Reprint from

RECENT ADVANCES
IN DOPING ANALYSIS
(3)

M. Donike
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1996

E. Nolteernsting, G. Opfermann and M. Donike:
1-(N,N-Diisopropylamino)-n-Alkanes: A New Reference System for Systematical
Identification of Nitrogen Containing Substances by Gas-Chromatography and Nitrogen
Specific or Mass Spectrometrical Detection
In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping
analysis (3). Sport und Buch Strauß, Köln, (1996) 369-388

E. Nolteernsting, G. Opfermann and M. Donike

1-(N,N-Diisopropylamino)-n-Alkanes: A New Reference System for Systematical Identification of Nitrogen Containing Substances by Gas-Chromatography and Nitrogen Specific or Mass Spectrometrical Detection

Institut für Biochemie der Deutschen Sporthochschule, Köln, Germany

This lecture was held during the Symposium "Progress in Dope Analysis"
(May 21, 1981), the first Cologne Workshop, initiated by Manfred Donike

Summary

Using an element specific detector like the nitrogen phosphorus specific thermionic detector (NPD) the gas chromatographic determination of retention indices is not possible using n-alkanes as a reference system since the ionisation mechanism of this type of detector suppresses the signals of hydrocarbons. With the goal to find an alternative nitrogen containing reference system 11 homologous series with tertiary amine, secondary and tertiary amide, N-alkyl-sulfonamide and nitrile functions were synthesized and their gas-chromatographic properties investigated. The 1-(N,N-diisopropylamino)-n-alkanes (DIPA-alkanes) proved to be superior to other classes. An additional advantage is that under electron impact ionisation the DIPA-alkanes produce a base peak in high intensity with m/z of 114, so this system can also be used to calibrate the GC-MS-systems. With this reference system in GC screening procedures using a NPD, nitrogen positive peaks may be localised in a gas chromatographic mass spectrometric system.

1.   Introduction

Many nitrogen containing compounds such as amines, amides and N-heterocycles are of biochemical, physiological, pharmacological and toxicological interest. A wide variety of these nitrogen containing substances are volatile enough to be analysed without derivatisation by gas-chromatography using a nitrogen specific detector. From alkaline solutions they can be isolated by a single solvent extraction step. This method first published in 1970 [1] is still the method of choice for the detection of the typical dope agents like the amphetamines and ephedrines.

A correct and reliable identification by gas-chromatographic means can be performed if (1) columns of sufficiently different polarity are used and (2) a retention parameter can be reproducibly measured. Preferable to retention times and relative retention times is the determination of retention indices correlated to reference substances. As a reference system to calculate retention indices for isothermal gas-chromatography Kovats proposed n-alkanes as early as 1958 [2]. Van den Dool and Kratz [3] applied n-alkanes as reference system for temperature programmed gas-chromatography. Retention indices are widely used and several papers have been published on this subject [4,5,6,7,8].

Former types of nitrogen selective detectors (alkali salts modified flame ionization detector) could be tuned in such a way that n-alkanes appeared as symmetrical peaks. Retention indices of nitrogen containing stimulants have been calculated using this type of detector [7].

The modern type of thermionic nitrogen phosphorus detectors (NPD), consisting of an electrically heated rubidium salt bead, do not detect n-alkanes, which fail to show up at all or appear as a slight positive and/or negative deviation of the baseline. If under such conditions a retention time is measured it will most probably not be the correct one as the true maximum of the peak cannot be recognized by the integrator or reconstructed manually. So retention time measurements of n-alkanes with a modern NPD and in consequence retention index calculations may be substantially incorrect.

Therefore it seemed logical and obvious to search for a homologous series of nitrogen containing reference substances to substitute the n-alkanes and to be able to continue identification of nitrogen containing substances by retention index measurements.

2. Experimental

2.1 Determination of tailing factors and Kovats retention indices of substances tested as reference system

GC: Hewlett Packard 5880
Column: glass, 0.9 m, 2 mm i.d., 2 % OV-101 on chromosorb
W-AW 80/100 mesh
Detector: NPD
Temperatures:
a) Oven (isothermal): 160°C
b) Injector: 280°C
c) Detector: 300°C

Column flow: 20 ml He / min.

Amount injected: 200 ng

2.2 Ratio between the logarithm of the net retention time and the carbon number of DIPA-alkanes

GC: Hewlett Packard 5880

Column: glasss, 0.9 m, 2 mm i.d., 2 % OV-101 on chromosorb W-AW 80/100 mesh

Detector: FID

Temperatures:

a) Oven (isothermal): 50, 100, 160, 220°C

b) Injector: 280°C

c) Detector: 300°C

Column flow: 20 ml He / min.

Amount injected: 1 μg

2.3 GC-conditions for the determination of Kovats retention indices of DIPA-alkanes and of nitrogen containing compounds and the determination of DIPA-retention indices of nitrogen containing compounds

GC: Hewlett Packard 5880

Column: glass, 0.9 m, 2 mm i.d., 2 % OV-101 on chromosorb W-AW 80/100 mesh

Detector: FID

Temperatures:

a) Oven: 60, 70, 110, 120, 140, 150, 170, 180, 210, 220, 230, 240°C

b) Injector: 280°C

c) Detector: 300°C

Column flow: 20 ml He / min.

Amount injected: 1 μg

All retention indices are the mean values of three determinations.

The dead volume time has been calculated from the retention times of three n-alkanes.

2.4 Determination of retention indices by temperature programmed GC

GC: Hewlett Packard 5880

Column: glass, 0.9 m, 2 mm i.d., 2 % OV-101 on chromosorb
W-AW 80/100 mesh

Detector: FID

Temperatures:

        a) Oven: 0 min., 80°C, 15 °C/min., 300°C

        b) Injector: 280°C

        c) Detector: 300°C

Column flow: 20 ml He / min.

Amount injected: 1 μg

The retention indices are average values from three determinations.

