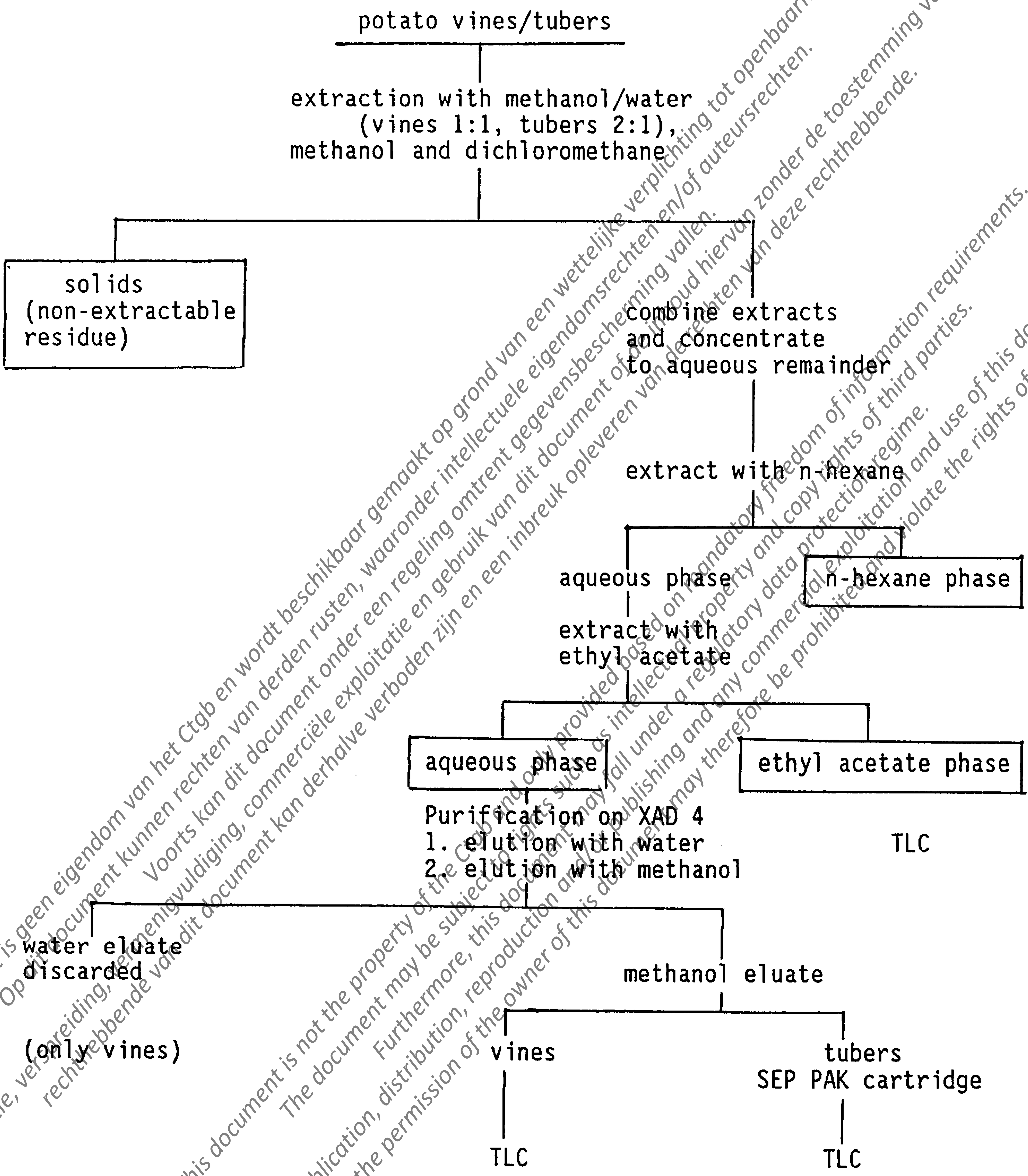


Figure 1:
Flow diagram for extraction, fractionation and purification for potato vines and tubers

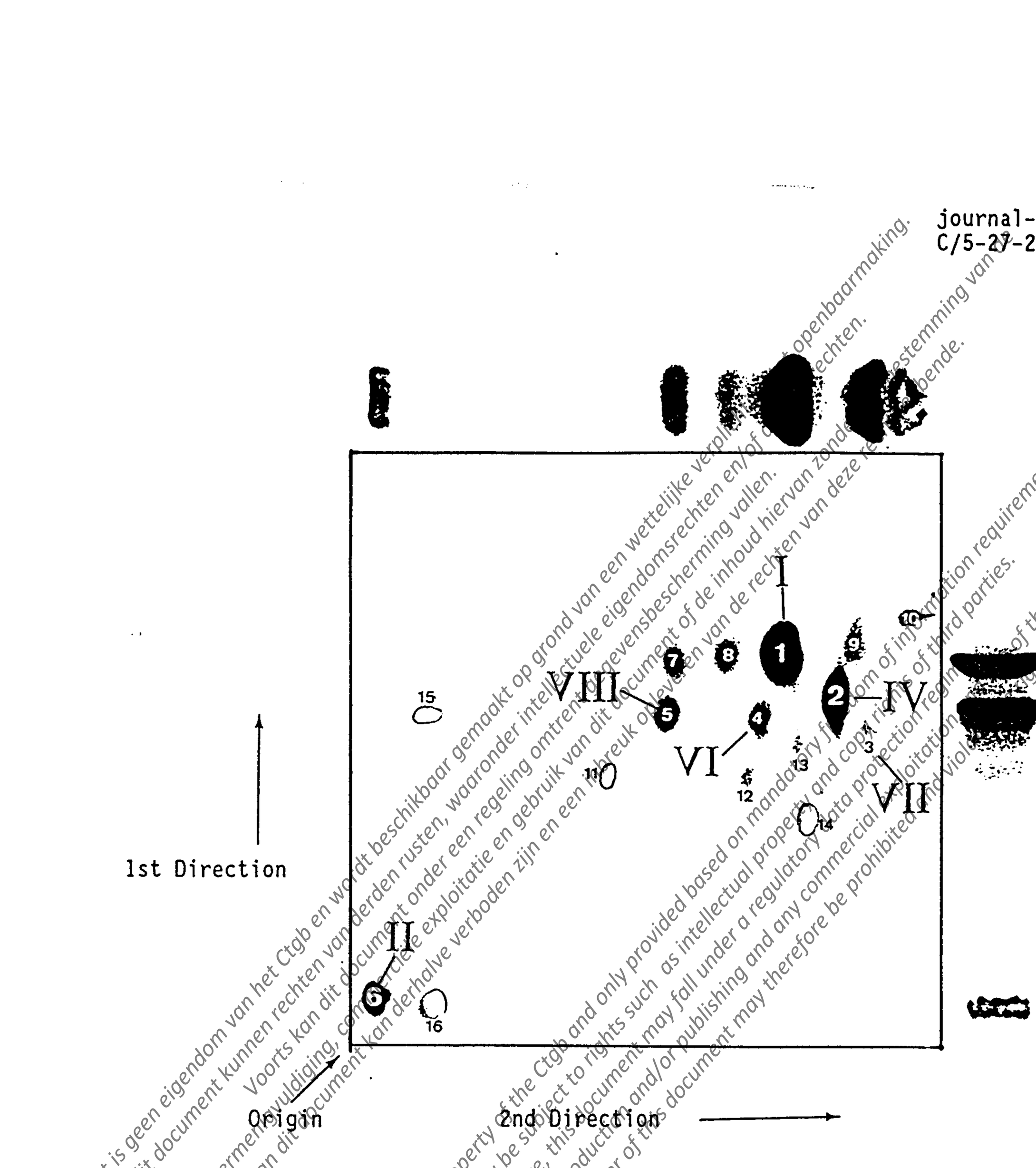


Figure 2:
Autoradiogram of two-dimensional and one-dimensional TLC of the ethyl acetate phase of potato vines

1st Direction: SS II = ethyl acetate/toluene/methanol/acetic acid 80:20:20:1
2nd Direction: SS I = ethyl acetate/i-propanol/water 65:23:12

Arabic numerals refer to the metabolites, roman numerals refer to reference compounds

