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Hing-Biu Lee and Yvonne D. Stokker

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IN NATURAL WATERS

by

Hing-Biu Lee and Yvonne D. Stokker

Analytical Methods Division
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario
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MANAGEMENT PERSPECTIVE

This paper describes a multi-residue method for the analysis of 11 common triazines at residue levels in natural water. Triazines are the most heavily used herbicides across Canada for control of broadleaf and grassy weeds in crops. This method was developed under the modular concept so that simultaneous analysis of triazines and other neutral herbicides and pesticides could later be incorporated into a time and cost efficient multi-class multi-residue method.

ABSTRACT

A gas chromatographic method was developed for the analysis of 11 triazine herbicides in natural waters. Triazine residues were extracted from water by methylene chloride and they were cleaned up by a 10% deactivated Florisil column using 3% methanol in benzene as eluant. Extracts were quantitated, in two separate runs, on an Ultrabond 20M and a 3% OV-1 column connected to a nitrogen-phosphorus detector. These extracts could also be analyzed on a 30 m DB-1 capillary column in a single run. This method has been validated at 10, 1 and 0.1 µg/L using fortified distilled water and Lake Ontario water, and was also validated at 1.0 µg/L with two other natural water samples. Recoveries of the 11 triazines at all levels of fortification were between 87 and 108% with the exception of simetone and simetryne which was about 80% recovered in a few cases. Detection limit for all 11 triazines in this method is 0.025 µg/L.

INTRODUCTION

1,3,5-Triazines are widely used as selective pre- and post-emergence herbicides for the control of broadleaf and grassy weeds in many agricultural crops. Because of high application rates and stability of many triazines, their residues were often found in the environment. In one report, the authors estimated that between 0.3 and 1.9% of the atrazine applied to farmland found its way into stream water (1).

In the past 15 years or so, many papers have been published on the gas chromatographic separation and detection of triazines using various detectors such as the alkali flame-ionization detector (AFID) (2-4), nitrogen-phosphorus detector (NPD) (5), Coulson conductivity detector (1,3,6), flame-ionization detector (FID) (4,7,8), electron-capture detector (ECD) (6,9), mass spectrometer (10), and photoionization detector (11). Analysis of triazines by ECD after conversion to their heptafluorobutyryl derivatives (12) or by FID after formation of the trimethylsilyl derivatives (13) were also reported. The use of high resolution capillary columns for improved separation of triazines in complex, multi-component mixtures was also demonstrated (4,7,14). Recently, liquid chromatographic methods using ultraviolet detector were applied to the analysis of some polar and non-volatile amino, hydroxy and nitro (15) as well as other (16,17) triazines. Five classes of triazine herbicides were also studied by combined high-performance liquid chromatography-mass spectrometry (HPLC-MS) (18).

Procedures for extraction and cleanup of triazine residues in water (5,11, 19-22) and in other matrices (23-25) were also reported. However, recovery data for triazines were only partially available in many cases. In this paper, we shall describe a validated procedure for the quantitative determination of 11 triazines (Table 1) in natural waters by a gas chromatographic technique using a nitrogen-phosphorus detector.

EXPERIMENTAL

Apparatus

(a) Gas chromatographs. (1)Hewlett-Packard model 5700A equipped with model 18789A nitrogen-phosphorus detector (NPD) and model 3392A reporting integrator. Operating temperatures (°C): injection port 250°, detector 300°, column oven see GC columns below. Flow rates, carrier (helium) 25 mL/min, hydrogen 4.0 mL/min and air 50 mL/min. Collector voltage adjusted to give 50% full scale deflection at attenuation 1x32 with electrometer zero off. (2) Hewlett-Packard model 5880A equipped with a NPD, split/splitless injection port, and Level IV terminals. Operating temperatures and flow rates same as model 5700A described above except for the following flow rates: carrier (helium) 1.5 mL/min and detector make-up gas (helium) 25 mL/min.

