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Note

Determination of aromatic amines in urine and serum

MICHAEL J. AVERY

113 Spedding Hall, Iowa State University, Ames, IA 50011 (U S A)

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Epidemiological studies have shown a strong correlation between chronic exposure to aromatic mono- and diamines and human bladder cancers [1]. Human exposure to this class of compounds occurs in the dye industry through inhalation or absorption through the skin. Workers involved in the production of substitute crude oils using the solvent refined coal (SRC) process may also be exposed to aromatic amines, since nitrogenous compounds may comprise up to 25% of the SRC end-product [2,3]. The measurement of exposure to this class of compounds is therefore of interest and requires advanced analytical procedures to measure accurately part per billion concentrations of amines without interference from the large numbers of matrix-related biological materials present in urine and serum.

Previous methods of determining the concentration of free aromatic amines in urine have utilized solvent extraction of samples up to 25 ml in size, multiple clean-up steps and high-performance liquid chromatographic separation to achieve 5–10 ng/ml sensitivity [4,5]. The addition of a derivatization step after the extraction has also been used in conjunction with gas chromatography–electron-capture detection [6,7], thin-layer chromatography [8] and gas chromatography–mass spectrometry [9] to achieve 1–10 ng/ml detectability.

The combined disadvantages of the existing procedures are: (1) sample volumes too large; (2) emulsions in the extraction step; (3) multiple steps to eliminate interferences; and (4) cumbersome. This paper describes a convenient procedure for the determination of aromatic amines in both urine and serum that circumvents these disadvantages while still achieving sensitivity better than the 1–10 ng/ml level. The procedure involves solid-phase extraction of the amines from small volumes of urine and serum using readily available cartridges containing octadecyl (C_{18}) bonded porous silica, derivatization of the isolated amines with fluorinated alkyl anhydrides and measurement by combined gas chromato-

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graphy–negative-ion chemical ionization mass spectometry (GC–NICIMS). The developed procedure was tested on samples of urine and serum spiked with varying amounts of 2-aminobiphenyl (AB) and 4,4′-diaminodiphenylmethane (DADPM).

EXPERIMENTAL

Materials and reagents

The derivatizing agents, trifluoroacetic anhydride (TFAA) (Aldrich, Milwaukee, WI, U.S.A.), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) (Pierce, Rockford, IL, U.S.A.), were used as received. Stock solutions of the aromatic amines AB (Fluka, Ronkonkoma, NY, U.S.A.), 9-aminoanthracene (AA) (Aldrich) and DADPM (Chemical Services, West Chester, PA, U.S.A.) were made up at 1.0 μg/ml in acetone and stored in a refrigerator. Working solutions of the amines were made up fresh daily by serial dilution of the stock solutions using acetone. Organic-free water (J.T. Baker, Phillipsburg, NJ, U.S.A.) was further purified by extraction with methylene chloride.

Methodology

For each test, a 1-ml C_{18} bonded porous silica cartridge (Analytichem International, Harbor City, CA, U.S.A.) was conditioned for solid-phase extraction by the passage of 1 ml of methanol followed by 1 ml of water.

Test samples were prepared by spiking 0.5-ml volumes of water, urine or serum with 1 μg of the internal standard AA and varying amounts of AB and DADPM. After sonication for 5 min, the pH was adjusted to 10 with 5 *M* sodium hydroxide and the sample passed through the conditioned cartridge, followed by a 1-ml water wash to remove water-soluble matrix components. The cartridge was dried by pulling room air through it with an aspirator for 5 min. The amines adsorbed on the C_{18} bonded silica were eluted with 200 μl of ethyl acetate and the eluate was evaporated to dryness with a nitrogen stream. A 20-μl volume of the derivatizing agent, either TFAA, PFPA or HFBA, was added to the vial and allowed to react with the amines for 10 min at 50°C. A 1-ml volume of hexane and 1 ml of 5% aqueous ammonia were added to the vial and the mixture was shaken for 30 s. The hexane layer was dried by passage through a 10-ml filtration column (J.T. Baker) containing 100 mg of anhydrous sodium sulfate and reduced in volume to about 100 μl with a nitrogen stream.