2.5 Mass spectra

GC-MS: Hewlett Packard 5995B

Column: 25 m fused-silica capillary crosslinked methyl silicone,
0.2 mm i.d., 0.11 μm film thickness

Temperatures:

        a) Oven: 0 min., 80°C, 15 °C/min., 280°C

        b) Injector: 280°C

        c) Transfer line: 300°C

        d) Ion source: 200°C

Ionisation: electron impact, 70 eV

Column flow: 1 ml He/min.

Split ratio: 1:10

3. Results and Discussion

3.1 Choice of the most suitable homologous series

The substances of the new reference system should meet the following requirements:

1. availability, either commercially or by simple synthetic methods
2. thermal stability
3. symmetric peak shape
4. stability in alkaline medium - necessary for alkaline extractions and alkaline modified columns
5. characteristic mass fragmentation pattern.

We tested 11 homologous series for their suitability as reference systems with the following nitrogen containing functional groups: tertiary amines, secondary and tertiary amides of carboxylic acids, N-alkyl-sulfonamides and nitriles (table 1).

All classes of substances have been synthesized from commercially available products. Nearly all proved to be stable under conditions normally used in gas-chromatography (helium or hydrogen as carrier gas, injector and oven temperature up to 300°C, detector or GC-MS interface temperature 300°C).

In preliminary experiments the tested homologous series showed large differences in peak symmetry. As symmetric peak shape is a prerequisit for reference compounds we determined the tailing factor at 10 % peak height (figure 1) on a packed glass column with a dimethylsilicone phase (OV-101) on chromosorb W. This type of column was chosen to evaluate peaksymmetry because most of the hitherto published data of substances of pharmaceutical and toxicological interest were measured using packed columns and a liquid phase of the dimethylsilicone type like SE 30, OV-1 or OV-101 [4,5,6,8].

To exclude effects which may be attributed to column overloading only 200 ng of each substance were injected. The tailing factors of selected members of the tested homologous series are summarized in table 1 for comparison. The homologues were selected by the criteria of similar retention times or similar Kovats retention indices. The most symmetric peaks were obtained for the classes of 1-(N,N-diisopropylamino)-n-alkanes and N-n-alkyl-heptafluoro-butyramides both with a tailing factor of 1.2, followed by the N-n-alkyl-trifluoroacetamides and the 1-(N,N-diethylamino)-n-alkanes with tailing factors of 1.8 or 2.1, respectively.

In spite of their symmetrical peak shape the series of the N-n-alkyl-heptafluorobutyramides and the N-n-alkyltrifluoro-acetamides are not suited as they proved to be very unstable

under alkaline conditions which may occur at the stage of the sample preparation when an alkaline extraction is performed or at the stage of gas-chromatography when alkali modified packed columns are used.

The homologous series of the 1-(N,N-diisopropylamino)-n-alkanes (DIPA-alkanes) can readily be prepared from diisopropylamine and 1-bromo-n-alkanes [9]. Compared to the also easily available 1-(N,N-diethylamino)-n-alkanes the DIPA-alkanes tail less (1.2 versus 2.1) and have the additional advantage of a more favourable fragmentation pattern in a mass spectrometer. The well known α-cleavage of the DIPA-alkanes leads to the base peak m/z 114 (figure 2), an ion which is less often observed as base peak in substances of biological, pharmaceutical and toxicological interest than the ion m/z 86, the base peak of the 1-(N,N-diethylamino)-n-alkanes.

At that stage of the investigation we decided to introduce the 1-(N,N-diisopropylamino)-n-alkanes as reference compounds and to synthesize the homologues of this series. DIPA stands as abbreviation for the functional group N,N-diisopropylamino.

3.2 Gas-chromatographic properties of DIPA-alkanes on methylsilicone phases (OV-101)

The relationship between the logarithm of the net retention time and the carbon number is linear except for the lower members of the series (fig. 3). In this aspect the DIPA-alkanes follow other homologous series. The relative strong deviation from linearity between DIPA-methane and DIPA-butane is negligible for practical use because nearly all nitrogen containing substances of interest are eluted later than DIPA-butane (Kovats index 990 units) [4,5,6,7,8].

The deviation from linearity for the higher homologues can best be demonstrated by calculating the Kovats-index increments both of the DIPA group (dI_{DIPA}) and the additional methylene group (dI_{CH2}). But at a first glance the Kovats retention indices of the higher members seem to deviate from the expected ideal properties. A thorough analysis of the data shows that this deviation is caused by the temperature increment of about 1.3 units per 10°C. After calculating the indices for a standard temperature of 150°C, the temperature dependency can be eliminated (table 2). For the lower homologues the increment for the DIPA group (table 2) is greater, e.g. 610 for DIPA-n-propane, than the increment for the higher homologues and approaches a constant value of 579 for the DIPA-n-decane. Corresponding to the decrease of the increment for the DIPA group with

growing chain length the increment for the methylene group increases from 90.5 for the pair DIPA-n-propane/ DIPA-n-butane to the theoretical value of 100 for DIPA-n-dodecane/ DIPA-n-tridecane.

3.3 Practical use

In order to test if DIPA-alkanes are suited as a common reference system, the Kovats- and the DIPA-retention indices of nitrogen containing substances differing widely in chemical structure (e.g. amines, amides and heterocyclic systems) were determined. DIPA-alkanes were used starting with DIPA-pentane up to DIPA-docosane and the n-alkanes from undecane till octacosane. The indices measured under the same chromatographic conditions are listed in table 3. Kovats- and analogously calculated DIPA-retention indices (formula 1) differ from 579 to 590 units. Thus both are interchangeable with an accuracy of +/- 6 units by adding or subtracting 585 units. This accuracy is acceptable in comparison to the inter laboratory variation of retention indices which is reported to be 50 [5]. Further the value of 585 is in excellent agreement with the values calculated for the DIPA increments (table 2).

