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Determination of aromatic amines at trace levels by derivatization with heptafluorobutyric anhydride and gas chromatography–electron-capture negative-ion chemical ionization mass spectrometry

Marco Longo*, Aldo Cavallaro

Presidio Multizonale di Igiene e Prevenzione, Unità Operativa Chimica, Via Juvara 22, 20129 Milan, Italy

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Abstract

A procedure for the simultaneous identification of seventy-three primary and secondary aromatic amines (including alkyl-, chloro- and nitro-substituted anilines, benzidines, aminonaphtalenes and aminobiphenyls) is described. The amines were derivatized by reaction with heptafluorobutyric anhydride to form the corresponding heptafluorobutyramides. The electrophoric derivatives were analyzed by gas chromatography combined with electron-capture negative-ion chemical ionization mass spectrometry. Linearity was satisfactory for all the compounds examined. Detection limits were in the range 0.3–66.3 pg injected in the full-scan acquisition mode and 0.01–0.57 pg injected in the selected ion monitoring acquisition mode. Application of the procedure to contaminated groundwater samples was also attempted.

Keywords: Derivatization, GC; Detection, GC; Amines

1. Introduction

Aromatic amines are widely used in many industrial processes including azo dyes, pesticides and pharmaceuticals manufacturing. The high toxicity and carcinogenic potential of some of these compounds are well established [1–4], hence a careful and sensitive monitoring of their presence in the environment, workplace and products is a matter of great interest.

A number of analytical procedures have been proposed for the determination of low levels of aromatic amines in various matrices, e.g. environmental samples, cosmetics, biological fluids, etc. Some of these are based on the determination of underivatized amines by either gas chromatography (GC) [5,6] or high-performance liquid chromatography [5,7–10]. In general these methods allow the determination of selected aromatic amines at ppb levels but are not selective enough for the simultaneous identification of a wide series of compounds. Furthermore, the direct GC determination of some underivatized aromatic amines, particularly nitro-substituted anilines, requires optimal deactivation of the GC system and careful choice of experimental conditions to obtain satisfactory peak shape and resolution.

Recently, a capillary zone electrophoresis method was proposed for the simultaneous determination of twenty-one underivatized aromatic amines in ground-

*Corresponding author.

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Table 1
Retention time, instrument limit of detection and characteristic ions under EC-NICI conditions of aromatic amine HFBA derivatives