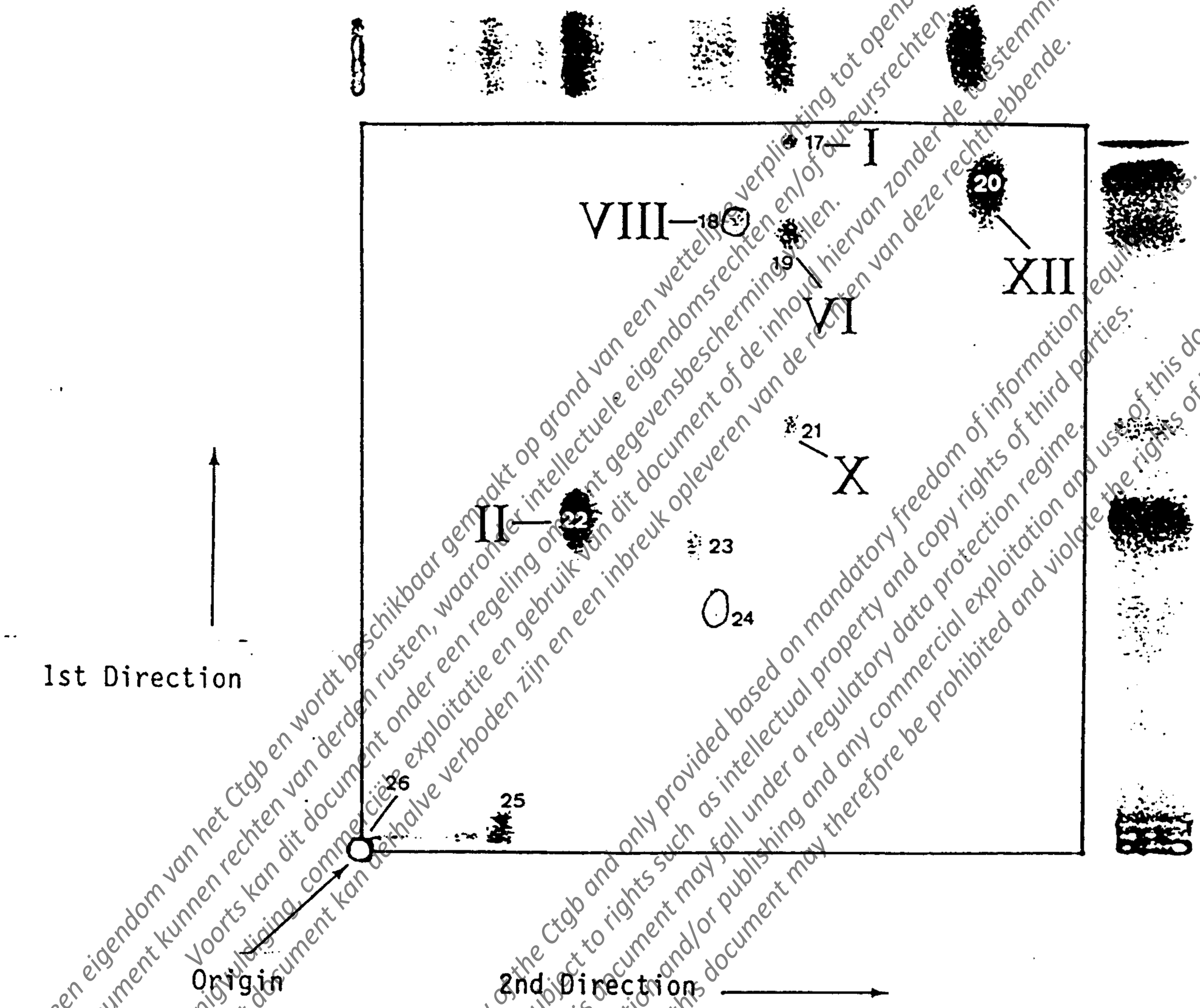


Figure 3:
Autoradiogram of two-dimensional and one-dimensional TLC of the aqueous phase of potato vines

1st Direction: SS II = chloroform/methanol/acetic acid/water 65:25:3.5:3.5
2nd Direction: SS I = n-butanol/acetic acid/water 80:20:20

Arabic numerals refer to the metabolites, roman numerals refer to reference compounds

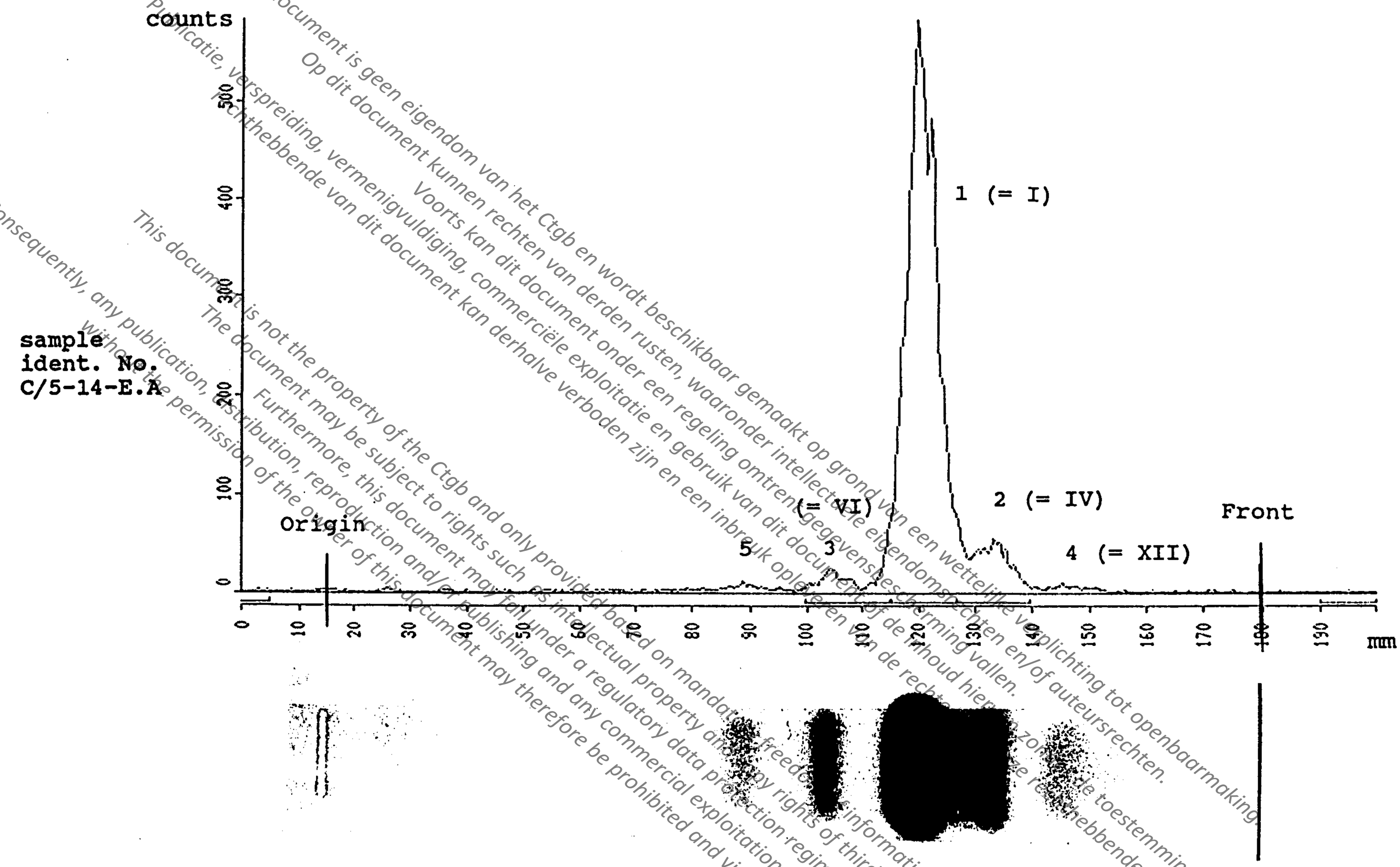


Figure 4: Radio-TLC and autoradiogram of the ethyl acetate phase of potato tubers
SS II = ethyl acetate/toluene/methanol/acetic acid 80:20:20:1

Arabic numerals refer to the metabolites, roman numerals refer to reference compounds

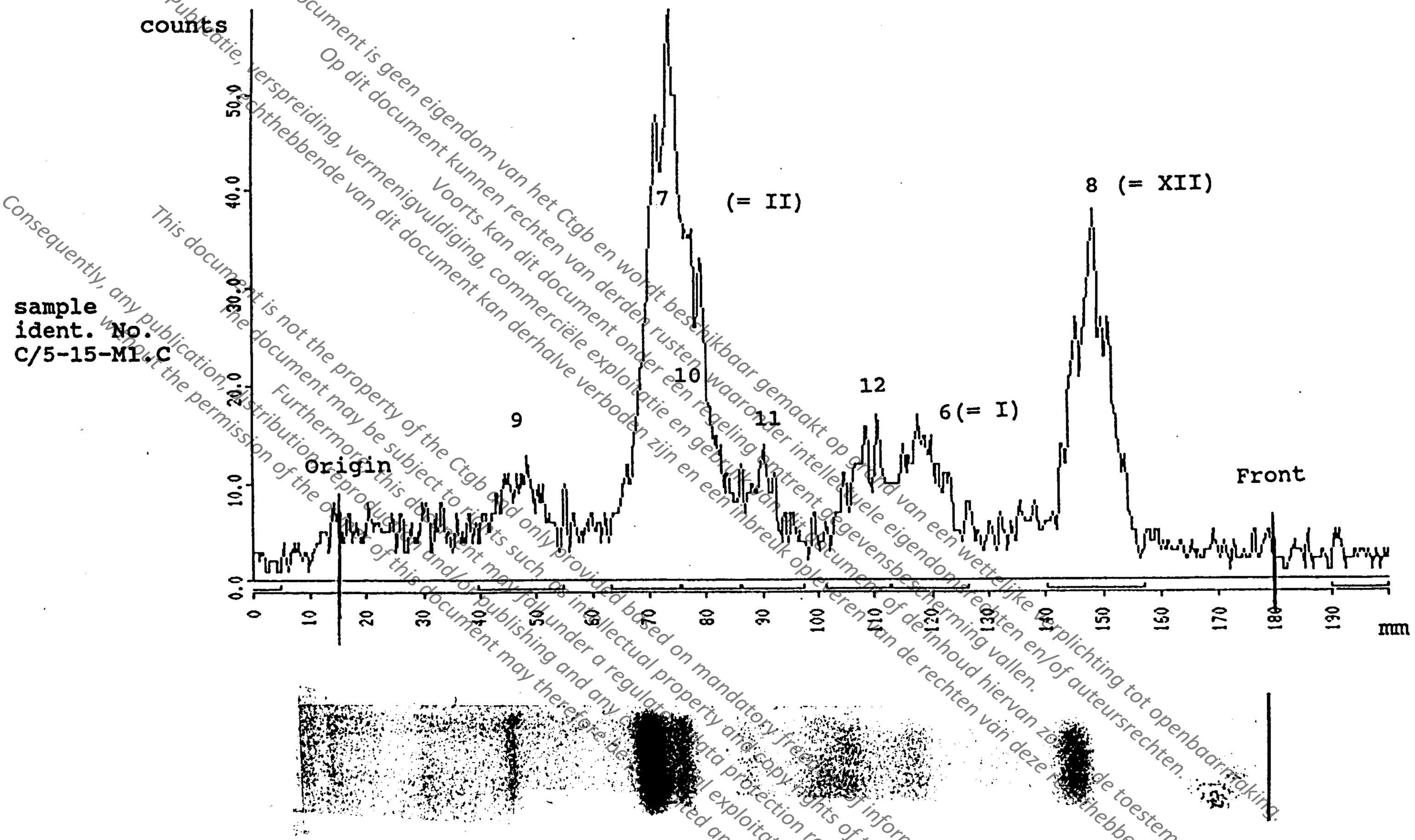


Figure 5: Radio-TLC and autoradiogram of the aqueous phase of potato tubers
SS III = n-butanol/acetic acid/water 80:20:20