In normal screening analyses often temperature programmed gas-chromatography is used. Table 4 enumerates retention indices calculated from temperature programmed runs which are calculated using first n-alkanes as reference system and second DIPA-alkanes as reference system (formula 2). The increment between both indices varies from 579 to 590 units for the different substances. This accuracy is once more acceptable in comparison to the inter laboratory variation of index determinations.

These experiments provide the evidence that both types of retention indices are exchangeable and can be used with the same level of accuracy. Literature values based on n-alkanes can be used for identification in isothermal as well as in temperature programmed gas-chromatography with nitrogen specific detection by substracting the increment of the DIPA-group. Alternatively DIPA-indices can be converted to Kovats-indices by adding the increment of 585.

The identification of a substance by GC-MS - detected in a screening procedure by a nitrogen specific detector - is also facilitated using retention indices. Due to the very intense base peak m/z 114 of the DIPA-alkanes less than 1 ng per compound and injection is sufficient to calibrate the GC-MS-system and to determine the region were the nitrogen specific detected substance is to be expected. Columns with the same polarity of the liquid phase should be used in both systems to compare the chromatograms. In figure 4 NPD chromatograms are reproduced a) without and b) with added DIPA-alkanes (1 ng each).

Figure 4d demonstrates the calibration of the GC-MS instrument using the reconstructed ion trace of m/z 114. The added amount of DIPA-alkanes will not interfere with the concentrations of dope agents as the total ion chromatogram (figure 4c) proves.

This procedure is very advantageous due to the fact that many non-nitrogen containing substances may interfere in samples of biological origin and the total ion trace is often crowded by non-nitrogenous compounds.

The conclusion is that the series of 1-(N,N-diisopropylamino)-n-alkanes is very helpful for identification of substances by gas-chromatography using nitrogen phosphorus detectors and mass specific detectors.

References

1. Donike, M., Jaenicke, L., Stratmann, D., Hollmann, W., J.Chrom., 52 (1970) 237.
2. Kovats, E., Helv.Chim.Acta, 41 (1958) 1915.
3. van den Dool, H., Kratz, D., J.Chrom., 11 (1963) 463.
4. Anderson, W.H., Stafford, D.T., J.High Res.Chrom.&Chrom.Comm., 6 (1983) 247.
5. Ardey, R.E., Moffat, A.C., J.Chrom., 220 (1981) 195.
6. Berninger, H., Möller, M.R., Arch.Toxicol., 37 (1977) 295.
7. Donike, M., Stratmann, D., Z.Anal.Chem., 270 (1976) 129.
8. Perrigo, B.J., Peel, H.W., J.Chrom.Sci., 19 (1981) 219.
9. Nolteernsting, E., Thesis, Cologne 1984.

Table 1: Tailing factors of 11 homologous series of nitrogen containing
homologous series

Substance	nC(R)	RT(min)	Index	BP m/z	Tailing factor	mean
1-(N,N-diethylamino)-n-alkanes	13	2.14	1755	86	1.9	
	14	3.26	1856	86	2.0	2.1
	16	7.63	2056	86	2.5	
1-(N,N-diisopropylamino)-n-alkanes	13	3.69	1885	114	1.2	
	15	8.66	2085	114	1.2	1.2
	17	20.41	2285	114	1.3	
1-(N,N-dimethylamino)-n-alkanes	14	1.87	1721	58	6.6	
	16	4.37	1925	58	4.5	5.6
N-(n-alkyl)-heptafluoro-butyramides	14	3.63	1881	43	1.2	
	16	8.47	2080	43	1.2	1.2
	18	19.84	2278	43	1.2	
N-(n-alkyl)-pivaloyl-amides	11	5.21	1966	57	3.3	
	12	7.97	2066	57	2.8	3.1
	13	12.27	2166	57	3.4	
N-(n-alkyl)-trifluoro-acetamides	14	3.16	1848	43	1.7	
	16	7.42	2049	43	1.8	1.8
	18	17.49	2249	43	1.8	
N,N-diethyl-carbonamides	9	1.98	1736	115	8.1	
	11	4.62	1938	115	9.8	8.8
	13	10.99	2141	115	8.5	
N,N-diisopropyl-carbon-amides	9	2.51	1793	86	2.7	
	11	5.82	1992	86	3.1	3.0
	13	13.70	2192	86	3.3	
N,N-dimethyl-carbonamides	11	3.00	1836	87	15.9	
	13	7.15	2040	87	22.4	18.3
	15	16.85	2240	87	16.6	
N-(n-alkyl)-N-methyl-methansulfonamides	10	3.47	1870	122	4.3	
	12	8.14	2071	122	4.5	4.3
	13	12.53	2171	122	4.1	
1-cyano-n-alkanes	14	2.41	1784	41	3.4	
	16	5.66	1986	41	3.9	3.8
	18	13.43	2188	41	4.0	

nC(R)	Carbon number of DIPA-alkane's n-alkyl chain
RT(min)	Retention time in minutes
Index	Kovats-Index: I_{DIPA} (formula 1) + 585
BP m/z	Base peak of the mass spectrum
Tailing factor	Tailing factor of the substance and the mean of the tailing factors of the measured homologues.

377

Table 2: Kovats retention indices of DIPA-alkanes, temperature dependency and increments for the methylene and DIPA group

nC(R)	$t_{(°C)}$	I_t	$dI_{10°C}$	$I_{150°C}$	dI_{CH2}	dI_{DIPA}
3	70	900.7	+1.0	910.1	90.5	610.1
4	70	990.2	+1.1	1000.6	92.3	600.6
5	70	1082.5	+1.1	1092.9	95.6	592.9
6	70	1178.1	+1.0	1188.5	96.2	588.5
7	110	1279.5	+1.2	1284.7	97.8	584.7
8	110	1377.3	+1.2	1382.5	98.8	582.5
9	110	1476.1	+1.2	1481.3	98.4	581.3
10	140	1578.4	+1.2	1579.7	99.3	579.7
11	140	1677.7	+1.3	1679.0	99.3	579.0
12	180	1782.0	+1.2	1778.1	99.9	578.1
13	180	1881.9	+1.3	1878.0	100.0	578.0
14	180	1981.9	+1.3	1978.0	99.9	578.0
15	180	2081.8	+1.4	2077.9	100.1	577.9
16	210	2185.8	+1.4	2178.0	100.0	578.0
17	210	2285.8	+1.4	2278.0	100.3	578.0
18	210	2386.1	+1.3	2378.3	100.15	578.3
20	240	2590.3	+1.3	2578.6	100.15	578.6
22	240	2790.6	+1.5	2778.9		577.9

