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DETERMINATION AND CONFIRMATION OF CHLORONITROBENZENES IN WATER AND FISH SAMPLES

by

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MANAGEMENT PERSPECTIVE

Chloronitrobenzenes are environmental pollutants that have been reported in fish samples in the United States and in Europe. However, up until now analytical methodologies for these compounds are still unavailable. This report describes a validated method suitable for the determination of 14 chloronitrobenzenes at low ppt levels for water and low ppb levels for fish samples. With only minor modification, this method can be included into the methodologies for organochlorine insecticides to improve productivity.

Dr. J. Lawrence
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PERSPECTIVE-GESTION

Les chloronitrobenzènes sont des polluants qu'on a trouvés dans des tissus de poisson aux États-Unis et en Europe. Cependant, il n'existe encore aucune méthode d'analyse applicable à ces composés. Dans ce rapport, on décrit une méthode validée qui peut servir au dosage de 14 chloronitrobenzènes en faibles concentrations de l'ordre des p.p. millier dans l'eau et de l'ordre des p.p. milliard dans les tissus de poisson. Adaptée par de simples modifications mineures, cette méthode peut être ajoutée à la méthodologie applicable aux insecticides organochlorés, ce qui améliore la productivité de l'analyse.

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SUMMARY

A method for the determination of 14 chloronitrobezenes (CNBs) in water and fish tissue samples was developed. CNBs were extracted by dichloromethane and the concentrated extracts were cleaned up by gel permeation chromatography (fish only) and/or by an activated Florisil column. Final analysis was performed by gas chromatography using an electron-capture detector (ECD). Because of the high ECD sensitivity of all CNB congeners, method detection limits of 2 ng/L for water and 5 ng/g for fish were achieved. Electron-impact mass spectral data were also acquired and summarized. In all cases, the intense molecular ion and the $[M-NO_2]^-$ ion are suitable for confirmation and quantitation purposes. This method has been applied to water and fish samples for the determination of octanol-water partition coefficients and bioconcentration factors in rainbow trout for the CNBs.

RÉSUMÉ

On a mis au point une méthode de dosage applicable à 14 chloronitrobenzènes (CNB) dans l'eau et dans les tissus de poisson. On a extrait les CNB au dichlorométhane, puis purifié les extraits concentrés par chromatographie par perméation sur gel (tissus de poisson seulement) ou par traitement sur colonne de Florisil activé. L'analyse finale a été faite par chromatographie en phase gazeuse avec détection par capture d'électrons (DCE). En raison de la grande sensibilité de la DCE à tous les congénères de CNB, la limite de détection était de 2 ng/L, pour l'eau, et de 5 ng/g, pour les tissus de poisson. On a aussi recueilli des données de spectroscopie de pertes d'énergie qu'on a résumées. Dans tous les cas étudiés, on a constaté que l'ion moléculaire intense et l'ion $(M-NO_2)^+$ peuvent servir à des fins de confirmation et d'évaluation quantitative. La méthode décrite a été appliquée à des échantillons d'eau et de tissus de poisson pour l'évaluation du coefficient de séparation octanol-eau et des facteurs de bioconcentration des CNB chez la truite arc-en-ciel.

1.0 INTRODUCTION

Chloronitrobenzenes (CNBs), produced by the nitration of chlorobenzenes, are used in the manufacture of nitrophenols, nitroanilines, chloroanilines and other dye intermediates. Some monochloronitrobenzenes are used for the production of benzidine derivatives. Although residues of CNBs are not frequently reported in the environment, they were found in fish samples of the Mississippi River [1] and the Rhine River [2]. In particular, residues of three monochloronitrobenzenes and two dichloronitrobenzenes from 0.05 to 0.2 µg/g were found in several species of fish in Mississippi River. Several CNBs were listed as priority pollutants by the European Economic Community (EEC).

In preparation for a study of the bioconcentration factors of CNBs in rainbow trout, a simple and reliable method for the determination of the above compounds in water and fish samples is required. This manuscript reports the successful validation of a method suitable for the determination of 14 CNBs in water at ng/L and in fish tissue at ng/g levels. Electron-impact mass spectral data are also included for the confirmation of such compounds.

