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Determination of hemoglobin adducts of arylamines in humans

Gabriele Sabbioni*, Armin Beyerbach

Institut für Pharmakologie und Toxikologie, Universität Würzburg, Versbacher Strasse 9, D-97078 Würzburg, Germany

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Abstract

Aromatic amines and nitroarenes are important occupational and environmental pollutants. It is therefore essential to have fast and reliable methods to monitor human exposure. In rat experiments it has been shown that several arylamines form hydrolyzable hemoglobin adducts. Hemoglobin adducts are dosimeters for the internal dose and possibly for DNA modification at the site of tumor formation. In the present paper we introduce a new efficient method to quantify hemoglobin adducts. Precipitated hemoglobin was hydrolyzed in base in the presence of the recovery standards d_5-aniline, d_4-2-methylaniline, d_4-3-methylaniline, d_4-4-methylaniline, d_3-2,4-dimethylaniline, $^{13}C_6$-4-chloroaniline, d_9-4-aminobiphenyl, and 4'-fluoro-4-aminobiphenyl. The hydrolysate was extracted with hexane, derivatized with pentafluoropropionic anhydride (PFPA) and analyzed by GC–MS with negative chemical ionization. In one run over twenty amines can be determined. The method has been applied to human studies.

1. Introduction

Aromatic amines and nitroarenes are important occupational and environmental pollutants [1,2]. It is therefore, essential to develop fast and reliable methods to monitor exposure of humans [3,4]. Urine metabolites indicate only recent exposures up to 48 h. Hemoglobin (Hb) adducts are an indicator of the exposure over the last 4 months, if the adducts are not less stable than the lifetime of the erythrocytes. The mechanism of adduct formation between aromatic amines or nitroarenes with Hb involves the reaction of the metabolite–nitrosoarene–with cysteine residues to form a sulfinic acid amide [5–8]. Evidence for such reaction in vitro has been obtained by Ringe et al. [6]. Sulfinic acid amide adducts are readily hydrolyzed under mild conditions, yielding the parent amine. Hemoglobin adducts are dosimeters for the internal dose and possibly for DNA modification at the site of tumor formation. In animal experiments it has been shown that hemoglobin adducts of carcinogenic compounds correlate with the amount of DNA adducts in the organ of tumor formation [9]. Quantitation of hemoglobin adducts of monocyclic arylamines and 4-aminobiphenyl in humans has been performed in a few laboratories [10–14]. The proposed method adapted for the analysis of several amines [10–13] is very labor intensive and not very precise ($\pm$ 20%) for alkyl-substituted anilines, since d_5-aniline was used as internal standard. Therefore, we synthesized additional internal standards and developed a method which is faster and more

* Corresponding author.

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precise for the quantitation of a larger set of hemoglobin adducts of arylamines in humans.