nC(R)   Carbon number of DIPA-alkane's n-alkyl chain

I_t Kovats-Index at temperature t

$dI_{10°C}$ Increase of Kovats-Index with an increase of 10°C oven temperature

$I_{150°C}$ Corrected Kovats-Index for oven temperature 150°C using an average temperature increment of $dI_{10°C}$ = + 1.3

$$I_{150°C} = I_t + (1.3 * 150 - t)$$

dI_{CH2} Increment for the methylene group calculated from $I_{150°C}$

dI_{DIPA} Increment for the DIPA group

$(I_{150°C} - DIPA-Index \ nC(R) * 100)$

Table 3: Kovats- and DIPA-Retention-Indices on OV-101

Substance	$t(°C)$	I_{alkane}	I_{DIPA}	dI_{DIPA}	$I_{calc.}$
Amphetamine	110	1129	540	589	1125
Methylamphetamine	110	1177	593	584	1178
Dimethylamphetamine	110	1238	657	581	1242
Nicotine	120	1326	746	580	1331
Ephedrine	120	1349	770	579	1355
Caffeine	160	1772	1192	580	1777
Phenylbutazone	220	2352	1766	586	2351
Diazepam	220	2410	1824	586	2409
Chlorpromazine	240	2504	1914	590	2499
Acepromazine	240	2693	2103	590	2688

$t(°C)$	Oven temperature
I_{alkane}	Kovats-Retention-Index
I_{DIPA}	DIPA-Retention-Index
dI_{DIPA}	Difference between I_{alkane} and I_{DIPA} = increment for the DIPA group
$I_{calc.}$	Calculated Kovats-Retention-Index by addition of 585 to the DIPA-Retention-Index

Table 4: Retention-Indices by temperature programmed
gas-chromatography (80°C to 300°C by 15°C / min)

Substance	I_{alkane}	I_{DIPA}	dI_{DIPA}	$I_{calc.}$
Amphetamine	1148	561	587	1146
Methylamphetamine	1179	597	582	1182
Dimethylamphetamine	1236	655	581	1240
Nicotine	1326	747	579	1332
Ephedrine	1367	788	579	1373
Caffeine	1784	1203	581	1788
Phenylbutazone	2358	1771	587	2356
Diazepam	2422	1835	587	2420
Chlorpromazine	2495	1908	587	2493
Acepromazine	2710	2120	590	2705

I_{alkane} Retention-Index

I_{DIPA} DIPA-Retention-Index

dI_{DIPA} Difference between I_{alkane} and I_{DIPA} = increment for
the DIPA group

$I_{calc.}$ Calculated Retention-Index by addition of
585 to the DIPA-Retention-Index

Formulas

(1) $\quad I_{DIPA} = 100\ z\ +\ 100\ \dfrac{\log t_x\ -\ \log t_z}{\log t_{z+1}\ -\ \log t_z}$

(2) $\quad I_{DIPA} = 100\ z\ +\ 100\ \dfrac{t_x\ -\ t_z}{t_{z+1}\ -\ t_z}$

I_{DIPA} DIPA-retention index of the substance x

z   Carbon number of n-alkyl chain of DIPA-alkane eluted before substance x

t_x Net retention time of the substance x

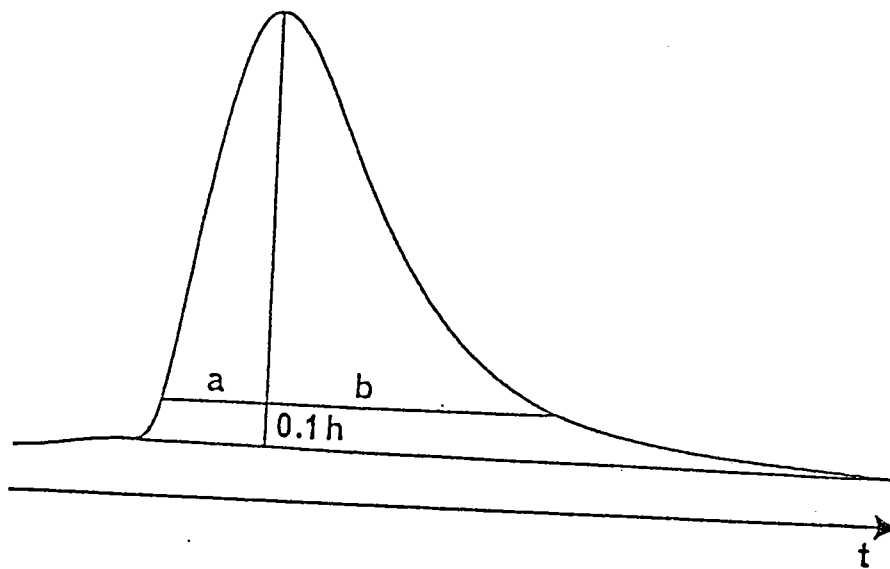t_z Net retention time of the DIPA-alkane with the carbon number z in the n-alkyl chain, eluted before the substance x

t_{z+1} Net retention time of the DIPA-alkane with the carbon number z+1 in the n-alkyl chain, eluted after the substance x