(b) GC columns. (1) 1.8 m x 2 mm id glass packed with 80/100 mesh Ultrabond 20 M (Ultra Scientific Co., Hope, R.I. 02831), column temperature 170°C. (2) 1.8 m x 2 mm id glass packed with 3% OV-1 on 100/120 mesh Gas Chrom Q (Chromatographic Specialties Ltd., Brockville, Ontario, Canada), column temperature 160°C. (3) 30 m x 0.25 mm id DB-1 fused silica capillary column (J&W Scientific Inc., Rancho Cordova, CA 95670), film thickness 0.25 micron. Temperature program (°C): initial 70°, hold 0.5 min, programming rate 1, 25°/min (70° to 195°), hold 8 min at 195°, programming rate 2, 10°/min (195° to 200°), hold 10 min at 200°. (4) 15 m x 0.25 mm id DX-4 fused silica capillary column (J&W Scientific Inc.), film thickness 0.25 micron. Temperature program (°C), initial 70°, hold 0.5 min at 70°, programming rate 1, 25°/min (70° to 200°), programming rate 2, 2°/min (200° to 220°), hold 5 min at 220°, programming rate 3, 20°/min (220° to 250°), hold 15 min at 250°. For columns 3 and 4, inject samples in splitless mode and keep splitless valve on for 30 sec.

Reagents

Use pesticide grade solvents only.

(a) Triazine standards. Obtain from manufacturers or Nanogens Inc. (Aptos, CA5003) and use without further purification (minimum percent purity better than 98%). Prepare stock solutions of each triazine in methanol at 1000 µg/mL. Prepare a mixture of all 11

triazines at 100 pg/μL each in 1+99 methanol/isooctane for GC calibration. Keep solutions at 4°C in the dark.

(b) 10% Deactivated Florisil. Activate Florisil PR (Supelco, Inc., Bellefonte, PA 16823) at 130°C for 16 hr. Deactivate adsorbent by adding 10.0 mL of reagent water to 90.0 g activated Florisil. Mix well on a shaker and keep in a tightly capped glass container overnight before use. Prepare fresh weekly.

Fortification of Water Samples

Spike 100 μL triazine mixture in methanol at appropriate concentrations to 1L water. Stir and equilibrate 30 min before extraction.

Extraction and Cleanup

Adjust pH of water sample (1L) collected in a 1.15 L or similar size long-neck whiskey bottle to about 6 if necessary. Stir sample with 50 mL methylene chloride using Teflon-coated stirring bar so that vortex formed almost reaches bottom of bottle. After stirring 30 min, transfer contents to 1 L separatory funnel. Drain organic layer to a 500 mL round bottom flask. Transfer aqueous layer back to original sample bottle. Repeat extraction twice. Discard water sample after last extraction. Filter combined organic extract through 5 cm anhydrous Na_2SO_4 in a sintered glass filter column. Apply vacuum

to dry column. Wash column with 50 mL fresh methylene chloride and apply vacuum to collect filtrate. Add 3 mL isooctane to extract and evaporate to ca. 10 mL on rotary evaporator under reduced pressure using a 35°C water bath. Add 50 mL hexane to concentrated extract and repeat evaporation until volume is reduced to ca. 3 mL.

Prepare cleanup column by filling 450 x 20 mm id tube with 20 g 10% deactivated Florisil. Top column with 1 cm anhydrous Na_2SO_4. Prewet column with 100 mL hexane and discard hexane. Quantitatively transfer sample extract to column with several hexane rinsings. Elute column with 200 mL toluene/hexane (25+75) and discard this fraction. Elute same column with 200 mL 3% methanol in toluene and collect. Add 3 mL isooctane to this fraction and evaporate to ca. 3 mL. Make up to 5.0 mL or 10 mL in 1+99 methanol/isooctane and mix well.

GLC Analysis

Inject 8 µL (packed column) or 2 µL (capillary column) of standard solution or sample extract onto the column. Inject one standard after every third sample.