2. Experimental

2.1. Chemicals and reagents

Chemicals were obtained from Aldrich (Steinheim, Germany): 4-aminobiphenyl (4ABP) (A4242-5, 98%), 2,3,4,5,6-d_5-aniline (d_5A) (17569-2, 99 + %), 3-chloro-4-fluoroaniline (3C4FA) (22858-3, 98%), 2-chloro-4-methylaniline (2C4MA) (12507-5, 98%), 2-chloro-6-methylaniline (2C6MA) (C5100-8, 99%), 3-chloro-4-methylaniline (3C4MA) (23632-2, 99 + %), deuterium chloride (DCl) (22707-2, 37% weight solution in D_2O, 99.5 atom% D), 2,4-difluoroaniline (24DFA) (D10140, 99%), 2,3-dimethylaniline (23DMA) (D14580-7, 99%), 2,4-dimethylaniline (24DMA) (24091-5, 99%), 2,5-dimethylaniline (25DMA) (10225-3, 99%), 2,6-dimethylaniline (26DMA) (39520, >98%), 3,4-dimethylaniline, (34DMA) (12637-3, 98%), 3,5-dimethylaniline (35DMA) (13786-3, 98%, distilled), 4-ethylaniline (4EA) (E1200-1, 99 + %, distilled), 4-fluoroaniline (4FA) (F380-0, 99%, distilled), 2-methylaniline (2MA) (18542-6, 99 + %), 3-methylaniline (3MA) (13201-2, 99%), 4-methylaniline (4MA) (23621-4, 99.9%), 4-(trifluoromethyl)-aniline (4TFA) (22493-6, 99%). Fluka (Neu-Ulm, Germany): 4-bromoaniline (4BrA)(16230, >99%), 5-chloro-2-methylaniline (5C2MA) (25090, >98%), 2-ethylaniline (2EA) (03060, >98%, distilled), 2,4,6-trimethylaniline (246TMA) (92290, >98%), and toluene (No. 89683). Pfaltz and Bauer (Waterbury, Connecticut USA): 2,4,5-trimethylaniline (245TMA) (P28660, 98%), 4'-fluoro-4-aminobiphenyl (4'F4ABP) (A22200, 98%). Riedel-de Haën (Seelze, Germany): aniline (A) (33029, >99.5%), 2-chloroaniline (2CA) (62285, 99%), 3-chloroaniline (3CA) (35824, 99%), 4-chloroaniline (4CA) (35823, 99%), 2,4-dichloroaniline (24DCA) (35829, >99%), 2,6-dichloroaniline (26DCA) (36703, >99%), 3,4-dichloroaniline (34DCA) (35827, >99%), 3,5-dichloroaniline (35DCA) (36704, >99%), and $^{13}C_6$-4-chloroaniline ([$^{13}C_6$]4CA) from Promochem (Wesel, Germany). The purity of the amines was checked by GC–MS.

Hexane (No. 34484) was obtained from Riedel-de Haën, pentafluoropropionic anhydride (PFPA) from Pierce (Oud-Beijerland, Netherlands), diethyl ether (No. 921), deuterium oxide (No. 13366, >99.8% deuteration grade), sodium hydroxide (No. 6498) and water (No. 15333) from Merck (Darmstadt, Germany), triethylamine (No. 90340), and anhydrous sodium sulfate (No. 71959) from Fluka (Neu-Ulm, Germany).

2.2. General procedure for the deuteration of aromatic amines

Several deuterated arylamines have been synthesized following a method previously published by Frischkorn et al. [15]. The purity of the products and the deuterium content were checked by ^{1}H NMR and GC–MS. For the synthesis of the deuterated amines the following protocol was followed: The amine (25 mmol) was dissolved in a mixture of 37% DCl (2 ml) and D_2O (5 ml), sealed in a glass ampoule and placed in an autoclave for 18 h at 250°C. Then the reaction solution was evaporated to dryness and redissolved in a mixture of 37% DCl (1 ml) and D_2O (5 ml), sealed in a glass ampoule and heated in the autoclave for 18 h at 250°C. The evaporation and dissolution procedure was repeated in the same manner. The resulting solution was made basic using aqueous sodium hydroxide and extracted with hexane. (In the case of 4ABP diethyl ether was used for the extraction.) The extract was dried over sodium sulfate, concentrated in a nitrogen stream and purified by Kugelrohr distillation at 13 Pa. The following deuterated aromatic amines were obtained: d_4-2MA (chemical yield 65%), colorless oil; d_4-3MA (69%), colorless oil; d_4-4MA (39%), white crystals; d_3-24DMA (77%), colorless oil; and d_9-4ABP (15%), white crystals.

The degree of deuteration was determined in $CDCl_3$ by ^{1}H NMR spectroscopy on a Bruker AC 250 (250 MHz) instrument. The signals of the methyl and amine protons were used as

reference for the integration of the residual ring protons. Toluene was added as an additional internal standard to determine the degree of deuteration in the methyl groups.

The purity and identity of the obtained products were checked by GC–MS under GC conditions described below. Exclusive deuteration of the aromatic ring was obtained. The percentages of deuteration for d_4-2MA, d_4-3MA, d_4-4MA, d_3-24DMA, and d_9-4ABP as determined by ^1H NMR were 99.0, 94.2, 97.0, 95.4 and 97.8%, respectively. For the PFPA-derivatives of d_4-2MA, d_4-3MA, d_4-4MA, d_3-24DMA, and d_0-4ABP the following relative abundance was observed for the m/z fragment of the d_0-amine compared to the m/z of the completely ring deuterated amine: 0.2, 0.2, 0.3, 0.3, and 0.2%, respectively.