Arabic numerals refer to the metabolites, roman numerals refer to reference compounds

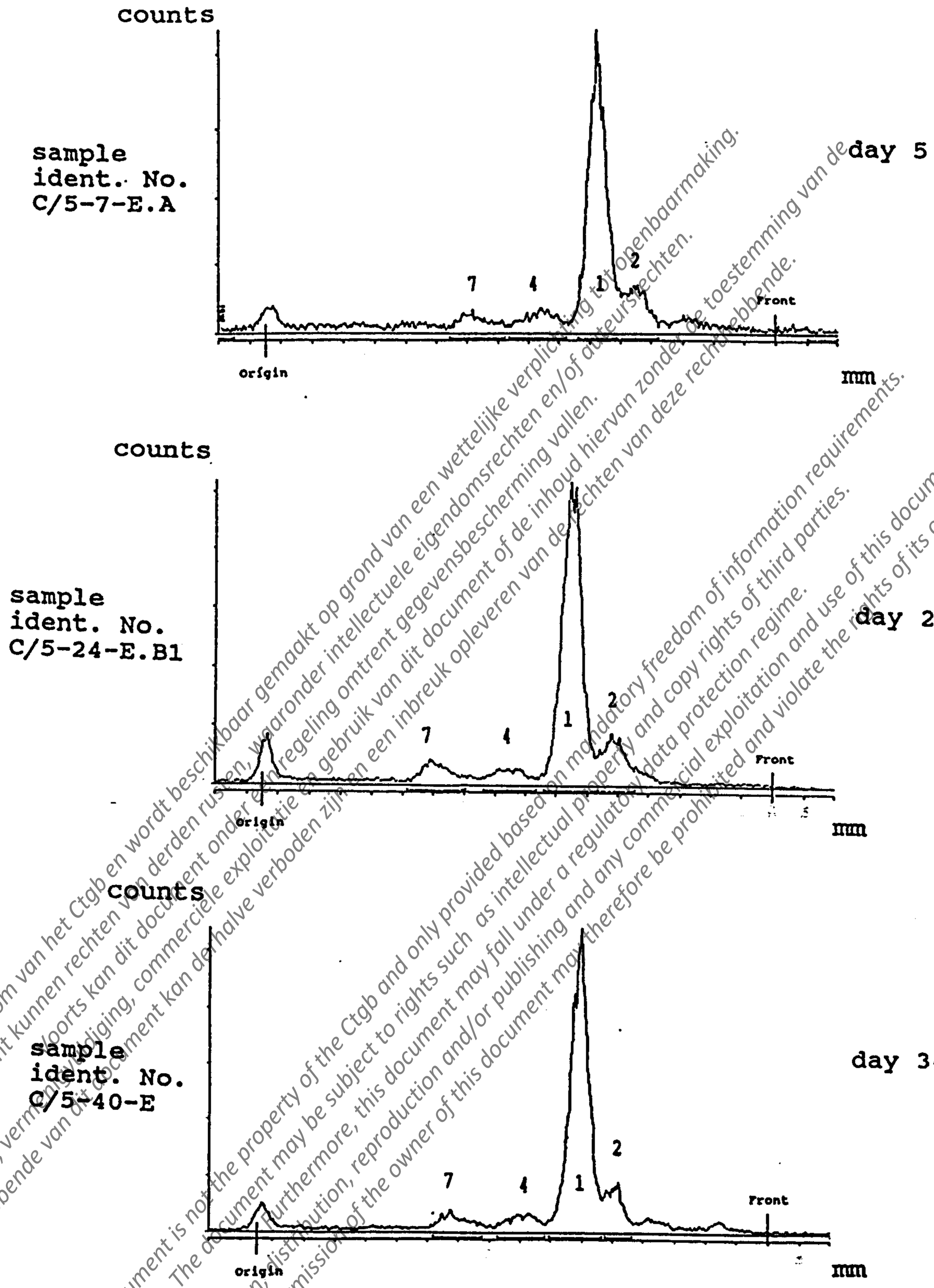


Figure 6:
Radio-TLC of the ethyl acetate phase of potato vines for the storage stability study.

SS II: ethyl acetate/toluene/methanol/acetic acid 80:20:20:1

The Arabic numerals refer to the metabolites

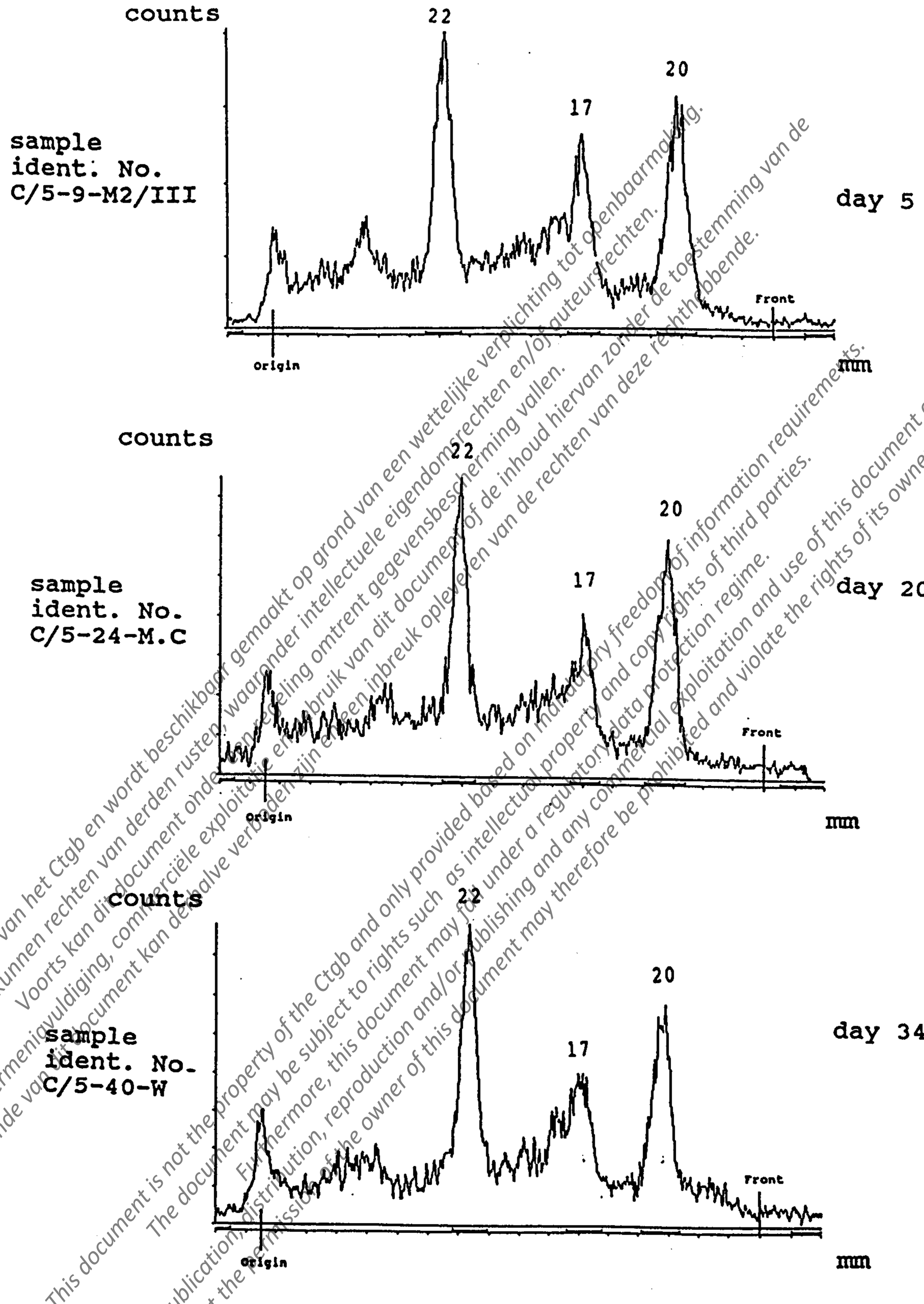


Figure 7:
Radio-TLC of the aqueous phase of potato vines for the storage stability study.