RESULTS AND DISCUSSION

GC Separation of Triazines

From the literature available, Carbowax 20M is the most popular and useful stationary phase for the GC analysis of triazines. Our initial attempt on the resolution of the present 11 triazines was made on an Ultrabond 20M packed column. Ultrabond 20M is a packing material prepared by bonding a monomolecular layer of Carbowax 20M to the specially treated diatomaceous earth. This material is then heat treated and exhaustively stripped with solvents to produce a system having 0.2% loading of unextractable liquid phase (26). With the Ultrabond 20M column operating at 170°C, 10 peaks with baseline resolution were observed for the 11 triazines (Fig. 1). Cyprazine and simetryne were not resolved and the retention time for cyanazine was long (>70 min) and the peak was broad under such conditions. No resolution of cyprazine and simetryne could be achieved on the Ultrabond 20M using lower column temperatures. On the other hand, operating the column at temperatures >200°C would significantly reduce the retention time of cyanazine at the expense of reducing resolution of the earlier triazines. Continuous operation of the column at such temperatures caused severe bleeding of column and thus caused rapid deterioration of the NPD collector as well as the detector sensitivity. Therefore, the Ultrabond column was not ~~quite~~ suitable for routine analysis of cyanazine. In order to save time in the

triazine analysis on the Ultrabond 20M column, four consecutive injections could be made before the cyanazine peak from the first injection started to elute. The column should then idle for another 60-70 min before injection of the fifth sample.

When this mixture of 11 triazines was chromatographed on a 3% OV-1 column at 160°C, many triazine peaks were merged (Fig. 2). However, quantitative analysis of cyprazine and simetryne was possible on this column since they are sufficiently well separated from each other and from other triazines. Also, retention time for cyanazine was relatively short. Therefore, all 11 triazines could be successfully quantitated on the combined chromatography on both the Ultrabond 20M and 3% OV-1 columns. It was estimated that the minimum detectable amount of each triazine on the packed columns tested was ca. 25 pg.

Several fused silica capillary columns were also evaluated for the resolution of these 11 triazines. Among the columns tested, the 12 m OV-1, 15 m DB-1701 and 30 m DB-5 columns did not resolve all 11 compounds on initial trials and therefore no further work on these columns was carried out. By far, the polar, 15 m DX-4 column gave the best resolution of the triazines since all 11 peaks were baseline resolved and were evenly spaced in the temperature programmed run (see the retention times in Table 2). However, a high column temperature was again required for the elution of cyanazine. Similar to the Ultrabond 20M packed column, such high column temperatures caused rapid deterioration of the DX-4 column. On the other hand, DX-4 would be the column of choice if cyanazine analysis is not required. In

this case, the third ramp in the temperature program (see Experimental) could be deleted to preserve the useful life of the DX-4 column. Although the 11 triazines were not as well separated by the 30 m DB-1 column as the DX-4 column, 11 resolved peaks were readily observed for reliable identification and quantification of each triazine. This column was also extremely stable under the operating conditions used and was therefore suitable for long-term application.

During the evaluation of various GC columns. it was observed that the responses of the 2-methoxytriazines (i.e. -tones) on the OV-1 and Ultrabond 20M packed columns were erratic between replicate injections. It was later found that the presence of 1 to 3% methanol in the standard solutions or sample extracts was required to give maximum responses of the 2-methoxytriazines on those columns.

Extraction and Cleanup

Methylene chloride was chosen for the extraction of triazines in water samples since the preliminary results using this solvent were quantitative. The same solvent was also proven suitable for the extraction of chlorinated insecticides, polychlorinated biphenyls, chlorobenzenes (unpublished results) and neutral herbicides (27), thus extraction of triazines could be incorporated with the above classes of compounds so that a multi-class multi-residue method could be generated. Extraction of the 11 triazines in waters was also attempted at pH $<$ 1 to evaluate the feasibility of simultaneous

extraction of the acidic herbicides and phenols in the same sample. It was found that the 2-chlorotriazines (i.e. the -zines) were quantitatively recovered under acidic conditions, while the 2-methoxy- and 2-methylthio- triazines were only 7 to 38% and 25 to 85% recovered, respectively. Because of the poor recoveries of the 2-methoxy- and 2-methylthio- triazines under acidic conditions, it was therefore not possible to include them into the multi-residue scheme involving the acidic compounds.