2.3. Isolation of hemoglobin from blood and determination of hemoglobin adducts

Freshly drawn, EDTA blood (4–6 ml) was centrifuged for 5 min at 2000 *g*. After removal of plasma, red blood cells were washed three times in equal volumes of 0.9% NaCl solution and lysed by the addition of 4 volumes of EDTA solution (10^{-4} *M*, pH 7.5). Cell debris was removed by centrifugation. Hemoglobin was precipitated with ethanol from lysed erythrocytes. The precipitate was washed with ethanol–water (8:2), ethanol, ethanol–diethyl ether (3:1) and diethyl ether. The dried hemoglobin (100–200 mg) was dissolved in 3.5–4 ml of 0.1 *M* NaOH in 20-ml tubes (22 × 100 mm). The hexane solution (20 μl) with the recovery standards was added to the basic hemoglobin solution (pH > 12). After 1 h in a shaking bath at room temperature 6 ml of hexane were added. The mixture was vortex-mixed for 3 min, centrifuged for 5 min at 3000 *g*, and frozen in liquid nitrogen. The thawed organic layer was passed through a pipette filled with 1 g of anhydrous Na_2SO_4. The Na_2SO_4 was rinsed with 1.5 ml of hexane. The dried organic phase was collected in a graduated tapered tube (98 × 15 mm). PFPA (5 μl) was added. After 10 min at room temperature 100 ng of 24DFA in 10 μl of hexane were added. The organic phase was then evaporated to 0.2 ml with a gentle stream of nitrogen at 30°C in ca. 20 min. The residue was then transferred to a micro-insert (200 μl) for 12 × 32 mm autosampler (Hewlett-Packard 7276) vials and evaporated very carefully under a stream of nitrogen at 25°C. At the disappearance of the last drop the stream of nitrogen was stopped. The residue was then taken up in 15 μl of ethyl acetate containing 1 ng of the PFPA derivative of 4-bromoaniline.

2.4. Quantitation by gas chromatography–mass spectrometry and identification of compounds

The analyses were performed on a Hewlett-Packard chromatograph (HP 5890II) equipped with an autosampler (HP 7276) and interfaced to a mass spectrometer (HP 5989A). The PFPA derivatives of the aromatic amines were analyzed by splitless injection on to a fused-silica capillary column (J&W, DB 1701, 15 m × 0.25 mm I.D., 1 μm film thickness) with a 1 m × 0.25 mm Methyl-Silyl retention gap (Analyt, Müllheim, Germany). In all cases the initial oven temperature, the injector temperature and the transfer line temperature were set at 50, 200 and 200°C, respectively. The oven temperature was increased at a rate of 50°C/min to 200°C held for 1.2 min and then heated at 50°C/min to 240°C and held for 3.2 min. Helium was used as carrier gas with a flow-rate of 1.5 ml/min. The elution order of the derivatized amines is listed in Table 1. For electron-impact ionization (EI), the electron energy was 70 eV, the emission current was 300 μA, and the source temperature was 200°C. For negative chemical ionization (NCI), with methane as the reagent gas, the source pressure was typically 160 Pa, the electron energy was 100 eV, the emission current was 300 μA and the source temperature was 200°C.

2.5. Derivatization with pentafluoropropionic acid anhydride (PFPA)

PFPA derivatives of all amines were synthesized on a large scale. To each amine (4 mmol) in dry ether (10 ml) and triethylamine (4 mmol), PFPA (0.9 ml, 6 mmol) was added at room

Table 1
Retention time, major EI- and CI-mass fragments for PFPA derivatives of aromatic amines and the relative response factor of the amines to the recovery standards