SS II: ethyl acetate/toluene/methanol/acetic acid 80:20:20:1

The Arabic numerals refer to the metabolites

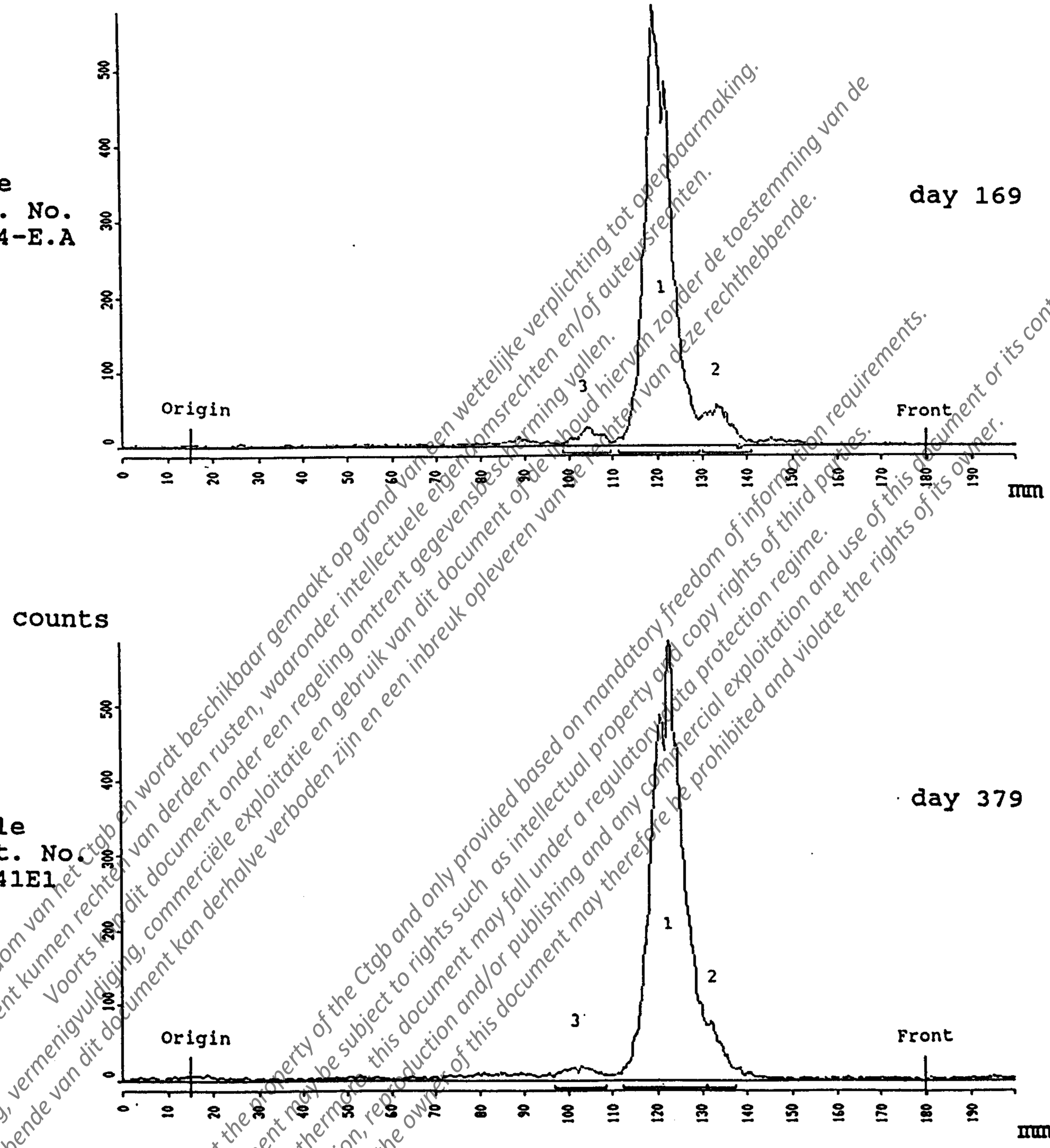


Figure 8 :

Radio-TLC of the ethyl acetate phase of potato tubers for the storage stability study.

SS II: ethyl acetate/toluene/methanol/acetic acid 80:20:20:1

The Arabic numerals refer to the metabolites

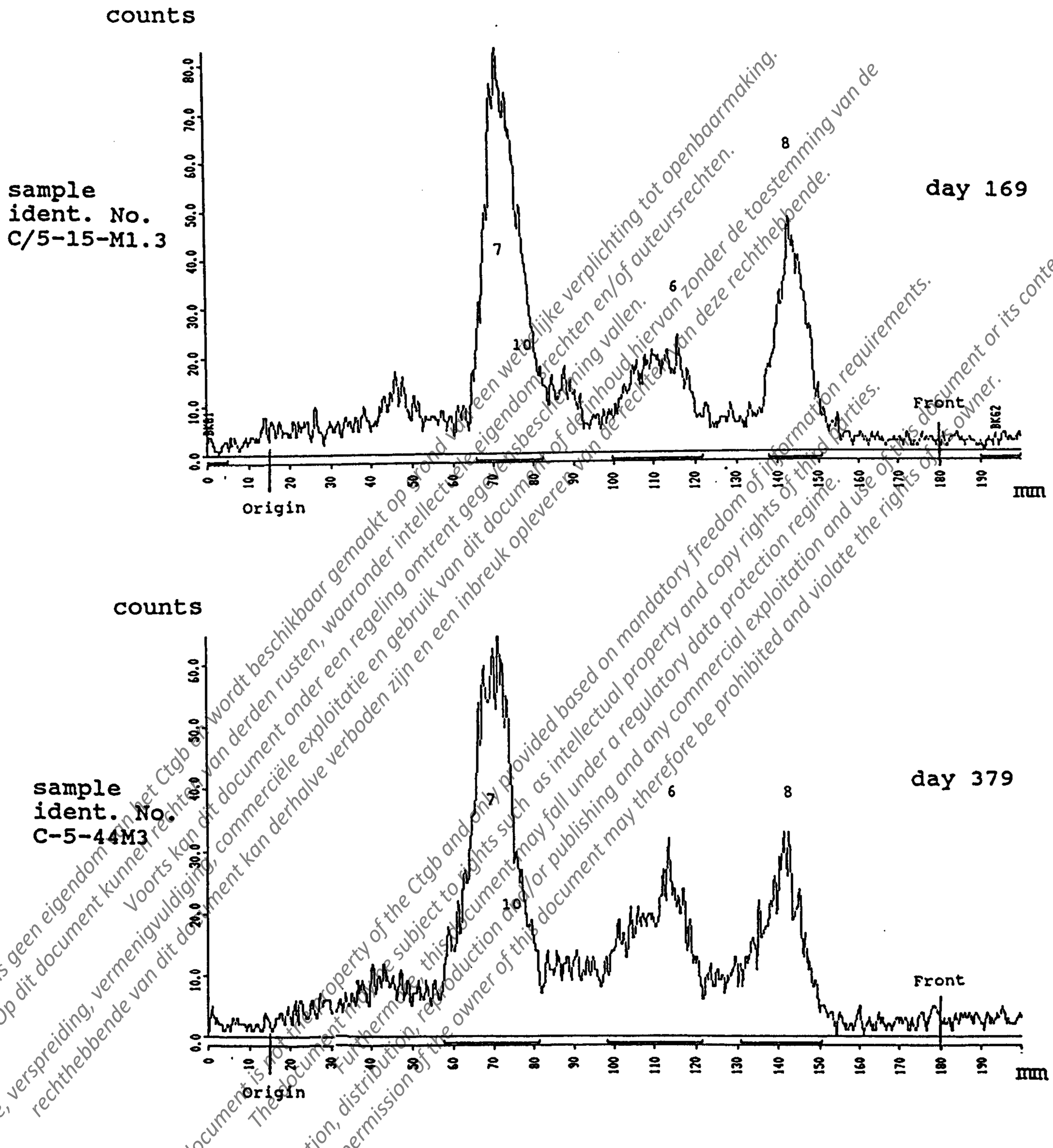


Figure 9 :

Radio-TLC of the aqueous phase of potato tubers for the storage stability study.

SS III: n-butanol/acetic acid/water 80:20:20

The Arabic numerals refer to the metabolites

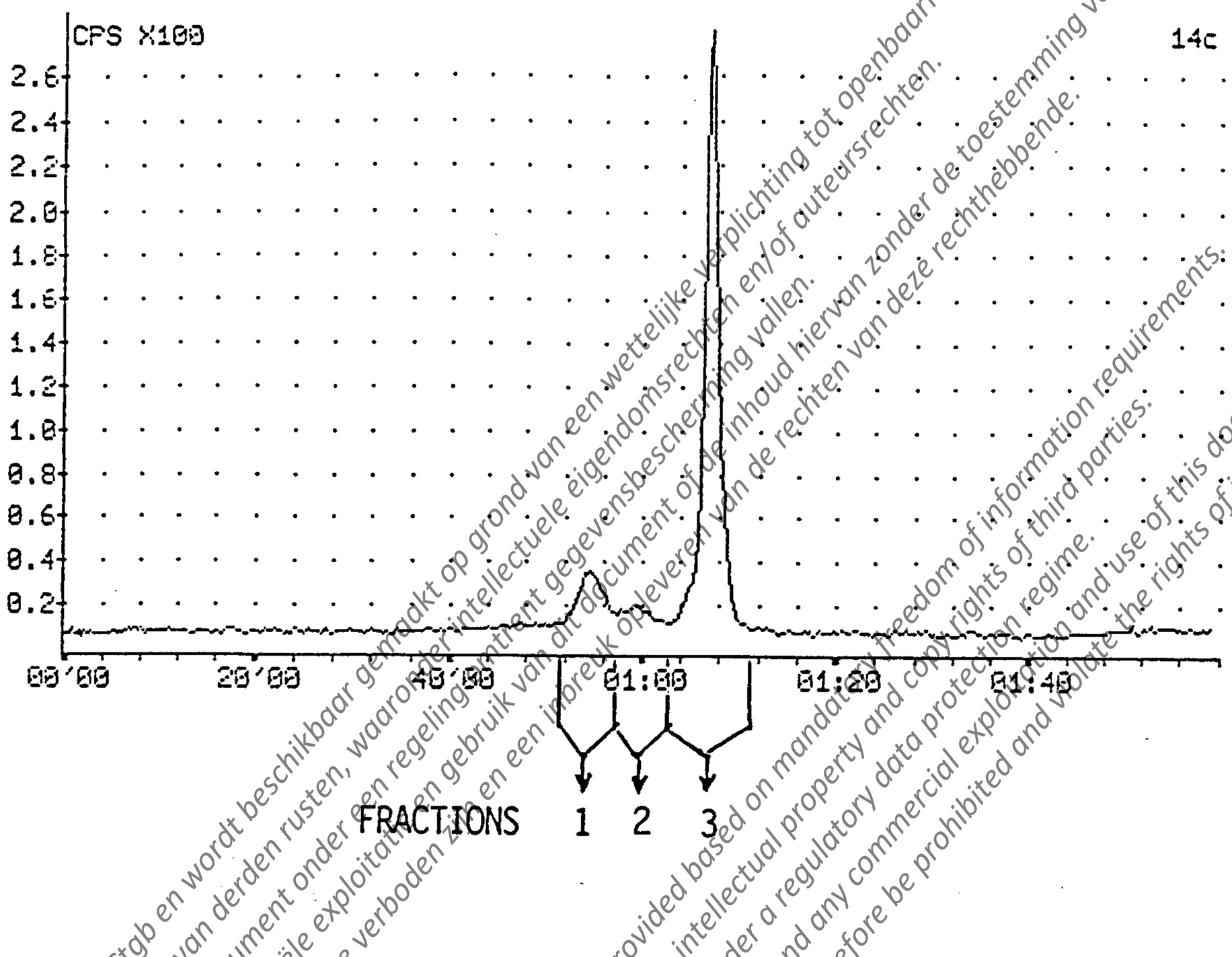


Figure 10 :