2.0 EXPERIMENTAL

2.1 Reagents and Chemicals

All solvents were distilled-in-glass grade supplied by Burdick and Jackson. Chloronitrobenzenes were purchased from Aldrich Chemicals Company and were used without further purification. Florisil

was obtained from Supelco Ltd. Anhydrous sodium sulfate purchased from BDH Chemicals was heated at 650°C for 8 hr before use.

2.2 Instrumentation

The chromatographic conditions and equipment used in the gas chromatographic electron-capture and mass spectral analyses of the CNBs are summarized in Table 1. Full scan MS data were obtained by scanning the mass selective detector from m/z 30 to 310 at a rate of 1.52 scans per second and a scan threshold of 1000. The electron energy and electron multiplier voltage were 70 eV and 2000 V, respectively.

2.3 Extraction and Cleanup of Water Samples

Water samples (1 L) in a suitable glass container was tested for pH and adjusted to pH 6 to 8 if required. In the validation experiments, the fortified samples were stored at 4° C for 16 hr before extraction. The sample was extracted by stirring with 50 mL of dichloromethane for 15 min. The organic layer was separated and water sample returned to the original container. The extraction was repeated twice with 50 mL aliquots of dichloromethane. After the last extraction, the combined dichloromethane fraction was dried through a column of anhydrous sodium sulfate. Following the addition of 3 mL of iso-octane as a keeper, the solvent was evaporated down to ca. 5 mL by means of a three-stage Snyder column. The concentrated sample with petroleum ether (b.p. 30° to 60°C) rinsings was transferred to a 15 mL test tube and the solvent was further evaporated to 3 mL.

The above iso-octane extract was then transferred to a 400 x 10 mm i.d. column containing 5.0 g of activated Florisil previously washed by 20 mL of petroleum ether. The column was then eluted with another 50 mL of the petroleum ether and this fraction was discarded. The column was further eluted by 75 mL of a 1:1 mixture of petroleum ether and dichloromethane. This fraction was collected and the solvent replaced by iso-octane and made up to 1.0 mL before the final analysis.

2.4 Extraction and Cleanup of Fish Samples

Fish samples were prepared by cutting the whole fish and blending in a mechanical blender. Typically, 5.0 g of the homogenized fish tissue was thoroughly mixed with 15.0 g of granular anhydrous sodium sulfate with a mortar and pestle. The mixture was extracted with 50 mL of dichloromethane for 2 min using a Polytron homogenizer. After layers separated, the organic layer was decanted into an Allihn filter containing 5 cm of Celite 545 and the filtrate collected. The extraction was repeated twice with 30 mL aliquots of dichloromethane. The combined fish extract was evaporated down as described before and the solvent replaced and made to 10.0 mL with chloroform.

Five mL of the fish extract was filtered through a disposable 0.5 µm Millex SR filter and 2.0 mL of the filtrate was injected into a 122 x 2.5 cm i.d. 60Å µStyragel column. The GPC column was eluted with chloroform at a flow rate of 9.0 mL/min. The first 270 mL of the eluate were discarded and the next 90 mL were collected. The latter fraction was again concentrated down and the solvent replaced by iso-octane. This extract was further cleaned up on an activated Florisil

column, as described above for the water sample, before final analysis.

3.0 RESULTS AND DISCUSSION

3.1 Chromatographic, electron-capture and mass spectral properties

All 14 CNBs were completely resolved on the DB-5 capillary column (Figure 1). The separation of the CNB congeners was generally better than those previously reported [1] using a 25 m OV-101 WCOT. The retention times of all CNBs chromatographed on the DB-5 column are listed in Table 2.

The electron-capture detector is very sensitive to all CNBs. Among the 14 congeners used in this study, their ECD relative response factors were within a factor of 2.5, with 4-chloronitrobenzene and 2,3,4,5-tetrachloronitrobenzene being the least and the most responsive congener, respectively (Table 2). Since the monochloro and dichloro benzenes have ECD sensitivities from a few thousand to a few hundred times lower than that of hexachlorobenzene, the enhanced responses of the monochloro and dichloro nitrobenzenes are undoubtedly due to the presence of the strongly electrophilic nitro group. The enhanced detector sensitivity enables us to have low instrument detection limits even for the monochloronitrobenzenes. Under our experimental conditions, the ECD has a linear dynamic range of at least 250 (from 1 to 250 pg) for all CNBs. Injection of 0.4 pg of 2,3,4,5-tetrachloronitrobenzene onto the DB-5 column produced a S/N ratio of 10.