No.	Parent amine	t_R (min)	LOD [a] (pg)	M (m/z) [b]	$[M-HF]^-$ (m/z) [b]	$[M-H_2F_2]^-$ (m/z) [b]	$[M-HCl]^-$ (m/z) [b]	Other ions (m/z) [b]
1	Aniline	11.72	0.5	—	269(100)	249(2)	—	—
[$^2H_5$]-1	[$^2H_5$]Aniline	11.68	ND [c]	—	274(100)	—	—	—
2	Diphenylamine	24.27	0.8	—	—	—	—	197(100),178(12)
[$^2H_{10}$]-2	[$^2H_{10}$]Diphenylamine	24.16	ND [c]	—	—	—	—	197(100),178(12)
3	*N*-Methylaniline	12.51	1.6	—	283(80)	263(28)	—	178(100),243(5)
4	2-Methylaniline	13.31	0.5	—	283(100)	263(2)	—	—
5	3-Methylaniline	13.94	0.5	—	283(100)	—	—	—
6	4-Methylaniline	14.35	0.6	—	283(100)	263(2)	—	—
7	1,2-Phenylenediamine	19.24	5.0	—	480(100)	—	—	462(3)
8	2,3-Dimethylaniline	16.72	0.8	—	297(100)	277(3)	—	—
9	2,4-Dimethylaniline	16.02	0.7	—	297(100)	277(2)	—	—
10	2,5-Dimethylaniline	15.52	0.8	—	297(100)	277(1)	—	—
11	2,6-Dimethylaniline	15.50	1.3	—	297(100)	—	—	282(4)
12	3,4-Dimethylaniline	17.38	0.6	—	297(100)	277(3)	—	—
13	3,5-Dimethylaniline	16.21	0.5	—	297(100)	277(2)	—	—
14	*N*-Ethylaniline	13.78	4.2	—	297(100)	277(57)	—	257(77),178(38), 282(4)
15	2-Ethylaniline	14.81	0.4	—	297(100)	277(4)	—	—
16	3-Ethylaniline	16.02	0.4	—	297(100)	277(2)	—	—
17	4-Ethylaniline	16.76	0.3	—	297(100)	277(2)	—	—
18	*N*-Ethyl-3-methyaniline	15.54	2.3	—	311(74)	291(100)	—	271(99),178(17)
19	2,4,6-Trimethylaniline	18.05	1.3	—	311(100)	—	—	296(4)
20	2-Methoxyaniline	15.96	0.6	—	299(100)	279(3)	—	—
21	4-Methoxyaniline	18.17	0.7	—	299(100)	279(2)	—	—
22	2,4-Dimethoxyaniline	22.14	1.2	—	329(100)	309(2)	—	—
23	2,5-Dimethoxyaniline	20.60	0.6	—	329(100)	—	—	—
24	3,4-Dimethoxyaniline	22.30	2.9	—	329(100)	—	—	314(11)
25	3,5-Dimethoxyaniline	22.32	1.0	—	329(100)	—	—	314(4)
26	2-Ethoxyaniline	17.19	0.7	—	313(100)	293(3)	—	—
27	2-Chloroaniline	13.24	0.3	—	303(100)	283(4)	—	—
28	3-Chloroaniline	16.09	0.4	—	303(100)	283(4)	—	—
29	4-Chloroaniline	16.44	0.4	—	303(100)	283(3)	—	—
30	2-Chloro-4-methylaniline	16.06	0.5	—	317(100)	297(1)	—	—
31	2-Chloro-5-methylaniline	15.50	0.4	—	317(100)	297(2)	301(1)	—
32	2-Chloro-6-methylaniline	16.56	0.6	—	317(100)	—	301(10)	281(8)
33	3-Chloro-2-methylaniline	17.62	0.6	—	317(100)	297(4)	—	—
34	3-Chloro-4-methylaniline	18.90	0.3	—	317(100)	297(2)	301(1)	—
35	4-Chloro-*N*-methylaniline	16.80	1.6	—	317(48)	297(37)	—	178(100),277(3)
36	4-Chloro-2-methylaniline	17.98	0.4	—	317(100)	297(3)	—	—
37	5-Chloro-2-methylaniline	17.95	0.4	—	317(100)	297(2)	—	—
38	2,3-Dichloroaniline	17.46	0.9	—	337(100)	317(2)	321(24)	—
39	2,4-Dichloroaniline	16.96	0.8	—	337(100)	317(2)	321(12)	—
40	2,5-Dichloroaniline	17.10	0.9		337(100)		321(9)	

41	2,6-Dichloroaniline	18.88	1.5	—	337(14)	—	321(100)	—
42	3,4-Dichloroaniline	20.94	1.1	—	337(100)	317(3)	—	—
43	3,5-Dichloroaniline	19.63	1.1	—	337(100)	317(2)	321(4)	—
44	2,3,4-Trichloroaniline	21.67	1.2	—	371(75)	—	355(100)	337(3)
45	2,4,5-Trichloroaniline	21.04	2.0	—	371(100)	—	355(35)	337(4)
46	2,4,6-Trichloroaniline	21.78	1.01	—	—	—	355(100)	337(3)
47	3,4,5-Trichloroaniline	24.76	2.3	—	371(100)	—	355(6)	337(2)
48	2-Nitroaniline	17.71	3.0	334(100)	—	—	—	266(57),284(18), 318(9),228(5)
49	3-Nitroaniline	22.17	18.7	334(2)	314(100)	—	—	284(75),264(13), 333(3)
50	4-Nitroaniline	22.90	11.7	—	314(100)	—	—	284(66),298(9)
51	*N*-Methyl-2-nitroaniline	21.06	2.0	348(7)	—	—	—	280(100),240(32), 213(29),281(13)
52	*N*-Methyl-4-nitroaniline	22.87	7.7	348(100)	—	—	—	178(28),197(19), 278(10),298(7)
53	2-Methyl-3-nitroaniline	21.99	5.0	348(1)	328(100)	—	—	298(32),332(5), 278(3)
54	2-Methyl-4-nitroaniline	23.97	10.8	—	328(100)	—	—	298(27),312(8)
55	2-Methyl-5-nitroaniline	24.40	7.1	348(1)	328(100)	—	—	298(40),278(10), 332(6)
56	2-Methyl-6-nitroaniline	20.09	5.1	348(100)	—	—	—	280(53),331(16), 332(8),262(5)
57	5-Methyl-2-nitroaniline	20.23	5.2	348(100)	—	—	—	280(57),298(14), 332(9),242(10)
58	4-Methyl-2-nitroaniline	20.51	2.3	348(100)	—	—	—	280(36),298(9), 332(9),242(3)
59	4-Methyl-3-nitroaniline	23.87	4.4	348(1)	328(100)	—	—	298(32),278(8), 332(8)
60	2,4-Dinitroaniline	25.10	66.3	379(100)	—	—	—	362(32),329(15), 349(11),281(5)
61	2-Chloro-4-nitroaniline	22.22	14.5	368(1)	348(44)	—	332(20)	318(100),178(9)
62	4-Chloro-2-nitroaniline	20.52	5.6	368(98)	—	—	—	300(100),318(54), 351(16),262(8)
63	2-Chloro-5-nitroaniline	22.96	17.4	368(3)	348(54)	—	332(29)	318(100),298(24)
64	5-Chloro-2-nitroaniline	20.79	12.6	368(100)	—	—	—	300(68),318(60), 351(20),282(9)
65	4-Chloro-3-nitroaniline	25.69	19.2	—	348(58)	—	—	318(100),298(24), 350(18)
66	1-Aminonaphtalene	23.80	1.4	339(2)	319(100)	299(21)	—	338(1)
67	2-Aminonaphtalene	25.15	0.9	339(1)	319(100)	299(2)	—	—
[$^2H_7$]-67	[$^2H_7$]-2-Aminonaphtalene	25.10	ND [c]	346(1)	326(100)	—	—	—
68	2-Aminobiphenyl	24.02	0.6	—	345(100)	325(4)	—	327(2)
69	4-Aminobiphenyl	28.49	1.0	—	345(100)	325(2)	—	327(3)
70	4-Phenylazoaniline	31.91	17.3	393(67)	373(100)	—	—	284(29)
71	Benzidine	35.11	47.5	—	556(100)	536(2)	—	508(4)
72	3,3′-Dimethoxybenzidine	41.27	18.0	—	616(100)	596(2)	—	555(7)
73	3,3′-Dichlorobenzidine	37.57	10.2	—	624(100)	604(2)	588(10)	—