Methylene chloride extracts containing triazines were partitioned with a 2% $KHCO_3$ solution as a feasibility test of a cleanup for removal of acidic coextractives. Unfortunately, recoveries of the three 2-methylthiotriazines were only 70 to 80% after the partitioning, although the recoveries of the other triazines were quantitative. Therefore a base partitioning cleanup or an extraction under basic conditions should not be attempted if the analysis of the 2-methylthiotriazines is also required.

Cleanup of triazine extracts was also tried on a 10% deactivated Florisil column. This specific column was used since successful cleanup of 7 neutral herbicides including atrazine has been demonstrated (27). Because of the relatively high polarity of these triazines, adsorbents of high degrees of deactivation and polar eluants must be used in order to have quantitative elution of these compounds. The use of mixtures of 1 or 2 instead of 3% methanol in benzene for column elution would cause lower recoveries of the 2-methylthiotriazines. Because of the selectivity of the nitrogen-

phosphorus detector, this column cleanup step is only required for the analysis of triazines at lower concentration levels or in very dirty water samples. However, the incorporation of column cleanup would be beneficial in a multi-class procedure since fractionation of other classes of compounds could then be possible.

No losses of triazines were observed during the evaporation of their solutions in organic solvents using a rotary evaporator.

Recoveries and Detection Limit

To determine the method recovery and precision, replicate reagent water samples were fortified to levels of 10, 1.0 and 0.1 µg/L for each of the 11 triazines. They were then extracted with methylene chloride and Florisil cleanup was applied to these extracts with the exception of the 10.0 µg/L samples for which no column cleanup was used. Recoveries of all triazines at all 3 levels of fortification were between 87 and 108% except for simetone and simetryne which were only 80% recovered at 0.1 µg/L (Table 3). Precision among replicate determinations was extremely good as the relative standard deviations were mostly between 2 and 5%.

This method was also validated with a Lake Ontario water sample at the above 3 levels of fortification and with 2 other natural waters at 1 µg/L. Again, only the samples fortified to 0.1 µg/L were subjected to the Florisil column cleanup. Recoveries of triazines

were again quantitative with slightly lower results for simetone at 1.0 or 0.1 µg/L levels (Table 4). Only small amounts of atrazine (0.08 µg/L) and simazine (0.04 µg/L) were found in the Lake Ontario water blank and their recoveries in this water were reported after blank correction.

The lowest validation level for the 11 triazines was 0.1 µg/L in this study. Since the worst case standard deviation was about 0.008 at this level and the student t-value at 99% confidence level was 3.36 for 5 degrees of freedom (6 replicates), a conservative estimate of the method detection limit (28) would be equal to the amount of S.D. x t or about 0.025 µg/L in this particular case.

TABLE 1 Common and chemical names of 11 triazine herbicides

Common Name	Chemical Name
Ametryne	2-Methylthio-4-ethylamino-6-isopropyl-amino-1.3.5-triazine
Atratone	2-Methoxy-4-ethylamino-6-isopropylamino-1,3,5-triazine
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine
Cyanazine	2-Chloro-4-ethylamino-6-(1-cyano-1-methyl-ethylamino)-1,3,5-triazine
Cyprazine	2-Chloro-4-cyclopropylamino-6-isopropyl-amino-1,3,5-triazine
Prometone	2-Methoxy-4,6-bis (isopropylamino)-1,3,5-triazine
Prometryne	2-Methylthio-4,6-bis (isopropylamino)-1,3-5-triazine
Propazine	2-Chloro-4,6-bis (isopropylamino)-1,3,5-triazine
Simazine	2-Chloro-4,6-bis (ethylamino)-1,3,5-triazine
Simetone	2-Methoxy-4,6-bis (ethylamino)-1,3,5-triazine
Simetryne	2-Methylthio-4,6-bis (ethylamino)-1,3,5-triazine