Low pressure chromatography of the ethyl acetate phase of potato vines.
For chromatographic conditions see chapter IV, F. 1

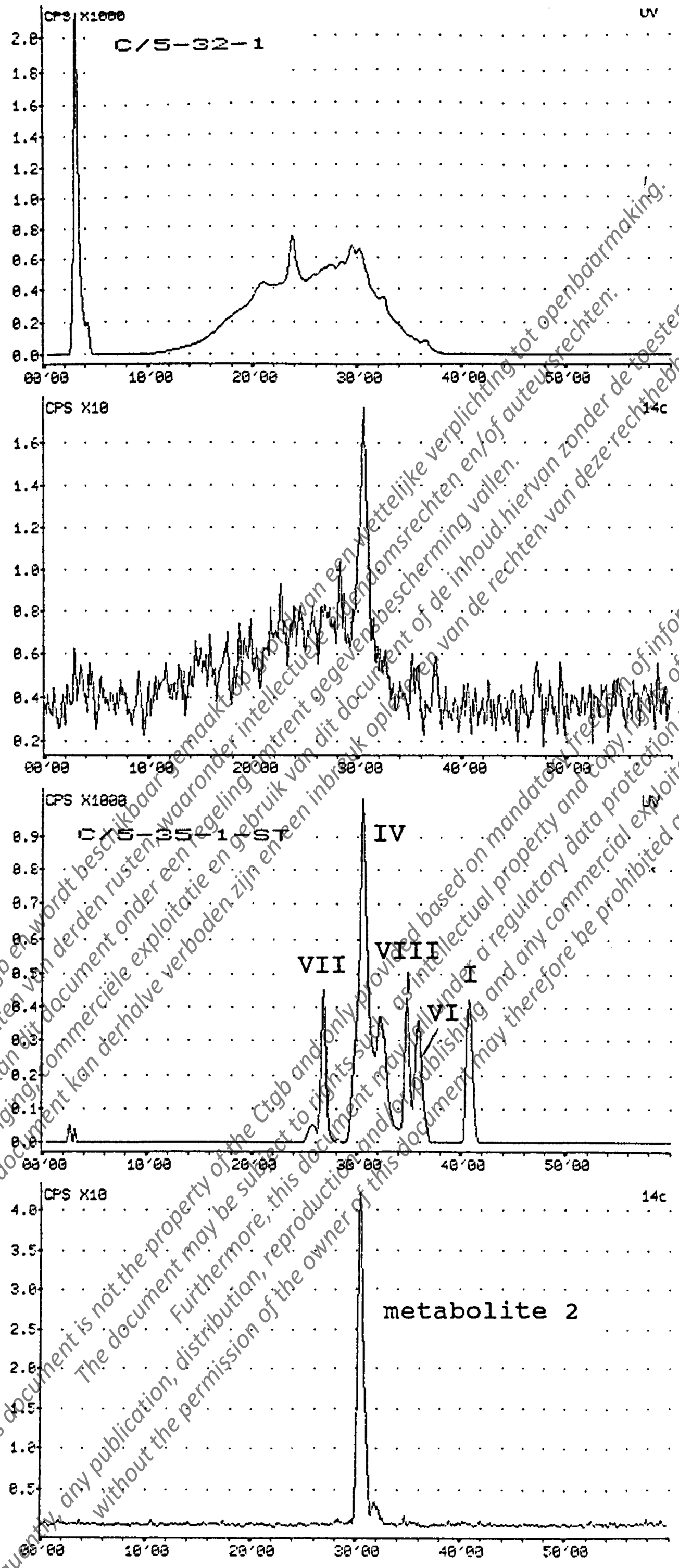


Figure 11:
HPLC-clean-up of the fraction 1 of the low pressure chromatography (potato vines, A and C) and identification with reference compounds (B).
For HPLC conditions see chapter IV.F.2
The Roman numerals refer to the reference compounds.

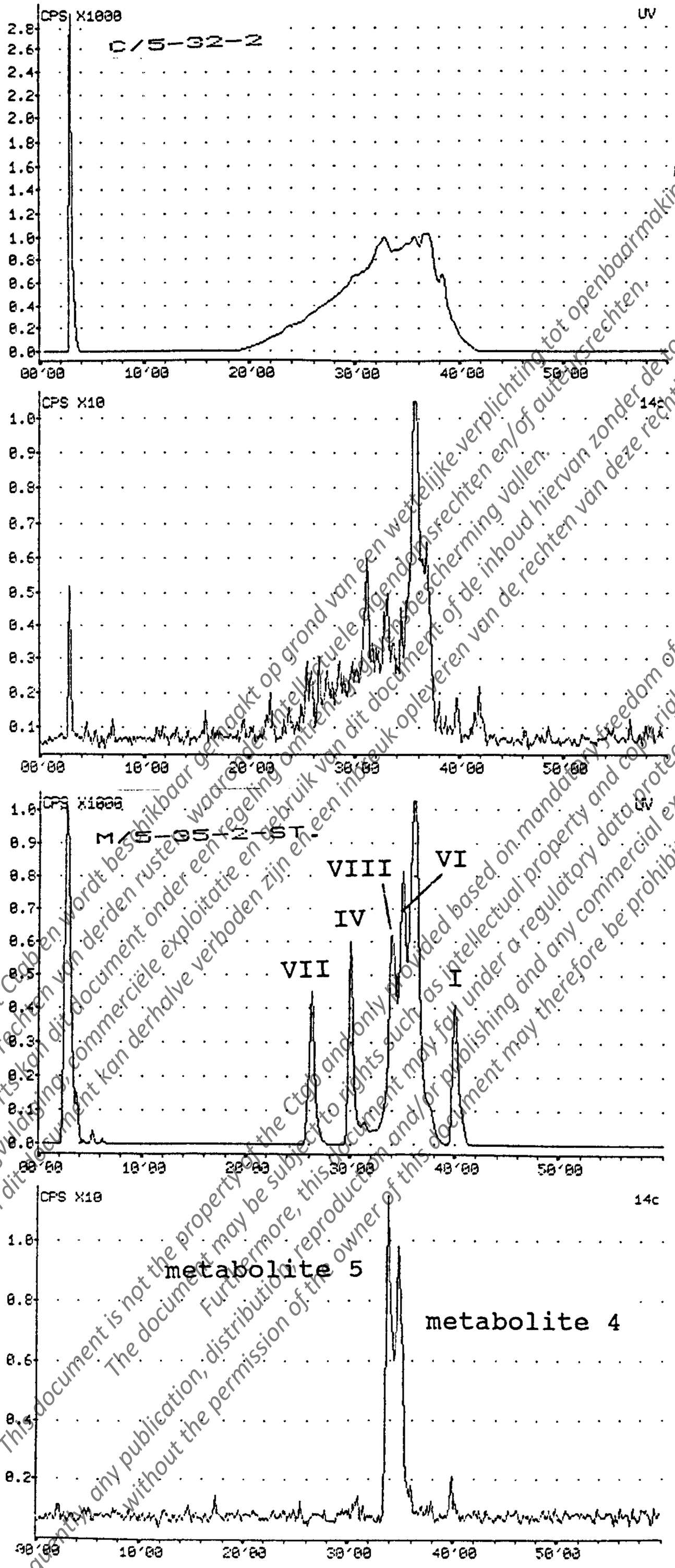


Figure 12:
HPLC-clean-up of the fraction 2 of the low pressure chromatography (potato vines, A and C) and identification with reference compounds (B).
For HPLC conditions see chapter IV.F.2
The Roman numerals refer to the reference compounds.