The best derivatizing agent was established using 10 ml of water spiked with 500 ng each of AB and DADPM. Three 1-ml aliquots of this sample were treated as above using a different anhydride for each aliquot. These derivatized extracts were then analyzed by GC–NICIMS. The best response was obtained using PFPA which was 15% better than HFBA and 20% better than TFAA.

Instrumentation

A Finnigan Model 4000 GC–MS apparatus with an INCOS 2300 data system (San Jose, CA, U.S.A.) was used for all analyses. The instrument was fitted with a DB-5 capillary column (J&W, Rancho Cordova, CA, U.S.A.) which led directly

into the mass spectrometer ion source; helium was the carrier gas at 25 cm/s linear velocity at 50°C. For each run, 1.8 μl of derivatized extract were injected splitless at 50°C for 30 s. The column temperature was held at 50°C for 4 min, then programmed to 265°C at 10°C/min. Full-scan mass spectral data were obtained with NICI using methane as the buffer gas at 0.30 Torr and an ion source temperature of 200°C. For quantitative studies, the $(M-HF)^-$ anions at *m/z* 295 for AB, 319 for AA and 470 for DADPM were monitored for 0.2 s each during a 1.0-s scan.

Quantitation

Response relative to concentration was determined using water solutions containing 2 μg/ml internal standard AA and 0.5–100 ng/ml AB and DADPM. Plots of the resulting data showed good linearity with correlation coefficients of 0.981 and 0.992 for AB and DADPM, respectively.

RESULTS AND DISCUSSION

The overall performance of solid-phase extraction, derivatization and GC–NICI IMS measurement is illustrated in Fig. 1, where a signal-to-noise ratio greater than 20:1 is obtained for 500 pg/ml DADPM spiked into pure water. Detection limits were about 50 and 100 pg/ml for AB and DADPM, respectively.

Excellent sensitivity was also obtained for spiked urine and serum. Figs. 2 and 3 illustrate the signals observed for AB and DADPM spiked into urine and serum at 5 ng/ml. The major difference between water and biological samples was an elevated background which would increase detection limits by about one order of magnitude over that achieved with water samples.

The NICIMS exhibited high sensitivity for the fluorinated compounds while producing minimal signals for any matrix compounds that might have been coextracted from the biological fluid. Spectra of the PFPA derivatives of AB and DADPM consist of mostly the $(M-HF)^-$ and $(M-2HF)^-$ anions.

Precision and accuracy for the method were obtained by analyzing three aliquots of a urine sample spiked at a concentration of 5 ng/ml. The measured concentrations of AB and DADPM were 4.73 ± 0.27 and 5.26 ± 0.21 ng/ml, respectively.

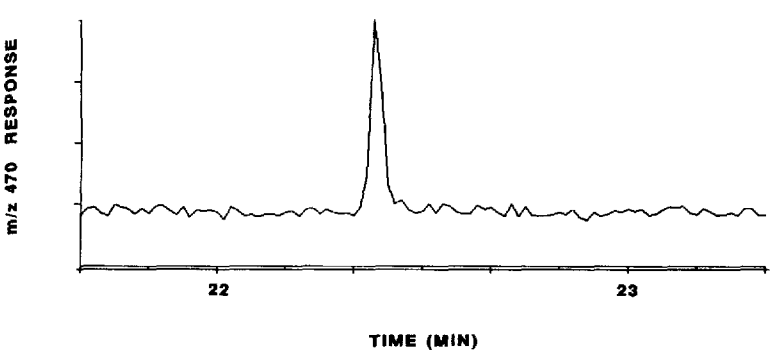

Fig. 1. GC–NICIMS response of PFPA derivatives of 0.25 ng of DADPM extracted from 0.5 ml water.