TABLE 2 Retention times (min) of 11 triazines on different columns. See Experimental for other chromatographic conditions

Column	Ultrabond 20M	3% OV-1	DB-1	DX-4
Column Temp (°C)	170°	160°	-	-
Triazine				
Prometone	4.20	4.55[2]	12.74	10.04
Atratone	5.12	4.35[3]	12.46	11.06
Propazine	5.62	4.55[2]	13.10	11.51
Simetone	6.28	4.15[4]	12.15	12.19
Atrazine	7.34	4.35[3]	12.87	13.04
Prometryne	8.86	8.79	17.22	13.76
Simazine	9.62	4.15[4]	12.59	14.77
Ametryne	11.19	8.38	16.92	15.43
Cyprazine	14.06[1]	7.17	15.79	16.93
Simetryne	14.06[1]	7.94	16.55	17.30
Cyanazine	>70	11.26	18.56	27.37

[1,2,3,4] Unresolved pairs.

TABLE 3 **Mean % recovery and standard deviation (in parentheses) of triazines from 1L fortified reagent water samples at pH 6 (n=6)**

	Fortification Level (µg/L)		
	10	1.0	0.1
Triazine			
Prometone	103(3.4)	96(1.7)	95(3.8)
Atratone	102(4.1)	96(2.4)	87(5.0)
Propazine	106(3.2)	100(2.4)	99(3.3)
Simetone	91(6.1)	95(6.0)	80(5.0)
Atrazine	103(5.0)	101(1.5)	108(8.1)
Prometryne	93(3.4)	97(1.9)	101(1.8)
Simazine	105(4.6)	97(2.4)	103(6.4)
Ametryne	90(4.5)	96(1.5)	94(3.4)
Cyprazine	102(4.2)	99(3.1)	96(3.4)
Simetryne	91(4.7)	97(3.5)	79(2.7)
Cyanazine	102(3.3)	97(3.6)	100(6.4)

TABLE 4 Mean % recovery and standard deviation (in parentheses) of triazines from 1L fortified natural water samples

	Lake Ontario				Saskatchewan	New Brunswick
Concentration, µg/L	10	1.0	0.1	1.0	1.0	1.0
pH	6	6	6	1	6	6
No. of Replicates	6	6	6	3	4	4
Triazine						
Prometone	102(3.6)	96 (1.0)	89 (3.6)	38	99	100
Atratone	101(3.3)	94 (1.3)	89 (6.9)	17	95	96
Propazine	104(3.2)	98 (1.8)	94 (3.1)	98	100	99
Simetone	98(4.9)	83 (1.7)	75 (6.6)	7	99	88
Atrazine	106(3.4)	99*(2.6)	103*(5.8)	98*	96	98
Prometryne	98(2.4)	94 (2.1)	101 (2.4)	85	100	94
Simazine	101(1.5)	95*(2.8)	90*(2.4)	91*	95	92
Ametryne	97(2.6)	102 (4.6)	97 (5.1)	67	93	94
Cyprazine	96(2.0)	96 (2.6)	91 (6.0)	96	100	95
Simetryne	95(1.7)	93 (2.9)	91 (4.6)	25	87	91
Cyanazine	97(2.9)	93 (2.6)	102 (5.9)	95	105	97

*Results corrected for blanks.

FIGURES

Figure 1 Chromatogram of the 11 triazine standards (ca. 1 ng of each injected) as chromatographed on the Ultrabond 20M packed column at 170°C. 1, prometone; 2, atratone; 3, propazine; 4, simetone; 5, atrazine; 6, prometryne; 7, simazine; 8, ametryne; 9, cyprazine; 10, simetryne; 11, cyanazine. Note that cyanazine (#11) was not shown here because of its long RT.