[a] Instrument limit of detection defined as the lowest amount of compound (expressed as parent amine) yielding a signal-to-noise ratio of 20:1.
[b] Relative abundance (%) reported in parentheses.
[c] Not determined.

water [11]. The detection limits of this procedure ranged from 0.06 to 1.8 mg l^{-1}.

Other methods are based on the derivatization of the aromatic amines followed by the analysis of the derivatives by GC coupled to electron-capture detection (ECD) [12,13] or electron-impact (EI) mass spectrometry (MS) [12,14]. The additional work necessary to carry out the chemical modification of the analytes is counterbalanced by the enhancement of GC performance (e.g. peak symmetry, resolution, peak height) and sensitivity. Acylation of the amino groups in the aromatic amines by perfluorocarboxilic acid anhydrides is often the reaction of choice when an electrophoric substituent, i.e. having a high electron affinity, must be introduced, e.g. for ECD analysis, even though fluoro-substituted benzil and benzoyl halides are also used for this purpose.

GC–MS is often the preferred approach when identification of compounds in a mixture is required. Unfortunately, the limited sensitivity of EI-MS analysis in the full-scan mode implies that selected ion monitoring (SIM) has to be used in order to achieve the detection of trace amounts of substance. In this case, a set of repeated analyses of the same sample has to be carried out for the determination of a broad spectrum of compounds.

In 1987, Trainor et al. [15] first reported a thorough investigation about the behaviour of several electrophoric derivatives of aniline and chloro-substituted anilines under conditions of electron-capture negative-ion chemical ionization (EC-NICI)-MS using methane as a moderator gas for the production of near-thermal energy electrons. In their study, they also demonstrated that GC–EC-NICI-MS is a suitable technique for the analysis at trace levels of some of these derivatives. Heptafluorobutyramides in particular showed a remarkable tendency to produce only one prominent analyte-specific ion via the facile elimination of hydrofluoric acid (HF). This feature, together with the intrinsically high sensitivity and specificity of EC-NICI-MS [16–18], suggests that this technique could be profitably extended to the analysis of other aromatic amines at trace levels. As a matter of fact, a GC–EC-NICI-MS method was proposed in 1989 for the determination of 2-aminobiphenyl and 4,4′-diaminodiphenylmethane in biological material after derivatization with pentafluoropropionic anhydride [19]. The reported detection limits for these compounds were about 50 and 100 pg ml^{-1}, respectively (0.25 and 0.50 pg injected).