Figure 1: Determination of Tailing Factors
t: retention time
0.1 h 10% of the total peak height
a: distance before the peak maximum measured at 10% of the total peak height
b: distance after the peak maximum measured at 10% of the total peak height
Tailing factor = $b \times a^{-1}$
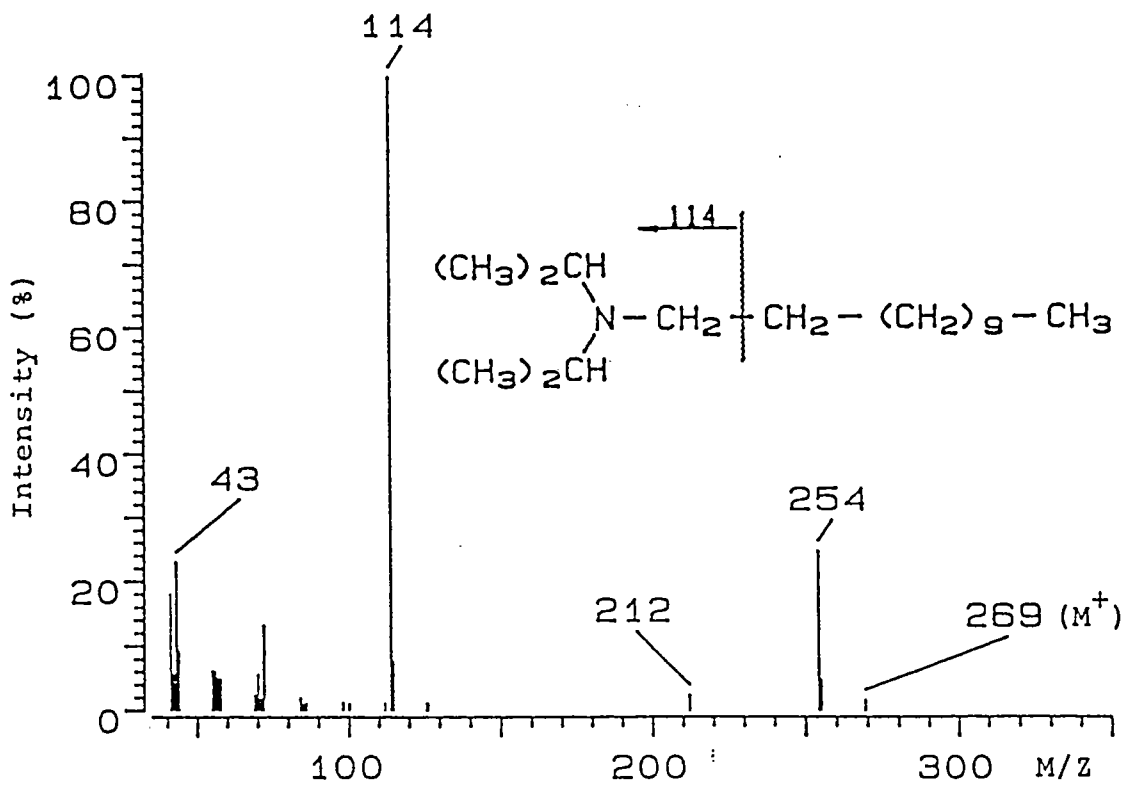
Figure 2: Mass spectrum of 1-(N,N-Diisopropyl)-dodecylamine
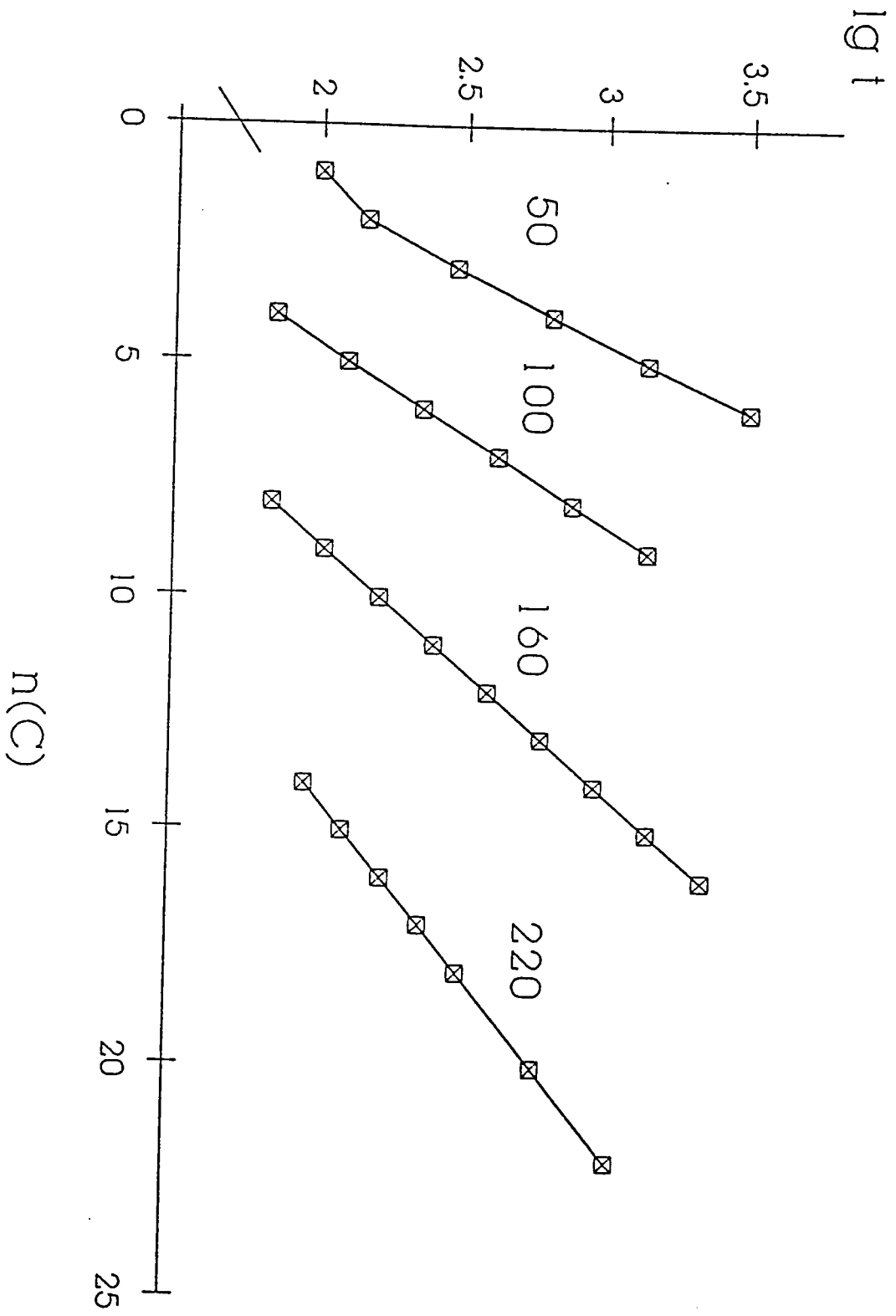
Figure 3:
lg: logarithm of the net retention time in seconds
n(C): carbon number of the alkyl chain of the DIPA-alkane 50,
100, 160, 220 oven
temperatures in centigrades