Figure 2 Chromatogram of the 11 triazine standards (ca. 1 ng of each injected) as chromatographed on the 3% OV-1 packed column at 160°C. See Figure 1 for peak identity.

Figure 3 Resolution of the triazine standards on a 30 m DB-1 fused silica capillary column (ca. 1 ng of each injected). See Figure 1 for peak identity.

Figure 4 A typical natural water sample extract as chromatographed on the Ultrabond 20M column. This sample was fortified to 1 µg/L for each triazine.

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Fig. 1

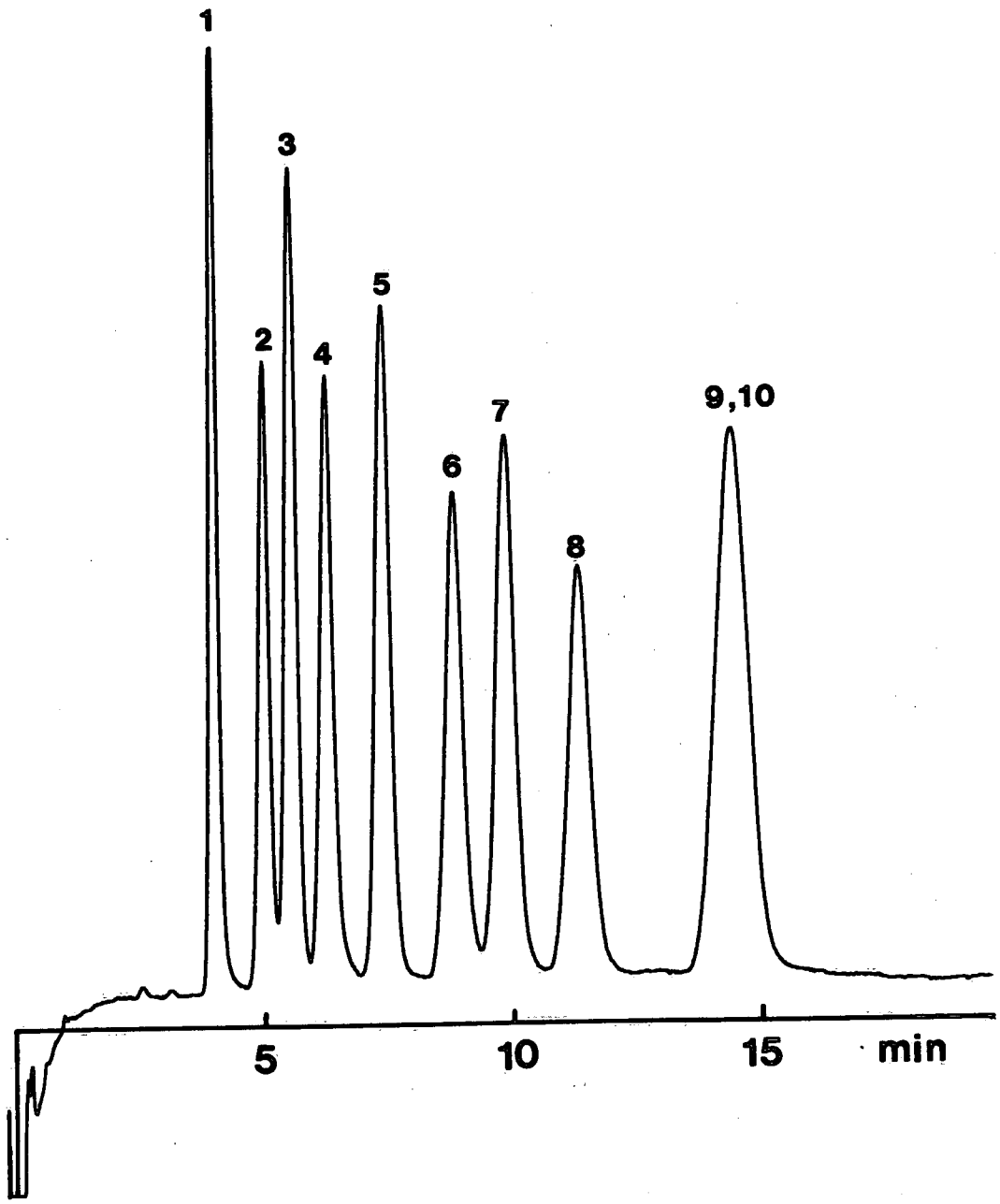
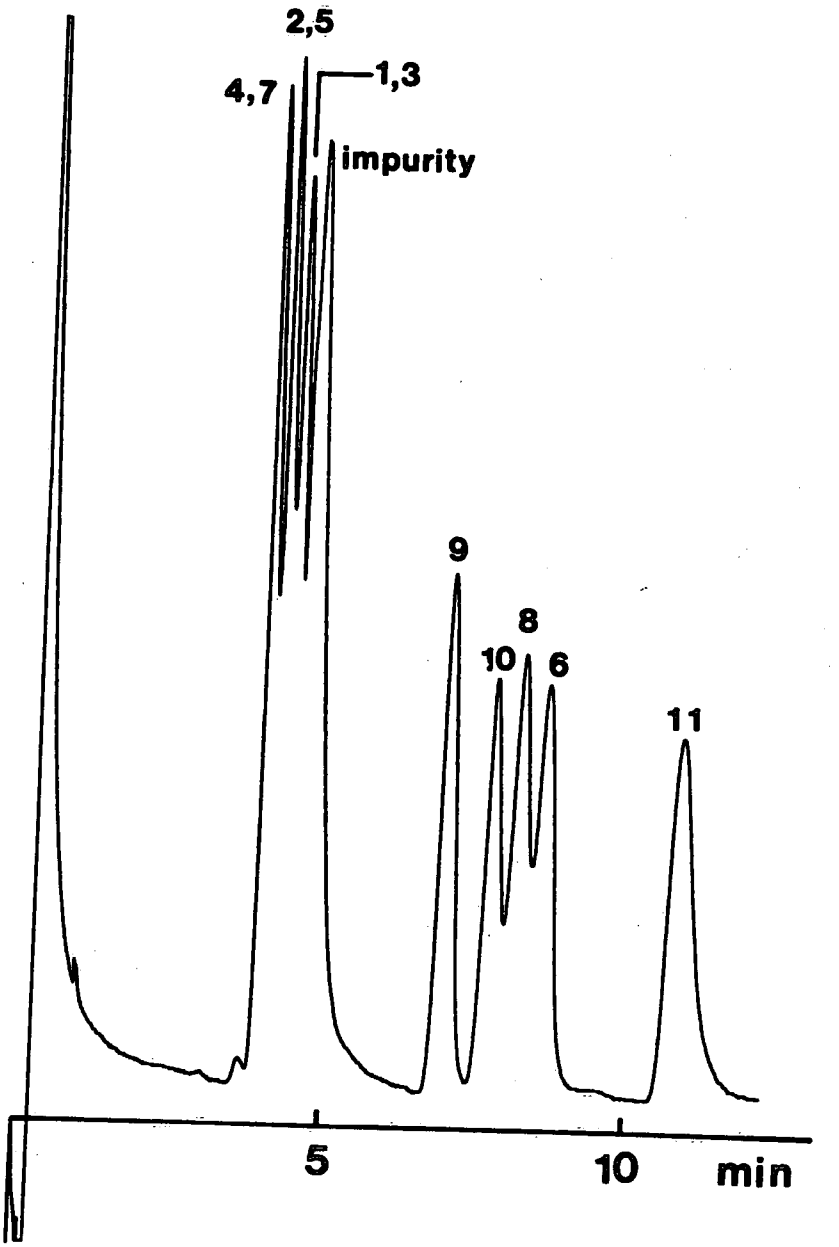