Under electron-impact conditions, CNBs fragmented extensively and produced a large number of characteristic ions of high intensity

(Table 3). All CNBs exhibited a strong m/z 30 peak due to the formation of a NO^- species from the parent compounds and thus it is a useful diagnostic ion for the CNBs. The molecular ions, $M^{-\cdot}$, with relative abundance of 40% or higher, were also useful for quantitation and confirmation in selected ion monitoring. In all mass spectra, ions resulted from the loss of a neutral NO molecule, $[M-NO]^{-\cdot}$ or (M-30), and loss of a NO_2 radical, $[M-NO_2]^-$ or (M-46), were observed. The latter subsequently lost chlorine atoms from the ring to produce $[M-NO_2-Cl_x]^-$ ions. Using pentachloronitrobenzene as an example (Figure 2), the following fragmentation scheme was observed:

$$M^{-\cdot} \longrightarrow [M-NO]^- \longrightarrow [M-NO_2]^- \longrightarrow [M-NO_2-Cl]^- \longrightarrow [M-NO_2-Cl_2]^-$$

m/z 293 263 247 212 177

$$\longrightarrow [M-NO_2-Cl_3]^- \longrightarrow [M-NO_2-Cl_4]^-$$

142 107

In addition, the $[C_5Cl_5]^-$ (m/z 235) species from pentachloronitrobenzene as well as the $[C_5H_{5-n}Cl_n]^-$ (for n = 1 to 4) species from the other CNBs were also dominant in the mass spectra.

3.2 Evaporative losses

Chloronitrobenzenes are volatile compounds and losses of such compounds occur during the solvent evaporation steps if the samples are not handled properly. It was found that about 70 to 85% of the CNBs were recovered if a rotary evaporator with a 40°C water bath was used for evaporation. On the other hand, evaporation with a 3-stage Synder

column improved the recoveries to about 90%, thus the latter was used in the evaporation of CNB solutions. The use of a more volatile solvent further minimized evaporative losses and hence substitution of hexane by petroleum ether in the Florisil cleanup was also recommended.

3.3 Cleanup

An activated Florisil column was used in this work to remove potentially interfering compounds in the sample extracts. All CNBs were quantitatively eluted by 75 mL of a 1:1 mixture of dichloromethane and petroleum ether. Less polar compounds such as PCBs and chlorobenzenes were effectively separated from the analytes by a pre-elution of the column with hexane while the more polar pesticides, phthalate esters, phenols, amines, fatty and carboxylic acids were left behind on the Florisil column. Presumably due to their difference in polarity, pentachloro-, 2,3,5,6-tetrachloro- and 2,4,6-trichloro- nitrobenzenes were the first CNBs eluted while 4-chloro- and 3,4-dichloro- nitrobenzenes were the last CNBs eluted during Florisil column cleanup. Thus, if the Florisil was deactivated either by atmospheric moisture or by the presence of polar solvents in the sample extract, the less polar compounds mentioned above would be lost in the hexane wash fraction. On the other hand, a reduction in the amount of the 1:1 dichloromethane and petroleum ether used in the elution of CNBs from 75 to 50 mL would cause low recoveries of the more polar compounds. Organochlorine insecticides, a common class of environmental pollutants coeluted with the CNBs, did not interfere with the final ECD analysis since they had much longer retention times.

For fish extracts, an automated gel permeation chromatographic cleanup step for lipid removal was included prior to the Florisil cleanup. Using a μStyragel column, all CNBs were successfully separated from the fish lipids and quantitatively recovered in the 270 to 360 mL fraction.

3.4 Validation Results

Distilled and surface water samples fortified to 100 and 10 ng/L for each CNB were analyzed and the recoveries of the replicates were summarized in Table 4. In nearly all cases, the recoveries were between 80 to 90%. As indicated earlier, lower recoveries of the less chlorinated CNBs were likely caused by losses in the evaporative steps.

Recoveries of CNBs in fortified fish homogenates were also better than 80% at 25 and 250 ng/g levels (Table 4). The efficiency of the Polytron extraction technique was examined by comparing with the exhaustive Soxhlet extraction method. Using fish samples which contained accumulated CNBs in bioconcentration studies as references, both extraction techniques provided the same recoveries of CNBs, indicating that the Polytron method is suitable for the extraction of fish tissues.