In this work, the GC–EC-NICI-MS approach is extended to the seventy-three compounds listed in Table 1, in order to obtain a procedure for the simultaneous determination of a broad spectrum of aromatic amines potentially present at trace levels in groundwater from a heavily polluted industrial area near Milan. Each compound has been characterized on the basis of its GC and MS properties for unequivocal identification.

2. Experimental

2.1. Chemicals

Chemical standards of the various aromatic amines were purchased from Aldrich (Milwaukee, WI, USA), except compounds 71 and 73 (Sigma, St. Louis, MO, USA), and were of the highest purity available. Deuterated analogues [$^2H_5$]-1, [$^2H_{10}$]-2 and [$^2H_7$]-67 were provided by Cambridge Isotope Laboratories (Woburn, MA, USA). The derivatizing agent heptafluorobutyric anhydride (HFBA) was supplied by Pierce (Rockford, IL, USA). Dichloromethane and isooctane, both of special-reagent grade, were obtained from Carlo Erba (Milan, Italy). Water used to prepare the phosphate buffer solution was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical-reagent grade and were obtained from local sources.

2.2. Derivatization procedure

Individual standard solutions each containing 1 mg ml^{-1} of a given aromatic amine in dichloromethane were prepared. A Reacti-Therm dry block derivatization system (Pierce) was used to heat reaction mixtures. A 100-μl aliquot of each solution was transferred to a different conical reaction vial (Pierce) containing 100 μl of isooctane. The solution was evaporated with a nitrogen stream to about 100 μl and after addition of 100 μl of HFBA the reaction vial was tightly sealed with a PTFE-laminated cap. The reaction mixture was allowed to react for 30 min at 60°C. After cooling, the excess of HFBA was eliminated by addition of 2 ml of a pH 8 0.5 *M*

phosphate buffer solution, then the mixture was immediately extracted with three 1-ml aliquots of dichloromethane. The choice of a slightly basic buffer was necessary to minimize the acid content of the final derivative solutions, since it was noted that residual heptafluorobutyric acid co-extracted with derivatives progressively damaged GC columns. The extract was dried by passing it through a glasswool-plugged Pasteur pipette containing 2 g of anhydrous sodium sulphate. The dried extract was evaporated to about 0.5 ml at room temperature using a nitrogen stream, then 1 ml of isooctane was added and the extract was finally concentrated to 1 ml. A 1-μl aliquot of this solution was injected into the GC–MS system operated in the EI mode to confirm the identity of the derivative and assess reaction completeness. In no case was unreacted amine detected.

In the course of the concentration steps, the solutions were never allowed to evaporate into dryness in order to avoid the loss of the most volatile compounds.

2.3. GC–MS analysis

A Model HP5890A gas chromatograph coupled to a Model HP5989A quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) was used. The samples were introduced via a splitless injector into a HP5 (Hewlett-Packard) fused-silica capillary column (50 m×0.20 mm I.D.; 0.5 μm film thickness). Helium was used as the carrier gas at a column head pressure of 250 kPa. The GC oven was kept at a temperature of 50°C for 0.5 min then heated to 110°C at 50°C min^{-1}, to 225°C at 5°C min^{-1} and to 280°C at 20°C min^{-1}, followed by a 15 min isothermal step. The injector and transfer line temperatures were 250°C and 280°C, respectively.

The ion-source temperature was 200°C; the filament emission current and electron energy were 300 μA and 150 eV, respectively. Methane filtered through an activated molecular sieve 5A moisture trap (Hewlett-Packard) was used as the moderator gas (1.0 Torr source pressure; 1 Torr=133.322 Pa). Optimization of the MS conditions was performed according to the indications of the manufacturer [20] to obtain adequate sensitivity. The spectrometer axis and the ion abundance were tuned using perfluorotributylamine (PFTBA); the GC–MS system performance was checked periodically by injection of a 1-μl aliquot of a solution containing 1 ng ml^{-1} octafluoronaphtalene (OFN) in isooctane: a signal-to-noise ratio (S/N) of 50:1, calculated on the extracted plot of the molecular anion at m/z 272, was considered the minimum sensitivity requirement. The presence of water, oxygen and residual PFTBA in the system was minimized to limit the well-known detrimental effect on EC-NICI sensitivity caused by side reactions of these contaminants with substrate molecules [18]. Since OFN gives rise, in the presence of oxygen, to significant formation of an anion at m/z 238, originated by the reaction $M^- + O_2 - F_2 - CO$ [17], monitoring of this ion after injection of OFN was used as a test to check the presence of air leaks in the GC–MS system.