Amine	time (min)	EI *m/z* (relative intensities)	NCI[a] *m/z*	Standard	Amount (ng)	Relative response[b]
24DFA	3.48	275(63), 156(42), 128(100)	255			
2CA	3.86	273(26), 238(100), 126(30)	253	d_4-2MA	2	1.6
A	4.08	239(100)	219	d_5-A	2	1.1
2MA	4.13	253(31), 134(100), 91(63)	233	d_4-2MA	2	1.2
2C4MA	4.22	287(28), 252(100), 140(40)	267			
2EA	4.27	267(21), 148(100)	247	d_4-2MA	2	1.9
4FA	4.32	257(65), 138(53), 110(100)	237			
3MA	4.33	253(93), 134(81), 91(100)	233	d_4-3MA	0.5	1.2
4TFA	4.36	307(60), 188(100), 145(79)	287	d_4-4MA	1	1.9
25DMA	4.37	267(56), 148(100)	247	d_4-24DMA	1	1.6
4MA	4.40	253(100), 134(44), 106(70)	233	d_4-4MA	1	1.1
24DMA	4.47	267(22), 148(100), 120(60)	247	d_3-24DMA	1	1.4
26DMA	4.53	267(56), 148(100)	247	d_3-24DMA	1	0.7
35DMA	4.59	267(60), 148(69), 105(100)	247	d_4-4MA	1	1.3
23DMA	4.61[c]	267(60), 148(100)	247	d_3-24DMA	1	1.2
3EA	4.62[c]	267(100), 252(55), 148(47)	247	d_3-24DMA	1	1.2
2C6MA	4.64	287(10), 252(100)	267			
4EA	4.75	267(63), 252(100), 107(51)	247	d_4-4MA	1	1.7
34DMA	4.84	267(100), 148(28), 120(58)	247	d_4-4MA	1	0.9
246TMA	4.89	281(41), 162(100)	261	d_3-24DMA	1	0.3
245TMA	4.92	281(29), 162(100)	261	d_3-24DMA	1	1.1
3CA	5.01	273(100), 154(91), 111(63)	253	[^{13}C]4CA	2	1.4
4CA	5.06	273(100), 154(51), 126(74)	253	[^{13}C]4CA	2	1.2
5C2MA	5.07	287(51), 168(100)	267			
26DCA	5.30	307(7), 274(33), 272(100)	271			
3C4FA	5.34	291(91), 172(67), 144(100)	271			
3C4MA	5.49	287(100), 168(47), 140(70)	267			
4BrA	5.64	317(100), 319(100), 172(74)	297			
35DCA	5.96	307(84), 309(53), 190(65), 188(100)	287	4'F4ABP	1	0.3
1NA	6.17	289(96), 142(54), 115(100)	269	4'F4ABP	1	0.4
34DCA	6.17	307(100), 309(63), 188(67), 160(81)	287	4'F4ABP	1	0.3
2NA	6.66	289(62), 142(54), 115(100)	269	4'F4ABP	1	0.7
3ABP	7.60	315(100), 153(61), 196(23)	295	4'F4ABP	1	1.3
4ABP	7.90	315(100), 168(72)	295	4'F4ABP	1	1.0
4'F4ABP	8.02	333(100), 186(77), 159(31)	313			

[a] These ions correspond to the molecular ion minus hydrofluoride: [M − HF] .
[b] The relative response was obtained from Hb solutions. The electron multiplier voltage was increased by 5% after 7 min.
[c] 23DMA and 3EA did not separate under the described GC conditions.

temperature. The reaction was monitored by TLC using silica-gel plates (Alugram, SIL G/UV_{254}; Macherey-Nagel, Düren, Germany) with hexane–ethyl acetate (7:3) as the solvents. When all the starting material had disappeared, the solvent and any excess reagent were evaporated on a rotary evaporator and the residue was redissolved in diethyl ether, washed with saturated sodium bicarbonate followed by water. The organic layer was dried over magnesium sulfate, filtered and evaporated to dryness, producing in all cases white needles. The mass

spectra of the products were obtained by GC–MS with EI. The major mass fragments have been summarized in Table 1.

2.6. Standard solutions

Stock 1% solutions of all amines were stored in hexane at −25°C. The final dilution of the standard mixtures were prepared fresh at the beginning of each week and stored at −25°C.