The method detection limit of CNB in natural water samples is ca. 2 ng/L based on a one litre sample and a concentration factor of 1000. For fish samples, the method detection limit was 5 ng/g based on a 2 g sample a final volume of 2 mL.

3.5 Application

The methods developed in this work have been used for the determination of the octanol-water partition coefficients as well as bioconcentration factors of CNBs in rainbow trout [3] . A typical ECD chromatogram of a tissue extract from a trout previously fed with CNBs is presented in Figure 3. Because of the similarity in extraction and cleanup procedures [4], CNBs can be determined alongside the organochlorine insecticides by a simple adjustment of the gas chromatographic conditions in the final analysis.

ACKNOWLEDGMENTS

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LIST OF FIGURES

TABLE 1

Chromatographic conditions for the analysis of CNBs.

	ECD	MSD
Gas chromatograph	Hewlett-Packard 5880A	Hewlett-Packard 5880A
Detector type	Ni-63	5970B mass selective detector
Column	J&W DB-5, 30m x 0.25 mm 0.25µ thickness	Supelco SPB-5, 30m x 0.25 mm, 0.25µ thickness
Temperature program		
initial	70° for 0.75 min	70° for 0.75 min
rate 1	30°/min (70 to 110°)	30°/min (70 to 120°)
rate 2	2°/min (110 to 140°)	2.5°/min (120 to 220°)
rate 3	10°/min (140 to 220°)	none
final	220° for 5 min	220° for 5 min
Detector temp.	300°	200°
Injector temp.	250°	250°
Splitless time	0.75 min	0.75 min
Column pressure	15 psi	4 psi
Injection volume	2 µL	1 µL
Carrier gas	helium	helium
Makeup gas	Argon/methane 95/5	none
and flow	25 mL/min	none
Septum purge flow	1.5 mL/min	1.5 mL/min

TABLE 2

Retention times (min) and relative response factors of CNBs

CNB	Retention time	Rel. response factor
3-	8.36	5.02
4-	8.62	4.09
2-	8.83	4.23
3,5-	11.42	6.83
2,5-	12.65	6.31
2,4-	12.95	5.84
3,4-	13.47	6.06
2,3-	13.85	7.04
2,4,6-	15.31	4.78
2,4,5-	17.23	5.91
2,3,4-	18.67	9.50
2,3,5,6-	20.18	7.75
2,3,4,5-	21.86	10.0
Penta-	24.59	7.06

TABLE 3

Mass number (m/z) and relative abundance (%) of some characteristic ions observed for CNBs

CNB	$M^{-\cdot}$	$[M-NO]^-$	$[M-NO_2]^-$	$[M-NO_2-Cl]^-$	Others
3-	157/59	127/9	111/100	75/72	99/20,50/34
4-	157/77	127/51	111/83	75/100	99/34,50/55
2-	157/79	127/33	111/74	75/100	99/49,50/43
3,5-	191/75	161/5	145/100	109/60	133/41,74/37
2,5-	191/79	161/12	145/76	109/86	133/95,74/46,30/100
2,4-	191/91	161/86	145/70	109/100	133/86,74/59
3,4-	191/97	161/50	145/100	109/95	133/67,74/55
2,3-	191/100	161/45	145/98	109/98	133/93,74/49
2,4,6-	225/74	195/100	179/56	143/64	167/85,109/56
2,4,5-	225/72	195/45	179/70	143/75	167/89,109/56,30/100
2,3,4-	225/79	195/86	179/84	143/87	167/88,109/63,30/100
2,3,5,6-	259/48	229/9	213/59	178/45	203/100,201/74,143/46
2,3,4,5-	259/53	229/26	213/64	178/51	201/73,143/49,30/100
Penta-	293/44	263/31	247/51	212/61	237/100,235/61,177/35