Figure 4:
GC: Hewlett Packard 5880
Column: 17 m fused-silica capillary crosslinked SE-54,
0.2 mm i.d., 0.33 μm film thickness
Temperatures:
    a) Oven: 0 min. 80°C, 20°C/min., 280°C
    b) Injector: 280°C
Column flow: 1 ml He/min.
Split ratio: 1:10

1 DIPA-butane	9 DIPA-decane
2 DIPA-pentane	10 DIPA-dodecane
3 DIPA-hexane	11 Caffeine
4 Methylamphetamine	12 DIPA-tridecane
5 Dimethylamphetamine	13 DIPA-tetradecane
6 DIPA-heptane	14 DIPA-pentadecane
7 DIPA-octane	15 DIPA-hexadecane
8 DIPA-nonane	16 DIPA-heptadecane
	17 DIPA-octadecane
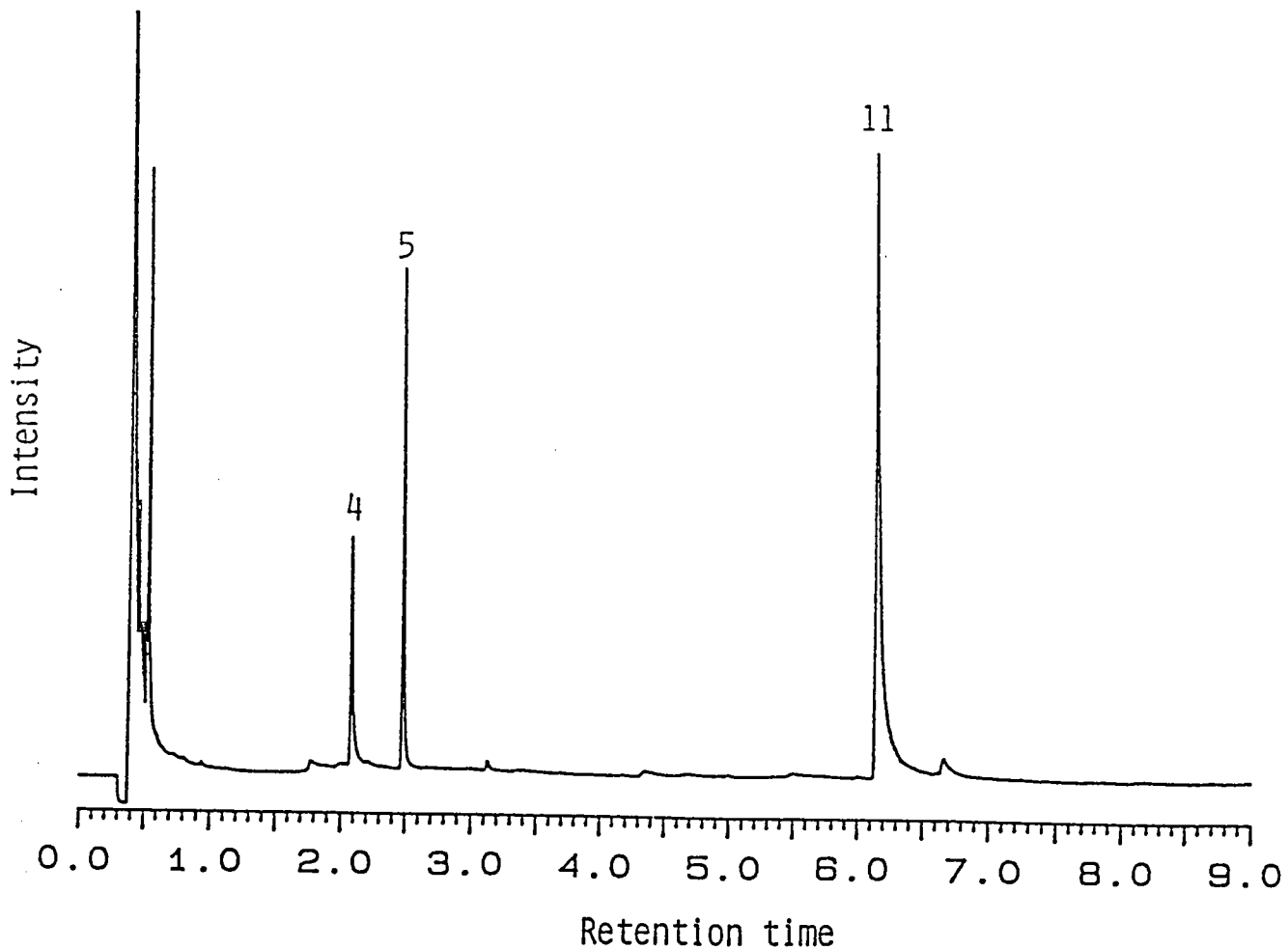
Figure 4a: Gas-chromatogram (NPD) of an urine extract containing methylamphetamine, dimethylamphetamine and caffeine
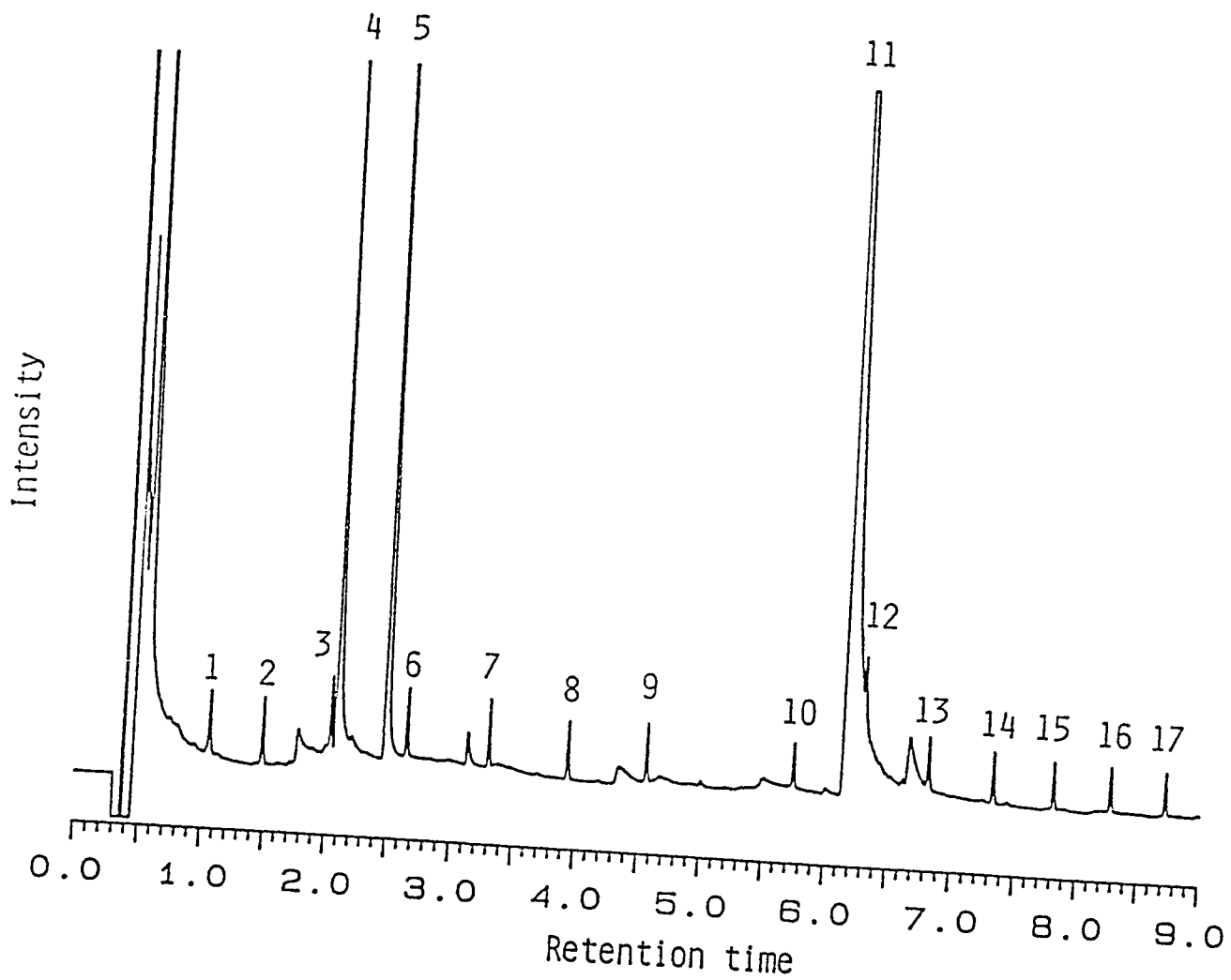