Full-scan mode acquisition was carried out in the range 50–650 u to determine the EC-NICI spectra of the derivatives, whereas the following steps were adopted to analyze the samples and determine the detection limits: 150–550 u (10–30 min); 150–650 u (30–42.45 min).

Approximate instrument limits of detection (LOD) were calculated using a slightly modified macro program supplied by Hewlett-Packard and they were arbitrarily defined as the lowest amount of compound yielding a S/N of 20:1.

The ions monitored for quantitative determination are those reported in Table 2.

2.4. Calibration

An internal standard (I.S.) solution containing 1 μg ml^{-1} of compounds $[^2H_5]$-1, $[^2H_{10}]$-2 and $[^2H_7]$-67 in dichloromethane was prepared.

Standard solutions containing a mixture of the parent amines of the compounds listed in Table 2 were prepared in dichloromethane at concentrations of 0.1 μg ml^{-1} and 1.0 μg ml^{-1} of each compound. Appropriate amounts of the latter solutions together with 100 μl of the I.S. solution were transferred into four separate reaction vials and subjected to the derivatization procedure described above in order to achieve final concentration levels of 1, 10, 50 and 100 ng ml^{-1} (referred to parent amines). A blank sample was prepared by similar treatment of 100 μl of the I.S. solution. 1 μl of each calibration sample was injected into the GC–MS system.

Table 2
SIM acquisition time programme, calibration parameters, limit of detection and precision for the quantitative determination of aromatic amine derivatives

Group No.	Start time (min)	Derivative No.	Ions (*m/z*)	Dwell-time (ms)	$b \pm$c.l. [a] ($\times 10^4$)	$a \pm$c.l. [a] ($\times 10^4$)	r	LOD [b] (pg)	w-d R.S.D. [c] (%)	b-d R.S.D. [d] (%)	F
1	11.00	1	269,274	100,100	104±3	86±177	0.99974	0.03	0.8	3.2	15.2
2	12.15	3	283	200	26.0±0.5	10±27	0.99980	0.10	2.2	12.1	30.6
3	13.00	27	303	100	72.1±0.2	36±86	0.99975	0.01	2.7	12.6	21.4
		4	283	100	73.2±0.6	6±29	0.99997	0.02	1.7	3.2	3.3
4	13.50	14	297,277,257	100,100,100	26.9±0.6	11±30	0.99977	0.12	2.3	9.8	17.7
		5	283	100	72.5±0.3	−7±15	0.99999	0.02	1.9	4.6	5.7
		6	283	100	79.5±0.3	−12±17	0.99999	0.01	1.7	4.0	5.8
		15	297	100	72.9±0.4	0±20	0.99999	0.02	1.7	3.4	3.8
5	15.50	20	299	100	49.7±0.2	−2±12	0.99999	0.02	1.9	4.7	5.8
		28	303	100	81.8±2.3	−61±117	0.99964	0.01	3.3	12.1	13.5
		29	303	100	94.4±2.6	−64±130	0.99966	0.01	3.5	12.3	12.6
6	16.70	39	337	100	55.1±0.7	17±37	0.99992	0.02	3.5	12.2	12.4
		40	337	100	58.9±0.5	10±27	0.99996	0.02	3.2	13.5	17.1
		38	337	100	55.2±1.1	10±56	0.99994	0.02	3.6	13.0	13.3
		33	317	100	78.4±5.1	−65±256	0.99938	0.01	3.2	12.8	16.0
7	17.80	19	311	100	68.9±1.6	20±80	0.99992	0.02	3.7	7.2	3.9
		21	299	100	152±5	39±271	0.99981	0.02	2.7	5.6	4.3
8	18.50	41	321	200	151±7	86±364	0.99967	0.03	2.3	16.3	50.6
9	19.50	62	368,300	100,100	37.7±3.6	41±181	0.99866	0.20	3.8	11.0	8.2
		64	368,300	100,100	34.5±3.2	35±163	0.99871	0.23	3.1	8.7	7.7
		42	337	100	171±6	78±314	0.99980	0.03	2.8	10.0	12.4
10	21.30	46	355	200	163±5	46±231	0.99988	0.03	3.2	11.9	14.0
11	22.00	61	348,332,318	100,100,100	66.4±1.5	8±74	0.99993	0.47	2.5	8.0	10.2
		63	348,332,318	100,100,100	43.2±0.6	4±31	0.99997	0.57	1.9	12.0	39.3
12	23.50	66	319	100	79.7±0.7	3±35	0.99999	0.02	1.5	3.5	5.5
		68	345	100	146±4	50±212	0.99988	0.02	1.9	6.1	10.4
		2	197	100	119±2	17±105	0.99996	0.02	0.6	0.7	1.1
13	24.50	67	326,319	100,100	115±3	70±171	0.99992	0.02	0.6	2.1	11.7
14	25.45	65	348,318	100,100	57.8±1.4	10±72	0.99991	0.46	2.1	11.9	32.3
15	27.00	69	345	200	127±2	32±112	0.99996	0.02	3.1	3.5	1.3