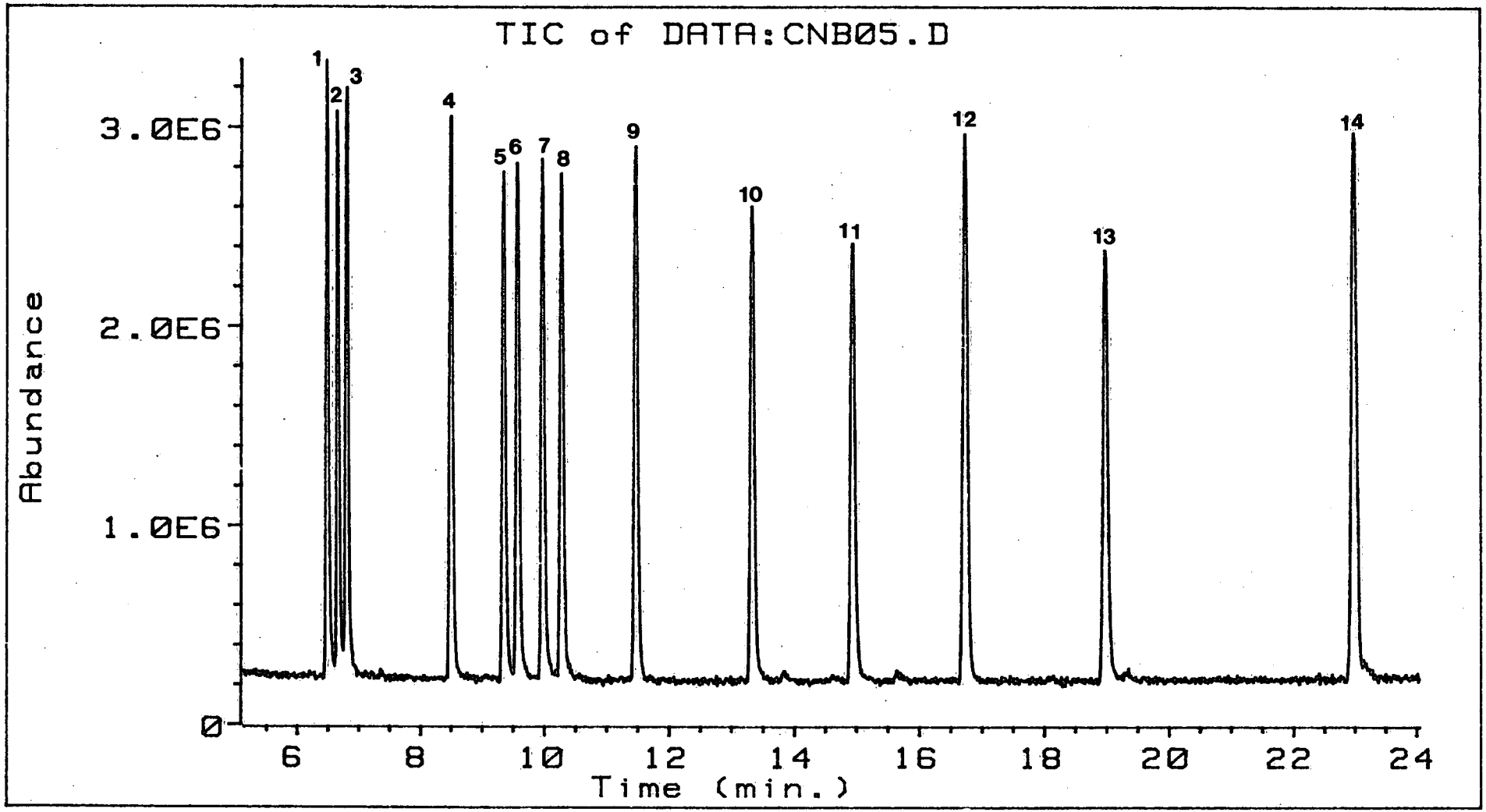
TABLE 4

Mean recoveries (%) and standard deviations of replicate determination of CNBs in fortified water and fish samples

(n = 4)

	Pure water		Lake water		Fish	
	100	10	100	10	250	25
CNB	ng/L		ng/L		ng/g	
3-	90 ± 5	83 ± 7	97 ± 6	103 ± 9	86 ± 8	82 ± 8
4-	92 ± 6	81 ± 9	98 ± 4	105 ± 6	85 ± 7	79 ± 7
2-	93 ± 5	80 ± 8	88 ± 5	85 ± 7	78 ± 6	76 ± 9
3,5-	93 ± 4	79 ± 6	92 ± 4	86 ± 6	83 ± 5	87 ± 8
2,5-	95 ± 5	83 ± 6	86 ± 5	79 ± 6	80 ± 6	89 ± 11
2,4-	91 ± 5	85 ± 8	89 ± 7	81 ± 7	86 ± 5	83 ± 8
3,4-	87 ± 7	77 ± 8	88 ± 6	82 ± 7	85 ± 6	85 ± 7
2,3-	89 ± 8	82 ± 5	85 ± 5	88 ± 9	82 ± 6	85 ± 10
2,4,6-	84 ± 4	81 ± 6	91 ± 5	78 ± 6	87 ± 5	90 ± 8
2,4,5-	96 ± 5	82 ± 6	89 ± 6	85 ± 9	86 ± 7	87 ± 9
2,3,4-	87 ± 4	80 ± 7	87 ± 5	86 ± 9	82 ± 8	83 ± 6
2,3,5,6-	90 ± 6	84 ± 9	89 ± 5	81 ± 7	79 ± 6	80 ± 8
2,3,4,5-	87 ± 6	79 ± 8	95 ± 6	--*	86 ± 5	85 ± 9
Penta-	83 ± 6	85 ± 10	88 ± 5	80 ± 9	81 ± 4	75 ± 7