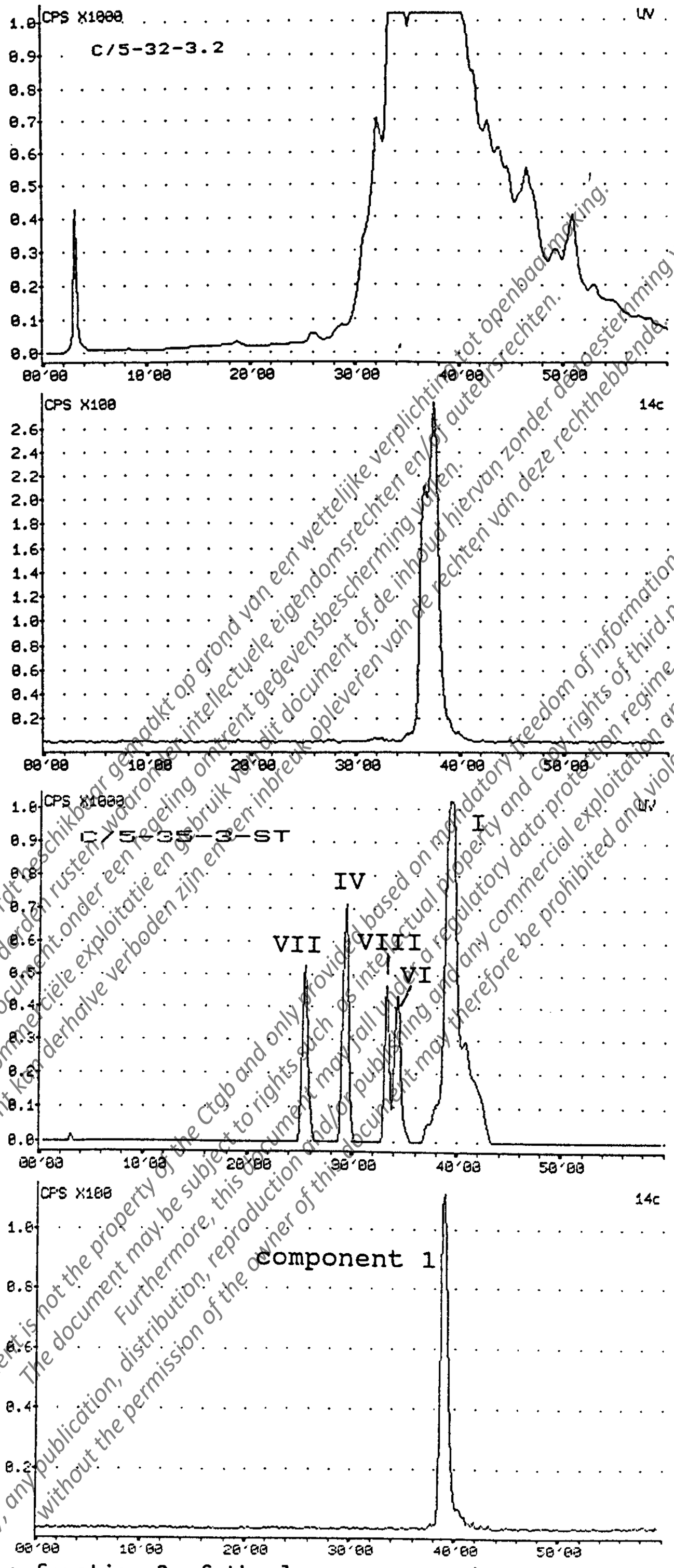


Figure 13:
HPLC-clean-up of the fraction 3 of the low pressure chromatography (potato vines, A and C) and identification with reference compounds (B).
For HPLC conditions see chapter IV.F.2
The Roman numerals refer to the reference compounds.

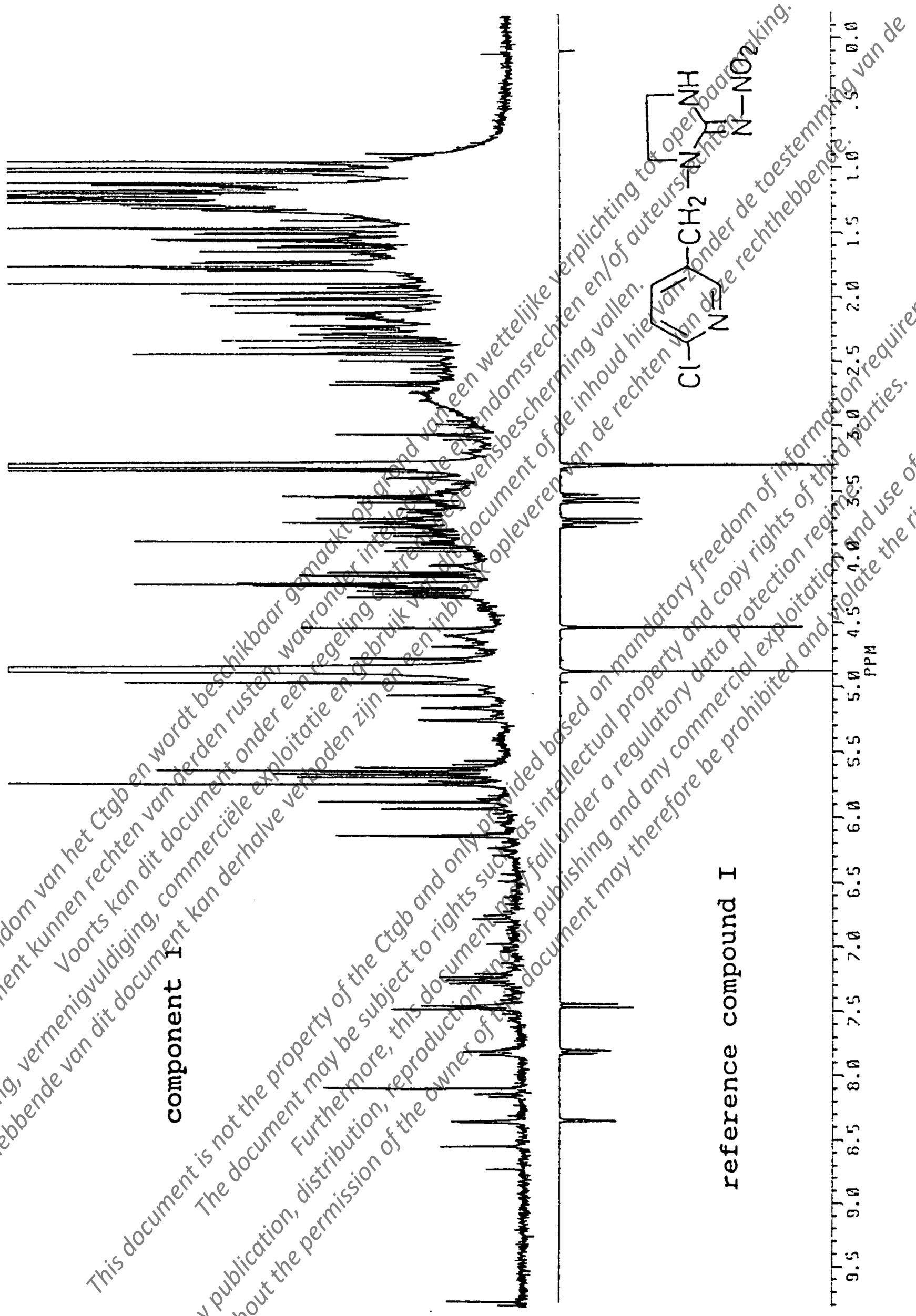


Figure 14:

^{1}H-NMR-spectra of component 1 isolated from the ethyl acetate phase of potato vines and of the reference compound NTN 33893 (I)

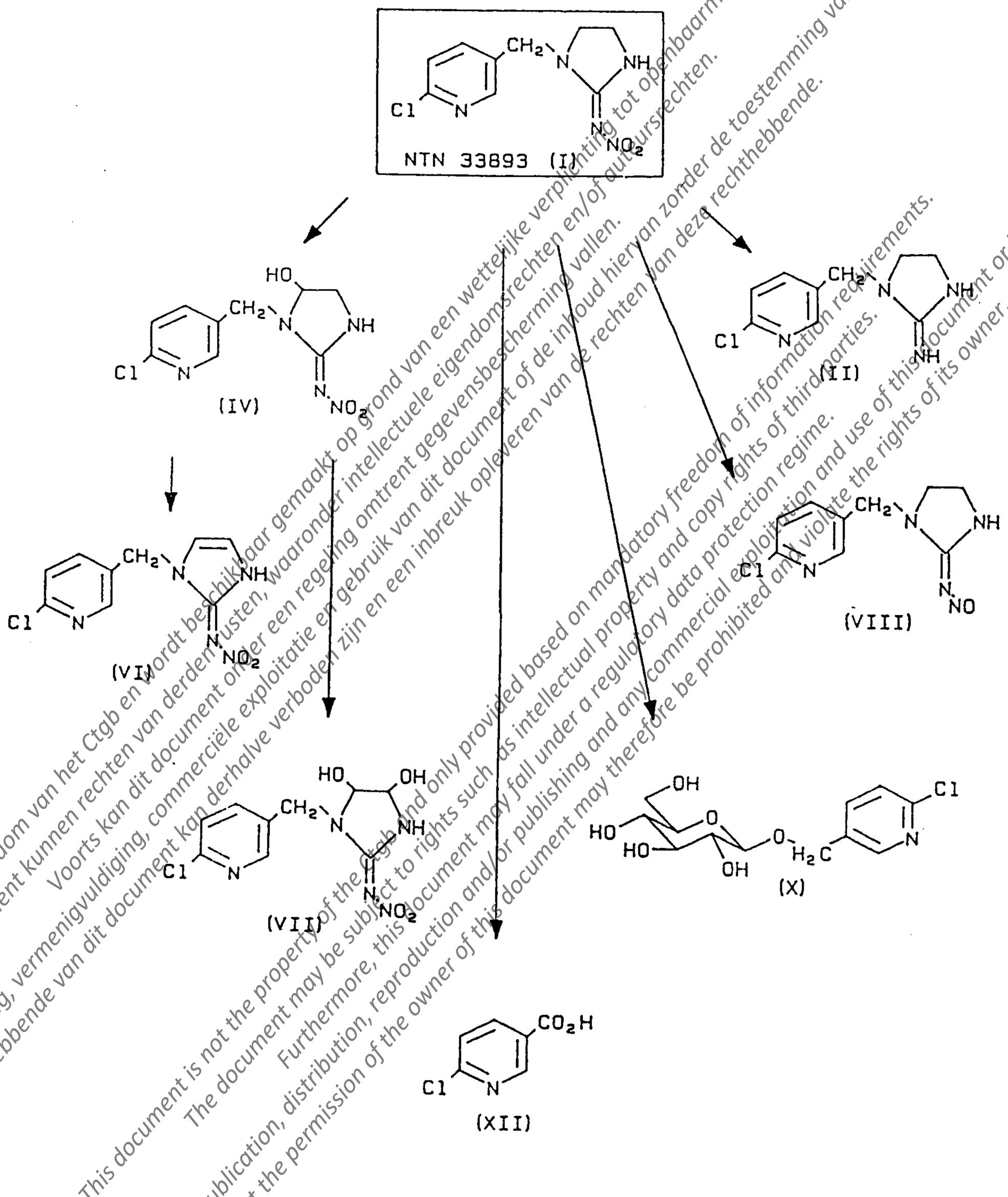


Figure 15:
Proposed metabolic pathway of NTN 33893 in potato plants following granular application