Figure 4b: Gas-chromatogram (NPD) of a co-injection of the extract used in 4a and a mixture of DIPA-alkanes (1 ng each)
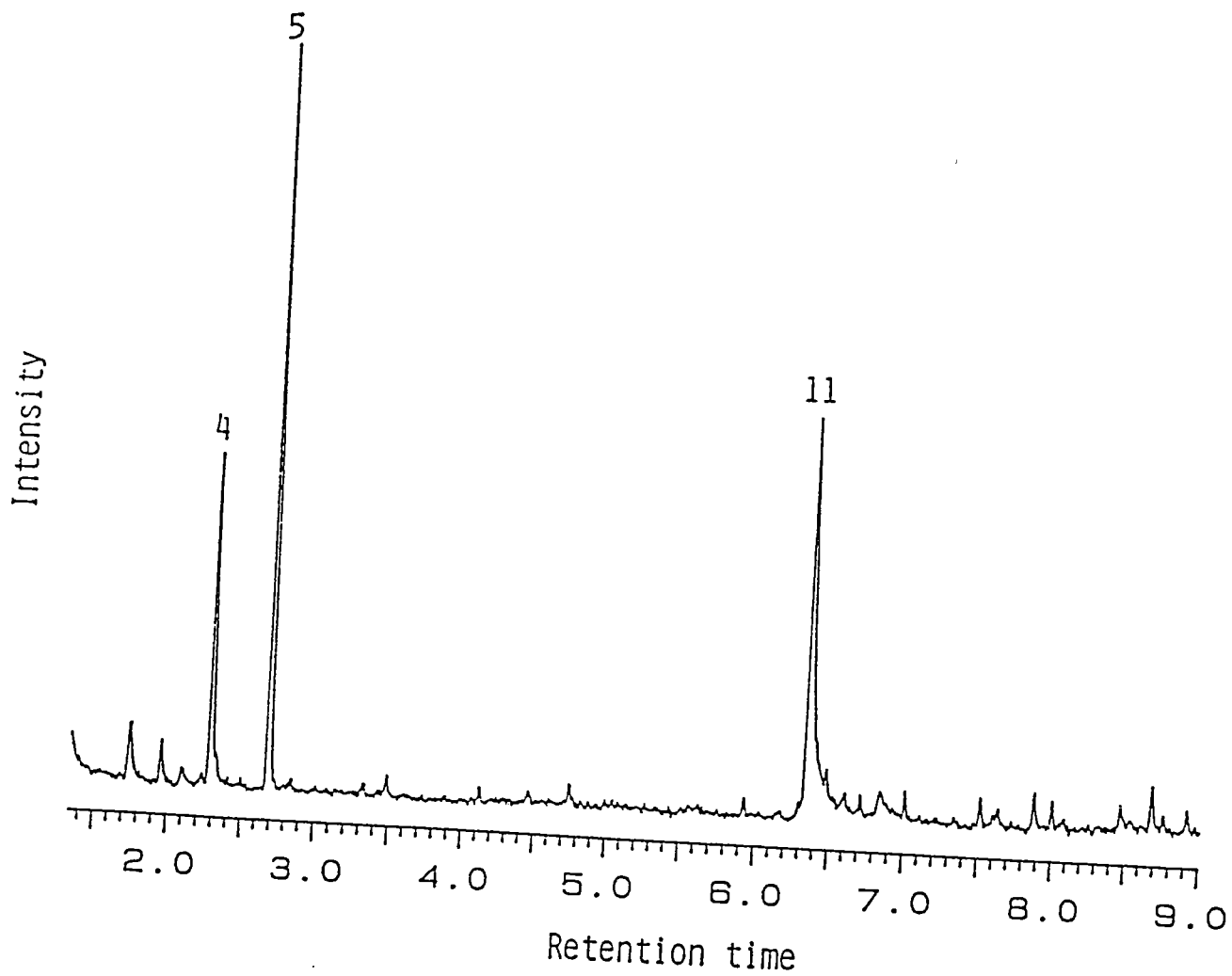
Figure 4c: Total ion chromatogram (MSD: Hewlett Packard 5970) of the mixture used in 4b
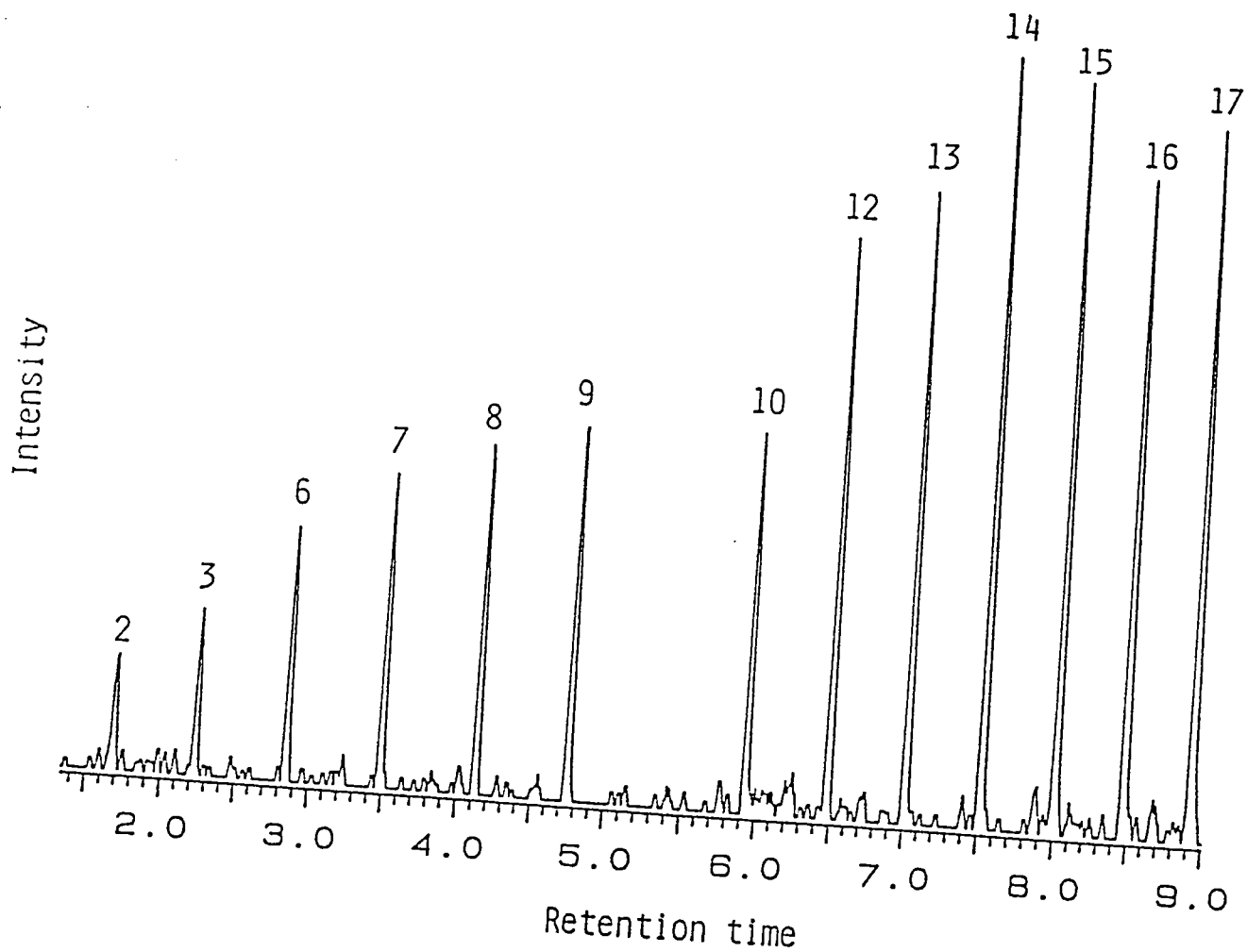
Figure 4d: Single ion chromatogram (m/z 114) extracted from the total ion chromatogram 4c.