* interference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7
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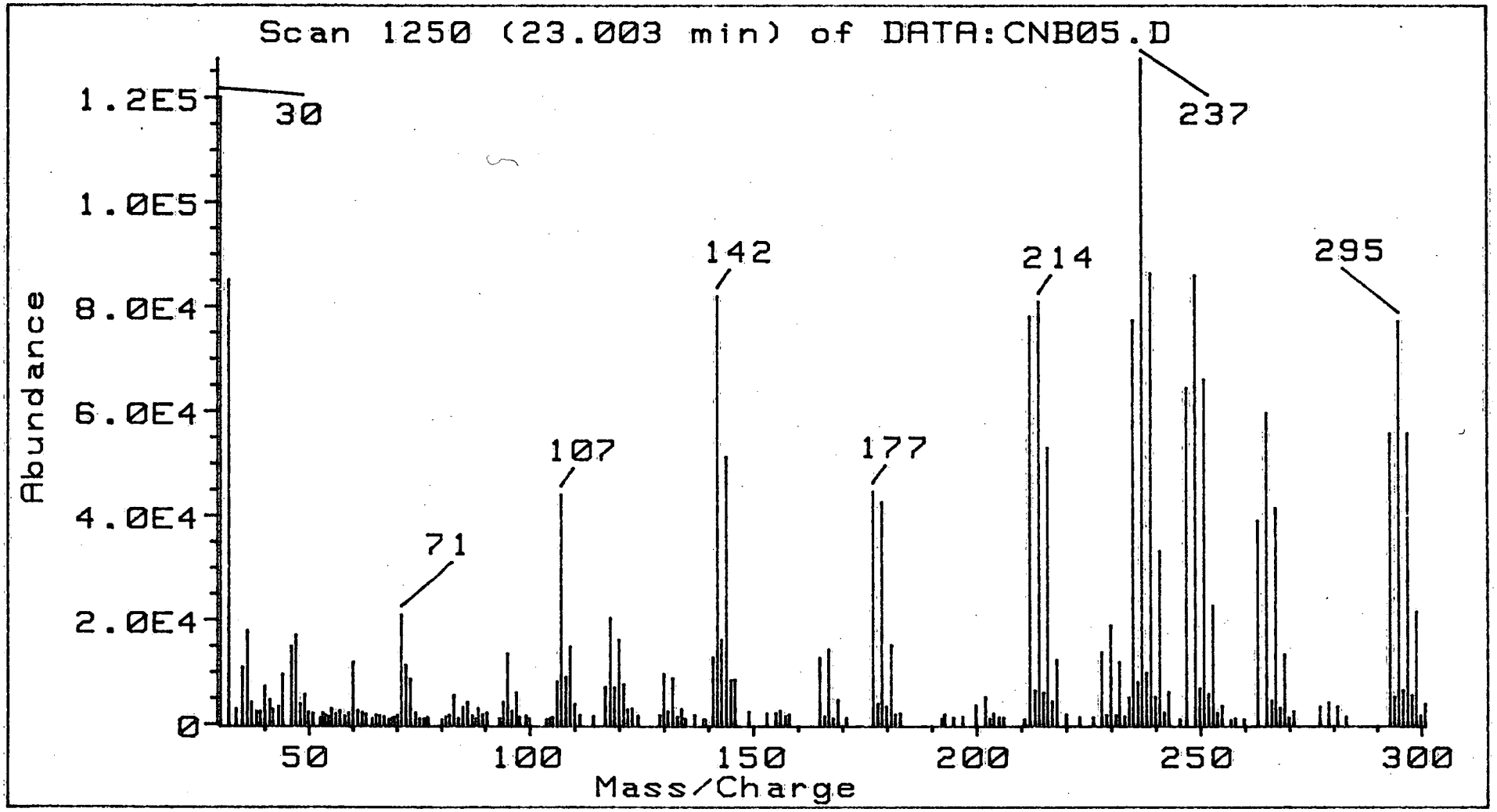
16
18
20
22
24
Time (min.)