XI. Appendices

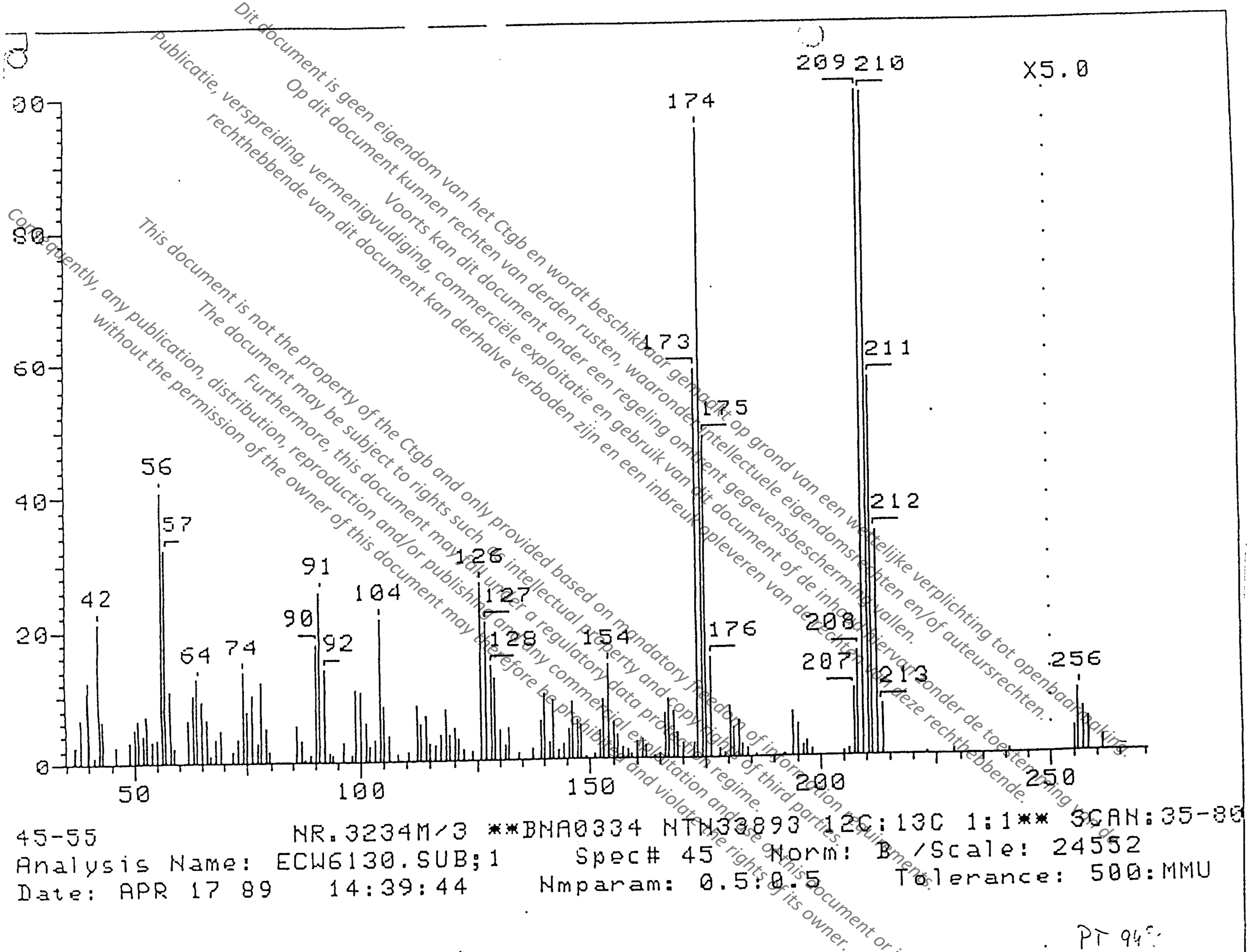
S A M P L E   C E R T I F I C A T I O N

[redacted]

Appendix I:
Radiochemical formulation details of the test substance

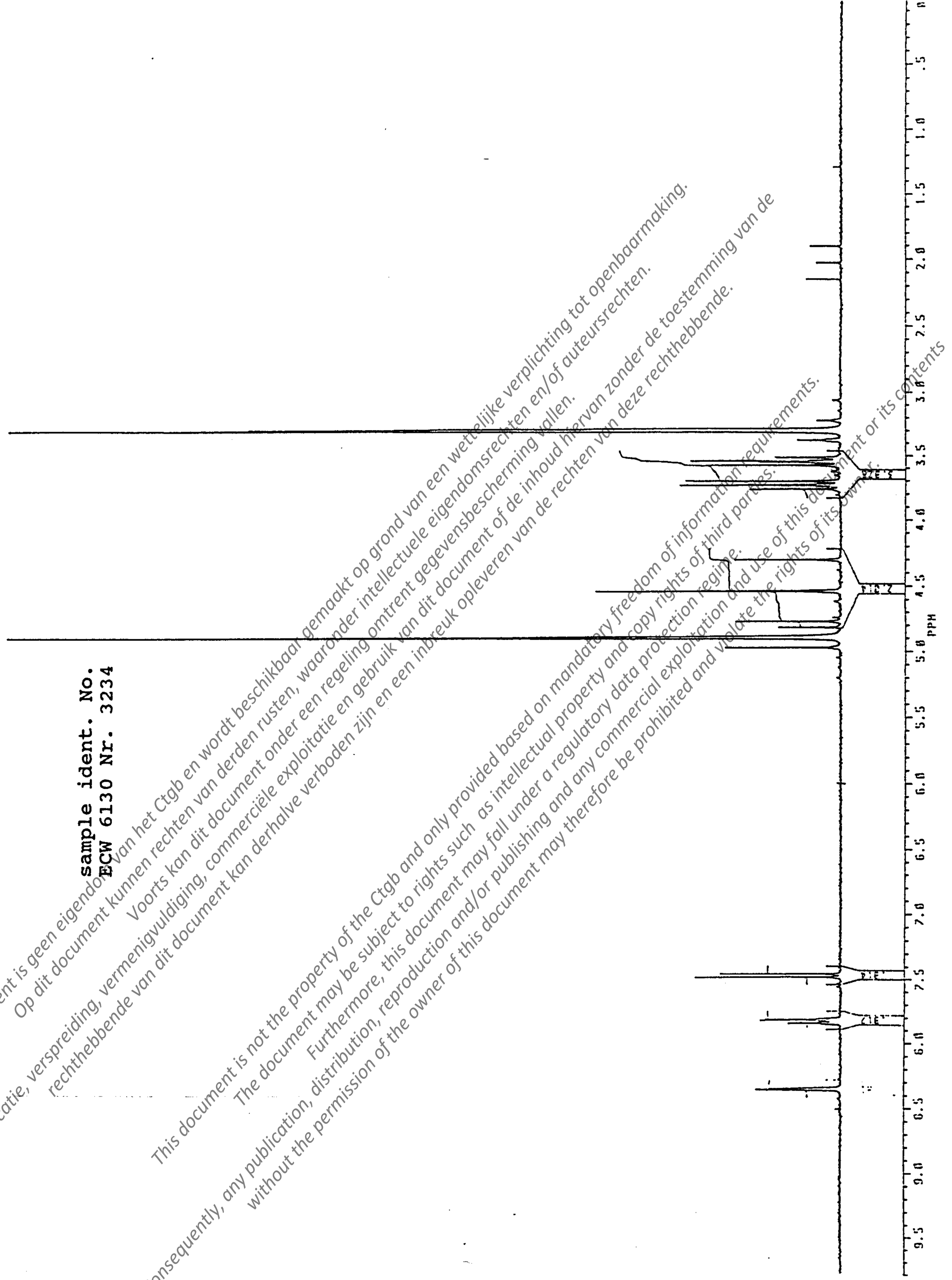
Project No. M 173 0297-9

Appendix I (continued):
MS-spectrum of the test substance



45-55 NR.3234M/3 **BNA0334 NTN33893 12C:13C 1:1** SCAN:35-80
Analysis Name: ECW6130.SUB;1 Spec# 45 Norm: B /Scale: 24552
Date: APR 17 89 14:39:44 Nmparam: 0.5:0.5 Tolerance: 500:MMU

PT 94%



Appendix I (continued):

^{1}H-NMR-spectrum of the test substance

Organic carbon [%]	1.40
microbial carbon/DS soil [mg/kg]	280
pH	4.73
Cation exchange capacity [mval/100 g]	7.5[1)]

Texture analysis according to DIN 19682 soil diagram:

sand [%]	51.9
silt [%]	32.7
clay [%]	15.4

Texture analysis according to USDA soil diagram:

sand [%]	56.4
silt [%]	28.2
clay [%]	15.4

Classification: Sandy loam soil

1) Analysis from "Landwirtschaftliche Untersuchungs- und Forschungsanstalt [redacted]", FRG

Appendix II:
Textural analysis of the soil "Monheim 1".
Conducted by [redacted] Institute for Environmental Biology, Bayer AG

Soil type : Monheim 1 (see appendix II)
container size : ca. 1 m^2
Filling date : 31.03.1987

Fertilization measures : 13.02.1989 1000 kg/ha
"Nitrophoska special"