3. Results and discussion

3.1. Synthesis of the internal standards

The H/D exchange was performed at pH < 1 and 250°C in an autoclave. The chemical yield and the degree of deuteration were generally high for anilines and methyl-substituted anilines. The main product for anilines with halogens (4-bromoaniline, 4-chloroaniline, 4-iodoaniline) or with a trifluoromethyl group as substituent was d_5-aniline. In contrast to Frischkorn et al. [15] we could not observe any exchange of the methyl protons. This was checked by NMR using toluene as an internal standard.

3.2. Work up and quantitation of hemoglobin adducts

The procedure was optimized for 100–250 mg Hb, as these were the amounts of Hb available from a study of workers exposed to aniline and 2MA (the details of this cooperative study will be published elsewhere). In order to reduce the amount of solvents the hemoglobin was dissolved in a minimum amount (3–4 ml) of 0.1 *M* NaOH. In control experiments it could be shown that hemoglobin of humans was not contaminated with compounds having the same retention time and mass fragments as the internal standards. For compounds of interest for occupational exposure, the procedure was tested with 0.020–7 ng of A and 2MA and with 0.005–2 ng for all the other amines at six concentration levels. For the present work we used 2 ng d_5A, 2 ng d_3-2MA, 0.5 ng d_4-3MA, 1 ng d_4-4MA, 1 ng d_3-24DMA, 2 ng [$^{13}C_6$]4CA, 1 ng d_9-4ABP and 1 ng 4'F4ABP as internal standards (= internal standard mixture). These were added in 20 μl of hexane to the hydrolysates. In order to reduce the possibility of contamination and to allow a large output of samples, the hemoglobin hydrolysates were extracted only once with 6 ml of hexane. To avoid loss of the amines during evaporation the derivatizing agent was added directly after drying the hexane phase over sodium sulfate. Before evaporation of the hexane phase 2,4-difluoroaniline was added as a control for the derivatization step. The evaporation step has to be carefully controlled as it is crucial to avoid loss of the volatile derivatives. The nitrogen flow should be stopped as soon as the last drop disappears in the microvials. The residue was taken up in ethyl acetate in the presence of the PFPA derivative of 4-bromoaniline (PFPA-4BrA) for the control of the overall recovery and of the performance of the GC–MS. The obtained peaks were quantified in relation to the relevant internal standards. The samples were analyzed overnight by GC–MS. In order to avoid contamination from the amines in the laboratory environment all the analyses were carried out in a room dedicated exclusively to analytical work. All the glassware was used only once without pretreatment. Pretreatment of the glassware with potassium hydroxide and ethanol yielded lower recoveries.

It has been shown by Bryant et al. [10] that hemoglobin adducts of arylamines are present in several species. We found no hemoglobin free of arylamines even from non-exposed, non-smoking individuals. Thus, for the quantitation of human samples the calibration curves were established from sodium hydroxide on the same day of analysis with three concentration levels of amines in the presence of a constant amount of the recovery standards. The extraction efficiencies and response factors obtained from sodium hydroxide were equivalent to those obtained from control human hemoglobin; except that 1NA is poorly extracted in the presence of hemoglobin.

In Table 1 the retention times and the response factor per ng of internal standard of all

tested compounds are listed. With the work up method given in the Experimental section all calibration lines were linear ($r^2 > 0.99$, except for 34DCA with $r^2 = 0.90$). We suggest to use d_5-aniline as an internal standard for aniline but not for other amines; including *ortho*, *meta* and *para* methyl-substituted anilines. For the methylanilines the corresponding deuterated compounds were used as internal standards. ^{13}C-4-Chloroaniline is an appropriate internal standard for 3CA and 4CA and it can be used for the quantitation of 35DCA and 34DCA. For the dimethylanilines, d_3-24DMA or d_4-4MA should be used as internal standard. Reproducible determination of 4TFA was performed with d_4-4MA as internal standard. For 4ABP, 2NA, 1NA, 34DCA and 35DCA the best results were obtained with d_9-4ABP and 4'F4ABP.

Table 1 contains the chromatographic and mass spectrometric properties of the PFPA derivatives of 2-chloro-4-methylaniline (2C4MA), 4-fluoroaniline (4FA), 2,4-difluoroaniline (24DFA), 2-chloro-6-methylaniline (2C6MA), 5-chloro-2-methylaniline (5C2MA), 2,6-dichloroaniline (26DCA), 3-chloro-4-fluoroaniline (3C4FA), 3-chloro-4-methylaniline (3C4MA). The presence of these compounds has only been qualitatively analyzed as the response factors have not been determined.