Figure 1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250
300
Mass/Charge

Figure 2

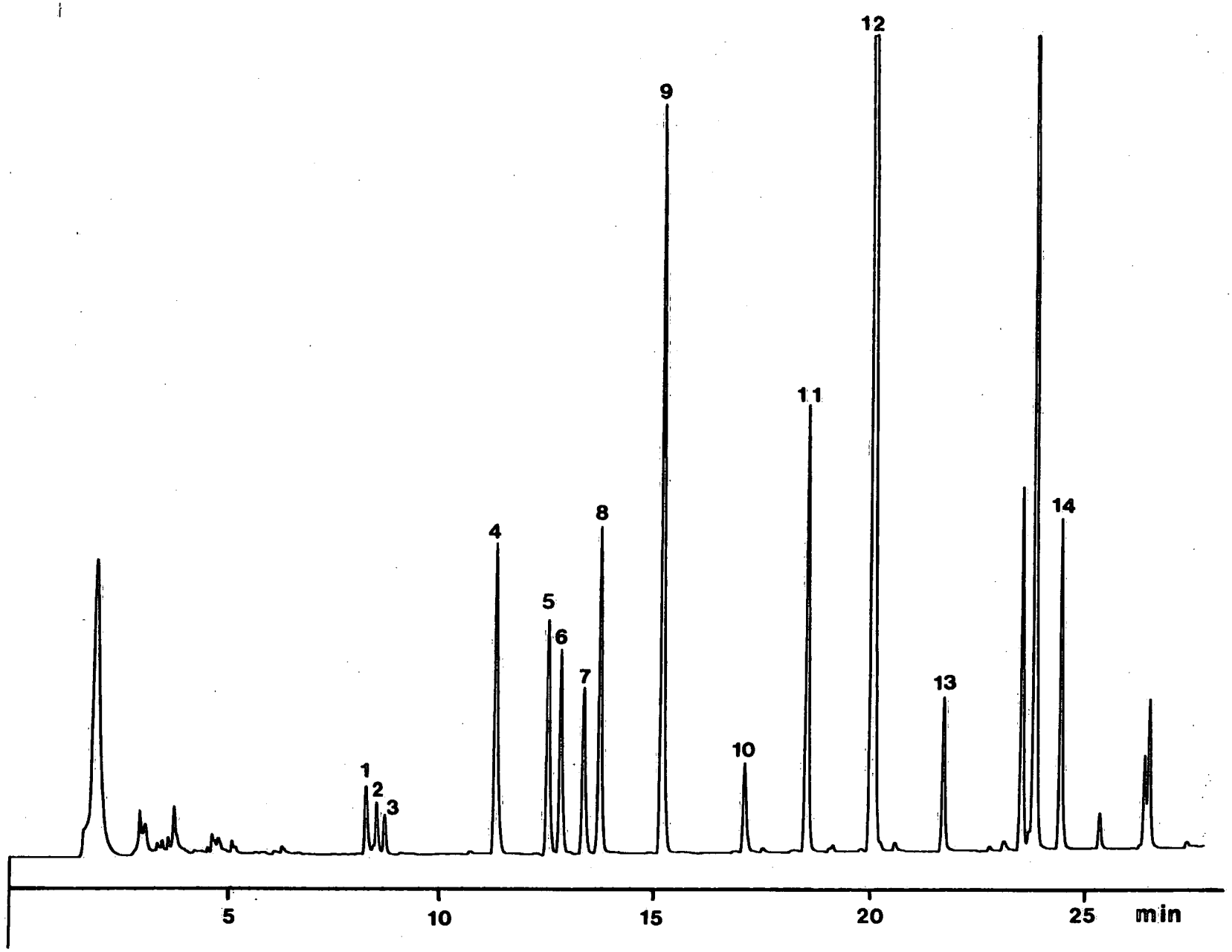

Figure 3