Fig. 2



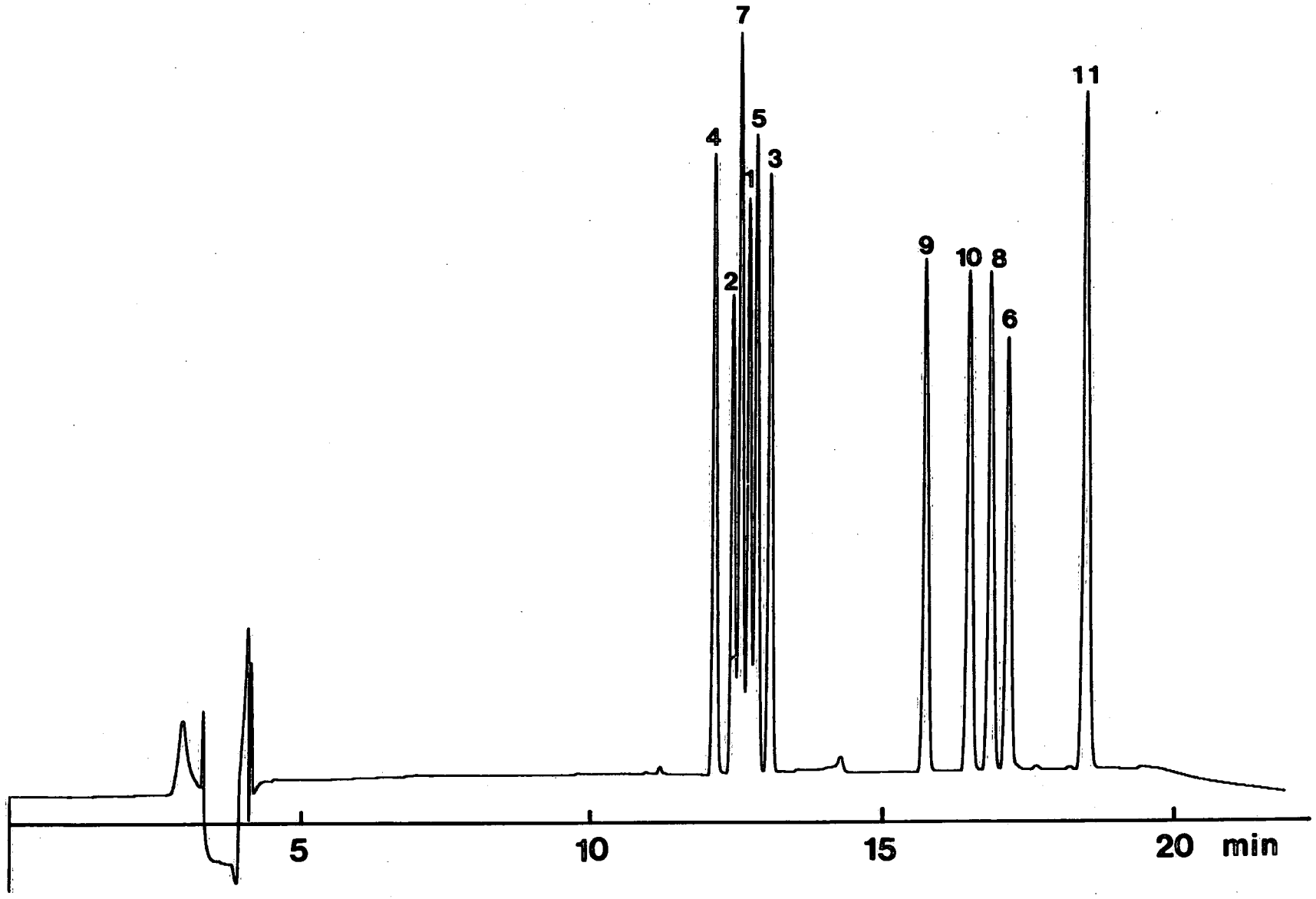

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Fig. 4