3.3. Recovery and precision

The precision and accuracy of the method was determined by spiking hemoglobin (120 mg) solutions ($n = 5$) with two concentrations of amine mixtures (Table 2). The first experiment was performed with 2 ng of A and 2MA; with 1 ng of 2CA, 25DMA, 4TFA, 4MA, 24DMA, 26DMA, 35DMA, 4EA, 34DMA, 3CA, and 4CA; and with 0.5 ng of 2EA, 3MA, 3EA,

Table 2
Recovery and precision of aromatic amines spiked into hemoglobin

Amine	Amount (ng)	Recovery (%)	R.S.D. (%)	Amount (ng)	Recovery (%)	R.S.D. (%)
2CA	1	110	7	0.2	108	3
A	2	110	6	0.4	149	8
2MA	2	98	4	0.4	120	11
2EA	0.5	97	4	0.1	101	3
3MA	0.5	98	3	0.1	121	3
4TFA	1	108	4	0.2	111	4
25DMA	1	97	4	0.2	98	8
4MA	1	93	3	0.2	104	3
24DMA	1	91	2	0.2	111	3
26DMA	1	103	2	0.2	110	3
35DMA	1	100	2	0.2	112	4
23DMA	0.5	99	4	0.1	103	2
3EA	0.5	99	4	0.1	103	2
4EA	1	95	3	0.2	109	3
34DMA	1	89	2	0.2	103	4
246TMA	0.5	98	6	0.1	121	15
245TMA	0.5	92	3	0.1	168	2
3CA	1	98	2	0.2	100	5
4CA	1	107	3	0.2	122	2
35DCA	0.5	110	4	0.1	106	2
1NA	0.5	48	2	0.1	80	10
34DCA	0.5	110	6	0.1	252	30
2NA	0.5	97	4	0.1	99	3
3ABP	0.5	110	3	0.1	108	5
4ABP	0.5	106	4	0.1	105	2

23DMA, 246TMA, 245TMA, 35DCA, 1NA, 34DCA, 2NA, 3ABP, and 4ABP (standard amine mixture). The second experiment was done with 1/5 of the standard amine mixture. The results are represented in the Table 2. The recoveries of the amines from Hb-solutions were determined with calibration lines obtained from amines and internal standards extracted from sodium hydroxide solutions without Hb. The recoveries and the relative standard deviations (R.S.D.) are presented in Table 2.

3.4. Precision of hemoglobin adduct determinations

The precision of the hydrolysis of hemoglobin adducts was determined by hydrolyzing 5 times 100 mg Hb of an exposed worker. The only amines present in significant amounts were A and 2MA, 10.6 ng and 24.9 ng/g Hb, respectively. The R.S.D.s of the determinations were ± 4.8 and ± 8.8%, respectively. The precision of hemoglobin adduct determination of other amines has been shown in animal experiments [3,4].

3.5. Covalent binding of arylamines to hemoglobin

In order to test if, with the given procedure for the isolation of hemoglobin, non-covalently bound arylamines were eliminated, hemoglobin was precipitated with and without the presence of the internal standard mixture and washed with the sequence of organic solvents presented in the Experimental section. This experiment was performed in duplicate. The precipitated hemoglobin was hydrolyzed in the presence of the stan-