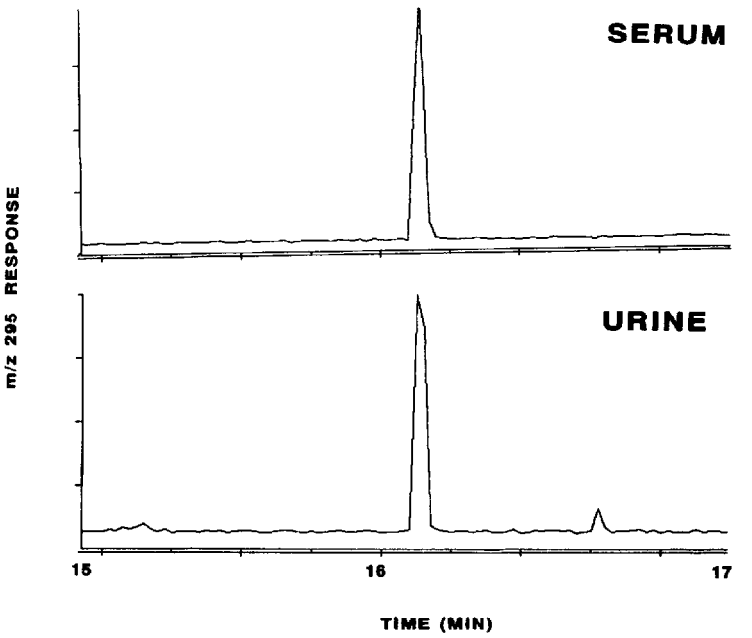

Fig. 2. GC–NICIMS response of PFPA derivatives of 2.5 ng of AB extracted from 0.5-ml samples of urine and serum.

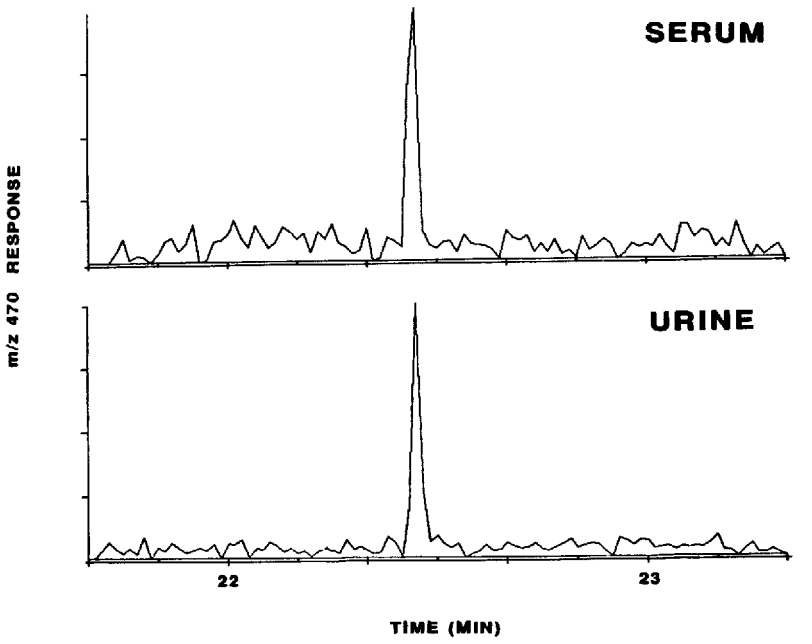

Fig. 3. GC–NICIMS response of PFPA derivatives of 2.5 ng of DADPM extracted from 0.5-ml samples of urine and serum.

The solid-phase extraction of the aromatic amines from the biological fluids was completely free of emulsions. A simple water wash of the cartridge removed endogenous compounds adsorbed from the urine and serum that could have been detrimental to subsequent separation and detection schemes. Additional benefits of the solid-phase extraction procedure are: a 100-fold decrease in the amount of organic solvent used; lower cost and reduced solvent-related hazards; more convenient; multiple extractions can be performed simultaneously; and the extraction can be automated.