[a] Confidence limits calculated for 3 degrees of freedom at the 95% confidence level ($t=3.18$).
[b] Instrument limit of detection defined as the lowest amount of compound (expressed as parent amine) yielding a signal-to-noise ratio of 20:1.
[c] Within-day relative standard deviation (14 degrees of freedom).
[d] Between-day relative standard deviation (6 degrees of freedom).

2.5. Sample extraction

Sample extraction was accomplished according to a previously reported procedure [5], with only minor modifications. A 1-l volume of groundwater water sample was adjusted to pH 11 by addition of 3 *M* sodium hydroxide solution. Three successive extractions with 60-ml aliquots of dichloromethane were carried out in a separating funnel. The fractions were passed through a glasswool-plugged chromatographic column containing 30 g of anhydrous sodium sulphate and directly collected in a 500-ml Kuderna–Danish evaporator (Supelco, Bellefonte, PA, USA). The extract was concentrated to about 1 ml by heating the concentrator tube at 60°C in a water bath and was then transferred to a reaction vial containing 100 μl of the I.S. solution and subjected to the derivatization procedure.

3. Results and discussion

3.1. EC-NICI-MS data

Examination of the mass spectrometric features of the heptafluorobutyramides under EC-NICI conditions was carried out in order to evaluate whether anions characteristic of the original analytes were produced in the fragmentation process, the presence of such anions being of great importance for the unequivocal identification of precursor amines. The complete list of the mass spectral data of the derivatives is reported in Table 1 (isotopic peaks were omitted).

All the compounds under investigation give rise to abundant analyte-specific ions, with the exception of compound 2, whose spectrum is characterized by the base peak at *m/z* 197 corresponding to the fragment ion $[C_3F_7CO]^-$ and a peak at *m/z* 178 corresponding to the fragment ion $[C_3F_6CO]^-$, both arising from the electrophoric moiety introduced by the derivatization as confirmed by the identical fragmentation of its deuterated analogue $[^2H_{10}]$-2. Most derivatives show spectra with one abundant ion $[M-HF]^-$ originating from the capture of a thermalized electron with subsequent loss of HF; a low abundance of ion $[M-H_2F_2]^-$ was also generally observed. The formation of the molecular anion M^- is not a favourite process for the compounds investigated, although the spectra of some derivatives (48, 52, 56, 57, 58, 60, 62, 64 and 70) exhibit abundant M^-.

It was noted that *N*-alkyl substituted derivatives 3, 14, 18 and 35 undergo a different fragmentation

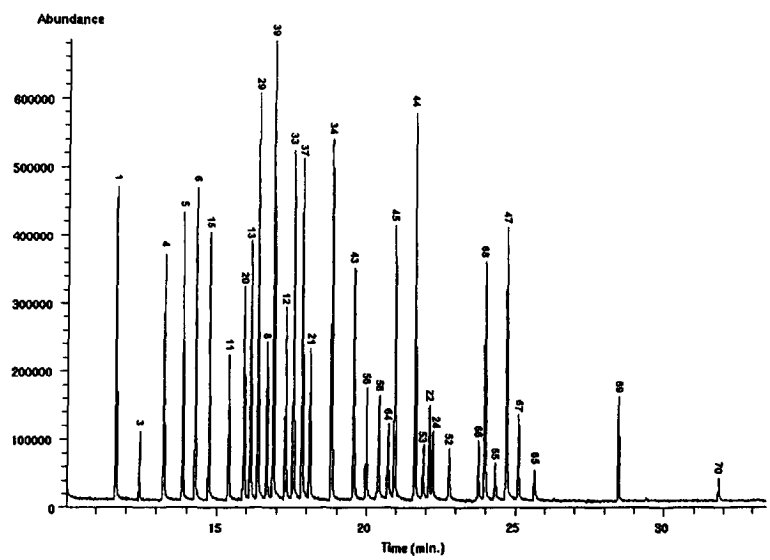

Fig. 1. TIC chromatogram of a mixture of HFBA derivatives under EC-NICI conditions. Numbers on the peaks refer to Table 1. The injected amount of each derivative corresponds to 50 pg of precursor amine.