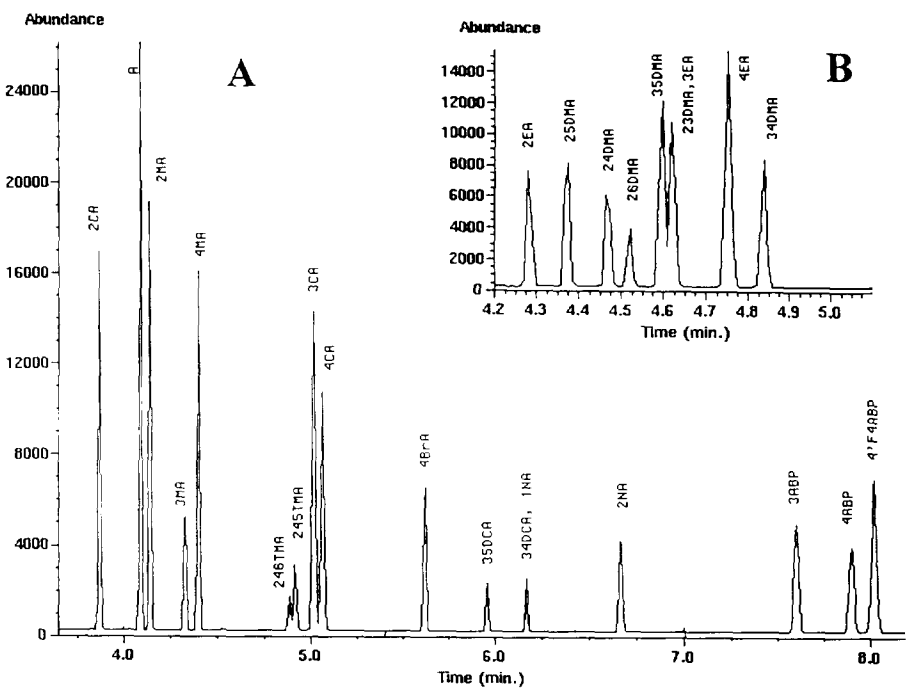

Fig. 1. Chromatogram of a hemoglobin hydrolysate spiked with 1 ng of each arylamine, except for A and 2MA with 2 ng and 2EA, 3MA, 23DMA, 3EA, 246TMA, 245TMA, 35DCA, 1NA, 34DCA, 2NA, 3ABP and 4ABP with 0.5 ng and the internal standards. Single ions were monitored in the negative chemical ionization (NCI) mode, with methane as the reagent gas (see Experimental). (A) Overlay of the single-ion chromatograms for the ions at *m/z* 219, 233, 261, 269, 287, 295, 297, and 313. (B) Overlay of the single-ion chromatograms at *m/z* 247. The peaks for the internal standards d_5A, d_3-2MA, d_3-3MA, d_3-4MA and d_3-24DMA with the single ions *m/z* 224, 237 and 250, respectively, are not represented in the Figure.

dard amine mixture. The levels of deuterated amines were not higher than in the control samples.

The same experiment was performed by precipitating hemoglobin from lysed erythrocytes with and without the presence of the standard amine mixture and washed with the sequence of organic solvents presented under the Experimental section. The precipitated hemoglobin of the spiked sample and of the control were hydrolyzed in the presence of the internal standard mixture. The levels of amines present in the control and spiked sample were the same. Therefore, the work-up procedure described for the isolation of hemoglobin eliminates non-covalently bound amines.

3.6. Detection limit and quantitation

The detection limits of the derivatized standard compounds are in the femtogram range for detection in the NCI mode. The determination limit from the present experiments was found to be 5–10 pg for all amines per analysis of 100–200 mg hemoglobin. For larger amounts of hemoglobin the determination limit should be re-evaluated. The limits might be the same or slightly higher.

The most problematic compound for quantitation was aniline. High background levels of up to 2 ng per sample were occasionally found in the control samples. The best results were obtained with the chemicals and glassware listed in the procedure. Furthermore only freshly opened PFPA should be used.

In Fig. 1 a typical chromatogram of an extract from human hemoglobin spiked with amines is shown. The single ions were monitored over a specific time period and not over the whole GC run in order to increase the dwell time per ion monitored. In Table 1 the fragment ions monitored are listed. For structural confirmation