CONCLUSION

The procedure should be applicable to other aromatic mono- and diamines. The use of high-resolution GC insures independent measurement of structural isomers. The major problem that might be encountered if other amines are determined is the possibility of interferences originating from the biological fluid matrix. NICIMS data obtained from blank urine and serum samples, shown in Figs. 4 and 5, illustrate the very few compounds detected by this procedure. At the retention times of the PFP derivatives of AB and DADPM at 16.2 and 22.4 min, no peaks are observed. This discrimination against endogenous materials means that the responses for other amine derivatives, obtained by monitoring single m/z values, would most likely be free of interferences.

The procedure measures the concentrations of free amines in the biological fluid. In some cases, where the total amine concentration is desired, any conjugated amines present must be cleaved prior to solid-phase extraction. If excessive protein precipitation occurs during the cleavage step, removal of the precipitate by centrifugation should be performed prior to the extraction to prevent plugging of the cartridge.

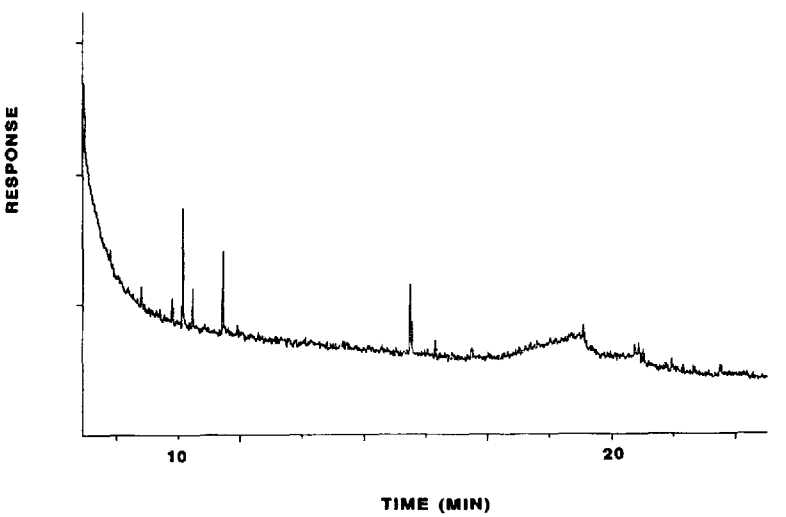

Fig. 4. GC–NICIMS reconstructed total-ion chromatogram (m/z 100–700) of a treated extract of a 0.5-ml blank urine sample.

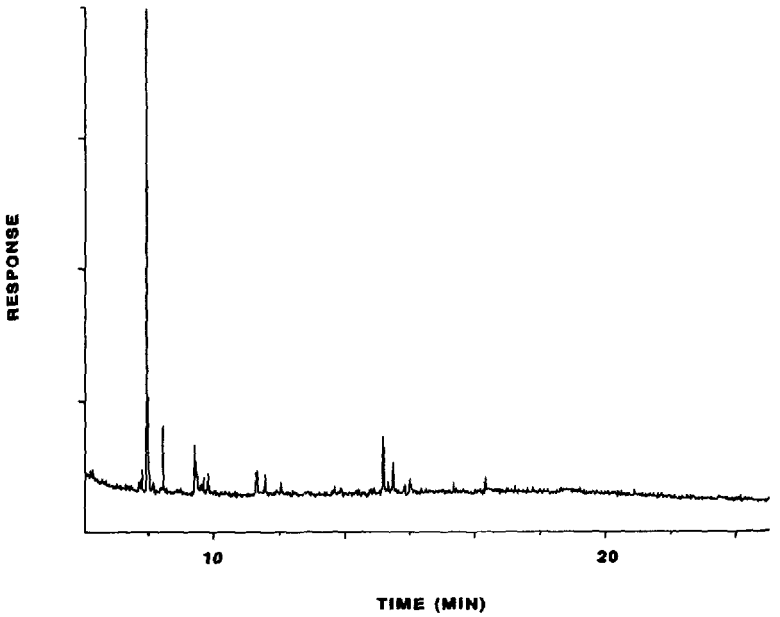

Fig. 5. GC–NICIMS reconstructed total-ion chromatogram (m/z 100–700) of a treated extract of a 0.5-ml blank serum sample.

ACKNOWLEDGEMENTS

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