From 15.05.1989 on one application a week
alternating between "Wuchsal" and "Fertisal",
2-4 liters of a 1.5 g/l solution applied

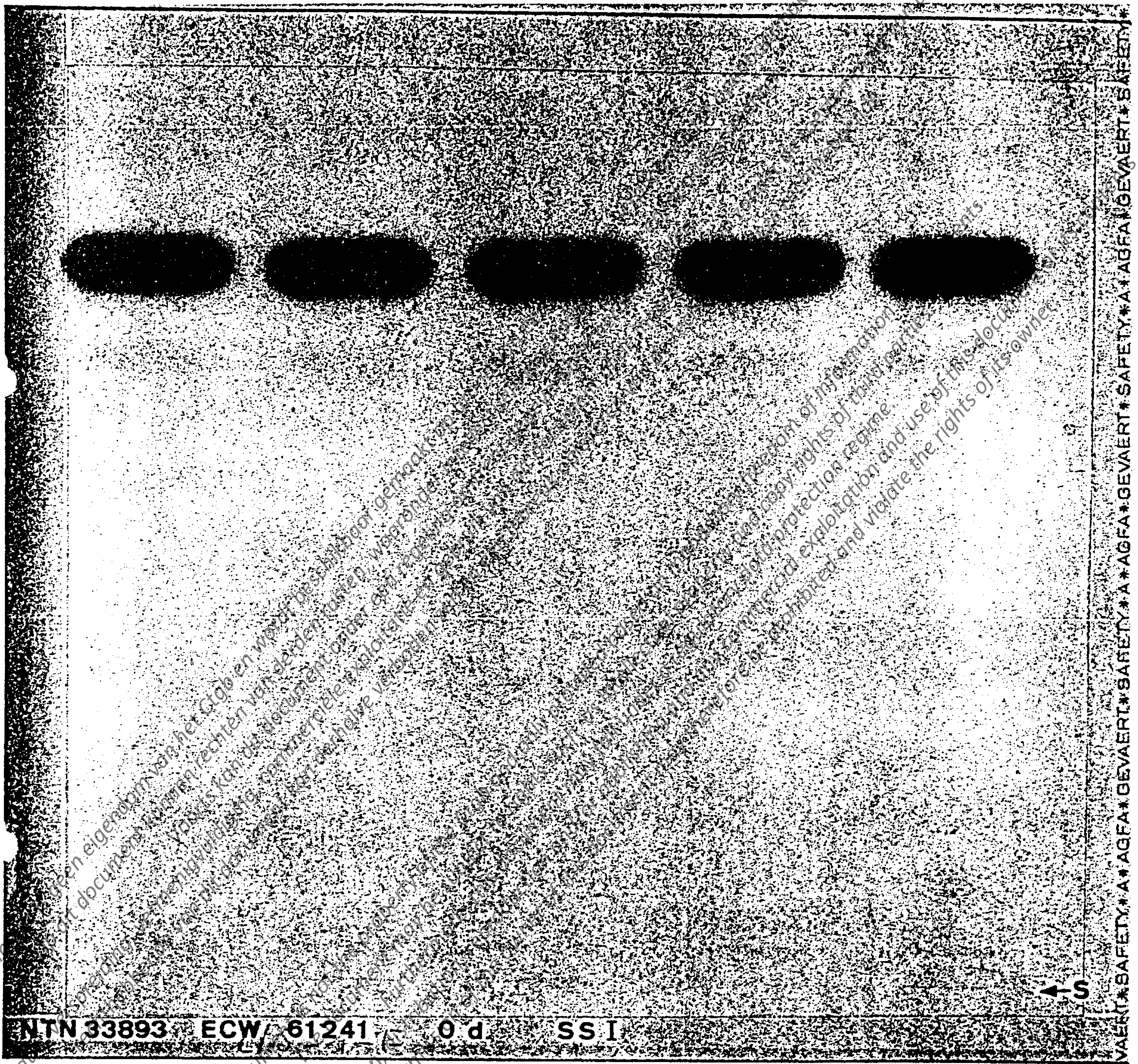
Plant protection measures:

Date	Chemical	% a.i.
01.06.89	Ridomil MZ	0.25
12.06.89	Cupravit OB21	0.50
	E combi	0.10
26.06.89	Antracol	0.20
04.07.89	Ridomil	0.25
12.07.89	Cupravit OB21	0.30
	E combi	0.15
28.07.89	Antracol	0.20
10.08.89	Shell Phosdrin	0.05

Average monthly temperature (measured at ground level) and hours of sunshine

: Month	Sunshine (h)	Temperature (°C)
April	82.75	8.3
May	290.7	15.3
June	231.0	19.1
July	214.0	21.4
August	209.0	19.3

Appendix III:
Growth and Environmental conditions



Appendix IV:
Radio-TLC stability check of the radioactive active ingredient in the 5% granular formulation

Solvent system I: ethyl acetate/i-propanol/water 65:23:12

Weight/Volume	Tubers	Vines
Weight at harvest (g)	3833	462
Weight of subsample (g)	500	100
Volume (ml)		
methanol/water		
(tubers 2:1, vines 1:1)	600	800
methanol	600	800
dichloromethane (2 x)	600	800
Volume used for extraction of the aqueous phase (ml)*		
n-hexane (2 x)	500	400
ethyl acetate (2 x)	500	400
Final volume after concentration (ml)		
n-hexane phase	200	220
ethyl acetate phase	200	240
aqueous phase	350	575
Solids (g)	75	54.5

* The aqueous phase was obtained after combination of the extracts and removal of methanol and dichloromethane

Appendix V:
Weights of potato samples and subsamples and volumes of solvents used for extraction and partitioning

Fraction	Vines radioactivity in analysis sample	Vines radioactivity in total sample (kBq)	Tubers radioactivity in analysis sample	Tubers radioactivity in total sample (kBq)
n-hexane phase	10.53 Bq/200 µl	11.6	2.35 Bq/3 ml	0.16
ethyl acetate phase	172.7 Bq/200 µl	207.3	393.4 Bq/3 ml	26.20
aqueous phase	62.8 Bq/200 µl	180.6	117.0 Bq/3 ml	13.65
Solids	2627 Bq/g	143.2	36.71 Bq/g	2.75
total		542.7		42.76

Appendix VI:

Raw data from the analysis of radioactivity in the different fractions in potato vines and tubers

Fraction	Radioactivity in analysis sample and in total sample		
	day 5	day 203	day 342
n-hexane	10.17 Bq/200 µl	10.53 Bq/200 µl	5.52 Bq/100 µl
phase	8.64 kBq/170 ml	11.6 kBq/220 ml	6.62 kBq/120 ml
ethyl acetate	91.67 Bq/200 µl	172.7 Bq/200 µl	79.3 Bq/100 µl
phase	220.0 kBq/480 ml	207.3 kBq/240 ml	158.6 kBq/200 ml
aqueous	72.5 Bq/200 µl	62.8 Bq/200 µl	77.5 Bq/100 µl
phase	188.5 kBq/520 ml	180.6 kBq/575 ml	155.0 kBq/200 ml
Solids	2129 Bq/g	2627 Bq/g	2457 Bq/g
	190.8 kBq/89.6 g	143.2 kBq/54.5 g	132.7 kBq/54 g

Appendix VII:
Raw data from the analysis of radioactivity in potato vines for the storage stability study.

Fraction	Radioactivity in analysis sample and in total sample	
	day 169	day 379
n-hexane phase	2.35 Bq/3 ml 0.157 kBq/200 ml	1.95 Bq/500 µl 0.078 kBq/20 ml
ethyl acetate phase	393.4 Bq/3 ml 26.2 kBq/200 ml	152.6 Bq/100 µl 30.52 kBq/20 ml
aqueous phase	117.0 Bq/3 ml 13.7 kBq/350 ml	36.0 Bq/ml 13.5 kBq/375 ml
Solids	36.7 Bq/g 2.75 kBq/75 g	56.4 Bq/g 4.78 kBq/84.7 g

Appendix VIII:
Raw data from the analysis of radioactivity in potato tubers for the storage stability study.

Liquid samples:

Number of aliquots	:	3
Amount per aliquot	:	0.1 - 7.0 ml
Instruments	:	1. PW 4700 (Philips/Raytest)
		2: Rackbeta 1219 Spectral (LKB)
Quench correction	:	External standard

Solid samples:

Number of aliquots	:	3-5 (normally 3)
Amount per aliquot	:	30-250 mg (normally 50-100 mg)
Instruments	:	Oxidizer 306 Tri-carb (Packard) and
		OX 300 (Harvey)

Statistics:

Reproducibility	:	± 1 - 2% (standard deviation of the mean value)
Comparability	:	± 1 - 2% (1 sample measured with different instruments)

Background radioactivity of the instrument (automatically subtracted from the measurement results):

1. Instant Scint Gel (7 ml) (Packard)	:	20 - 30 cpm
2. Carbosorb (8 ml)/Permafluor (10 ml) (Packard)	:	19 - 37 cpm
		(guaranteed < 40 cpm)

Appendix IX:

Measurement of Radioactivity

Measuring time of samples:

Generally between 10 sec. and 40 min. depending on the amount of radioactivity in the sample.
The measurements are stopped after reaching a 2-sigma error of 0.7%. If this error is not reached within 10 min. the measurement is stopped and the 2-sigma error of the cpm-value (PW 4700, Philips/Raytest) or the error of the dpm-value (Rackbeta 1219 Spectral, LKB) reached at that time is printed out. The error of the dpm-value is calculated from the 1-sigma error of the cpm-value and the error of the quench correction curve.

Detection limit:

2 x background

Counting efficiency:

Instrument:	1. PW 4700 (Philips)	=	84-93%
	2. Rackbeta 1219 (LKB)	=	48-96%

Appendix IX (continued):

Measurement of Radioactivity