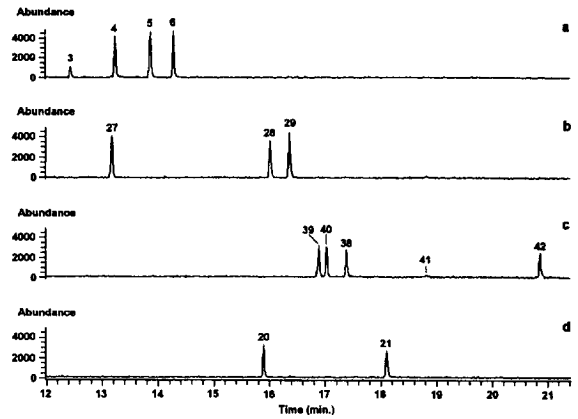

Fig. 2. Extracted ion plots corresponding to: (a) methyl- (*m/z* 283); (b) monochloro- (*m/z* 303); (c) dichloro- (*m/z* 337), and (d) monomethoxy- (*m/z* 299) substituted heptafluorobutyramides. The injected amounts are equivalent to 2 pg of each parent amine.

process yielding ions arising from the multiple loss of HF and the formation of $[C_3F_6CO]^-$. This difference is clearly related to the lack of the hydrogen atom on the amide group. As previously reported [15], this atom is involved in the mechanism leading to the formation of the fragment ion $[M-HF]^-$.

The abundance of ion $[M-HCl]^-$ is roughly related to the degree of chlorination to the aniline ring, although significant differences for positional isomers were observed. As a matter of fact, loss of hydrochloric acid (HCl) occurs preferentially for those compounds where chlorine atoms are located in the positions *ortho* to the amide group.

The spectra of nitro-substituted aniline derivatives are more complex by far than those of the compounds considered above. It was noted that the fragmentation pattern for these substances is highly structure-dependent. Indeed, the spectra of the *ortho*-nitro isomers 48, 56, 57, 58, 60, 62 and 64 are characterised by abundant M^- and the absence of $[M-HF]^-$. Except for 60, in all of these spectra we also observed an abundant ion at *m/z* [M−68].

Conversely, *meta*- and *para*-nitro isomers 49, 50, 53, 54, 55, 59, 61, 63 and 65 undergo preferentially an alternative fragmentation pathway leading to the loss of HF and the formation of a prominent peak at *m/z* [M−50], probably due to the fragment ion $[M-HF-NO]^-$.

3.2. Full-scan GC–MS analysis

Although complete GC separation of all derivatives was not achieved (Table 1), unequivocal identification of coeluting compounds was still possible owing to the different ions originated, with the exception of couples of coeluting isomers 8–17, 9–16, 10–11, 24–25 and 36–37. A representative total ion current (TIC) plot relative to the injection of a mixture of selected compounds is reported in Fig. 1.

All the LOD reported in Table 1 were calculated on the extracted ion plot corresponding to the base peak for each compound, except for compounds 3

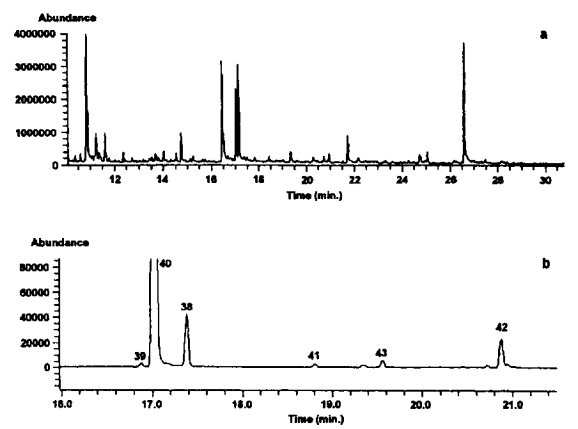

Fig. 3. (a) TIC plot, and (b) *m/z* 337 ion plot from a groundwater sample found to contain dichloro-substituted anilines.