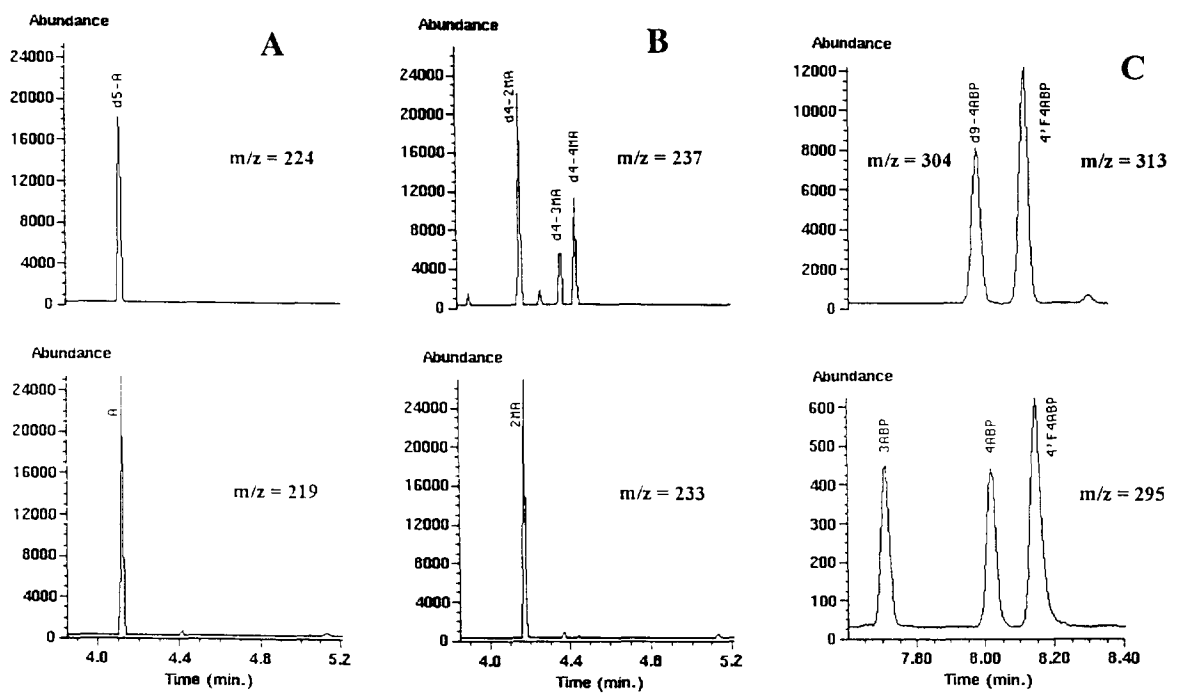

Fig. 2. Chromatogram of an hemoglobin extract of an exposed worker. Single ions were monitored in the negative chemical ionization (NCI) mode, with methane as the reagent gas (see Experimental). (A) Single-ion chromatogram at *m/z* 219 and 224, (B) *m/z* 233 and 237, (C) and *m/z* 295, 304 and 313. These peaks correspond to 1.6 ng aniline, 2 ng d_5-A, 2 ng 2MA, 2 ng d_4-2MA, 1 ng d_4-4MA, 0.5 ng d_3-4MA, 16 pg 3ABP, 19 pg 4ABP, 1 ng d_9-4ABP, 1 ng 4'F4ABP per 150 mg hemoglobin.

some samples were analyzed by monitoring the corresponding ions (Table 1) in the EI mode. Because of the enhanced selectivity of the negative-ion chemical ionization mode and because of its sensitivity this method was chosen for the quantitative determination of the hemoglobin adducts. With the given conditions and column length 3EA and 23DMA co-chromatograph and therefore, could not be distinguished. If these two amines are of concern we suggest the use of a 30-m GC column instead of a 15-m column. All the other compounds can be separated easily.

Typical chromatograms from an exposed worker are presented in Fig. 2. The levels of A and 2MA of the workers were up to 200 ng/g hemoglobin. The levels of the other arylamines (3MA, 4MA, 26DMA, 35DMA) were up to 10 times lower than the values published previously [10–12], except for 2NA, 3ABP and 4ABP, which were in the same concentration range (10–300 pg/g Hb).

4. Conclusions

The procedure presented here has been successfully applied to the biomonitoring of a group of over 70 workers exposed to A and 2MA. The simplicity of this method will make it possible to routinely monitor Hb adducts from people living or working in contaminated areas. If very low exposures are expected more hemoglobin could be used for the analyses. The volumes of the solvents and the glassware used in the present procedure should be increased accordingly.

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