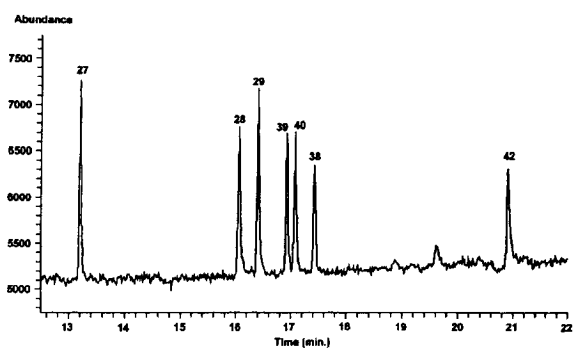

Fig. 4. SIM chromatogram of a mixture containing monochloro- and dichloro-substituted heptafluorobutyramides. The injected amounts are equivalent to 20 fg of each parent amine.

and 35, whose LOD were calculated on the ion plots at m/z 283 and 317, respectively, with the aim of better specificity.

As expected, LOD values were generally lower for those compounds which originate only one prominent ion, whereas higher LOD were found for derivatives which undergo more extensive fragmentation, particularly nitro-substituted heptafluorobutyramides.

Examples of extracted ion plots are reported in Fig. 2: each m/z value corresponds to a different class of derivatives, namely methyl- (m/z 283), monochloro- (m/z 303), dichloro- (m/z 337) and monomethoxy- (m/z 299) substituted heptafluorobutyramides. It is noteworthy that the injected amounts of each compound were equivalent to as much as 2 pg of the respective underivatized amine.

The analysis of real groundwater samples revealed that the proposed approach was suitable for the selective identification of aromatic amines in this matrix, despite the quite complex appearance of the TIC plot (Fig. 3a). Indeed, it was found that other compounds did not interfere when ion plots corresponding to the various classes of derivatives were extracted from the TIC plot, as shown in Fig. 3b for dichloro-substituted heptafluorobutyramides.

3.3. Quantitative analysis

The thirty aromatic amines investigated for quantitative analysis were chosen mainly on the basis of their occurrence in the groundwater samples examined. Quantification was performed by means of time-programmed SIM acquisition (Table 2).

After preliminary trials, deuterated analogues [$^2H_5$]-1 and [$^2H_7$]-67 were chosen as the I.S. for the compounds eluting in the intervals 10–18 min and 18–29 min (except for compound 2), respectively. As previously noted, [$^2H_{10}$]-2 gives rise only to non-specific fragment ions, hence its use as an I.S. was discarded in order to minimize the risk of interference. However, [$^2H_{10}$]-2 was judged to be the most suitable I.S. for compound 2, owing to the different t_R of the two isotopomers and in spite of the identity of their spectra.

The calibration curves were constructed by plotting the peak-area ratios of analyte and the appropriate I.S. versus the known amounts of analyte in the calibration samples and performing least-square regression analysis. Slope (b), intercept (a) and correlation coefficient (r) for each calibration curve are listed in Table 2. Linearity in the range 1–100 pg injected was satisfactory for all the compounds examined.

Consistent with the high sensitivity of the EC-NICI-MS technique, the LOD calculated on the SIM plot were in the range 10–30 fg injected for most compounds and about ten times lower than those reported for aniline and chloro-substituted anilines HFBA derivatives by GC–ECD [12]. A representative SIM plot corresponding to the injection of a mixture of monochloro-substituted and dichloro-substituted amine derivatives is reported in Fig. 4. Amounts equivalent to 20 fg of each precursor amine were injected.

Reproducibility of the instrumental measurements was assessed by three replicate injections of the 50 ng ml^{-1} calibration sample for a period of seven days. Analysis of the variance was performed to determine the contribution of within-day and between-day variation to the overall error of the procedure (Table 2). While the within-day relative standard deviation (R.S.D.) was satisfactory, high values of the between-day R.S.D. were generally obtained. After comparison of between-day and within-day variance by means of a one-sided F-test, significant differences were found for almost all compounds. As a matter of fact, the calculated value of F exceeded in most cases the critical value of F tabulated at the 95% confidence level ($F_{6,14}=2.848$). Hence, it was concluded that frequent re-calibration of the GC–MS system was necessary to perform correct measurements.

4. Conclusions

The proposed procedure proved to be suitable for the simultaneous determination of a large number of aromatic amines at trace levels in terms of selectivity, sensitivity, reproducibility and linearity. With respect to sensitivity, the EC-NICI-MS technique allowed us to obtain LOD even lower than those achieved by means of ECD. The application to real samples showed that extraneous peaks did not interfere. Although groundwater was the only matrix

tested, the EC-NICI-MS approach could probably be extended to different types of samples whenever specific and sensitive identification of aromatic amines is required.

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