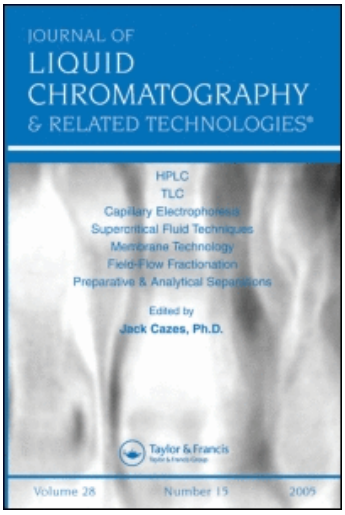

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High-Pressure Liquid Chromatography of N-(2-Propyl)-N'-Phenyl-p-Phenylenediamine (Ippd) and N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (Dbpd) and its Application to the Biomonitoring of Exposed Individuals

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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF N-(2-PROPYL)-N'-PHENYL-p-PHENYLENEDIAMINE (IPPD) AND N-(1,3-DIMETHYLBUTYL)-N'-PHENYL-p-PHENYLENEDIAMINE (DBPD) AND ITS APPLICATION TO THE BIOMONITORING OF EXPOSED INDIVIDUALS.

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ABSTRACT

A rapid method based on reverse-phase high-pressure liquid chromatography (HPLC) is described for the separation and quantitation of N-(2-propyl)-N'-phenyl-p-phenylenediamine (IPPD) and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (DBPD) in urine. Sample preparation is based on a simple extraction procedure and analysis is carried out on a chromatographic system using a LiChrosorb RP 18 column and buffered aqueous methanol as the mobile phase. The relationship between peak heights and amount injected was linear over a range of 0.05-5 µg for both compounds. Retention times and peak heights were highly reproducible. Detection was very sensitive, allowing quantitation of 5 ng of either compound. The application of the techniques for biomonitoring body fluids as an indicator of exposure to aromatic amines is discussed.

INTRODUCTION

The detection and determination of trace concentration of aromatic amines is of extreme importance, as many of these compounds have been demonstrated during the past few decades to be carcinogenic (1).

The development of a specific and sensitive method for their determination in the environment and in body fluids is therefore important and necessary.

Several procedures for quali-quantitative determination of aromatic amines, which provide a varying degree of sensitivity (TLC,GLC,HPLC) have been published (2-7).

IPPD and DBPD are two of the aromatic amines most widely-used as antioxidant agents in rubber industries.

The approach taken in this study was to develop a system which exploits the HPLC method to detect,in the nanomole range,aromatic amines in the urine of the exposed individuals.

MATERIALS AND METHODS

Standard IPPD and DBPD (Bayer) of known purity (greater than 98%) were used without further purification.The stock solution of 5 µg/ml was prepared by dissolving 5 mg of each standard,exactly weighed on a Cahn electrobalance mod. G2,in water purified by a MILLY-Q-System (Millipore Corp.,Bedford,Mass., U.S.A.) and diluting to volume into a 1000 ml volumetric flask.

Standard solutions were analyzed immediatly after preparation and kept in refrigerator (4 °C) to retard amines oxidation.

An appropriate quantity of aromatic amines standards were added to urine samples to yield urine standards containing 0.005,0.01,0.1,1,2 µg/µl.

These urine samples (100 ml) were extracted in order to isolate aromatic amines by shaking with analytical grade diethyl ether (50 ml) into a separatory funnel.The extraction was repeated twice,then the combined extracts were,if necessary,centrifuged (5 minutes at 2000 r.p.m.).The diethyl ether extracts were dried over anhydrous sodium sulfate,then the anhydrous extracts were eva-

porated to dryness under reduced pressure in a rotary evaporator.The residues were redissolved in methanol (LiChrosolv,Merck),transferred to 10 ml volumetric flasks and brought to volume with methanol.The volume of solution injected ranged from 1 to 10 µl.

HPLC analyses were run on a Perkin-Elmer Series 3B liquid chromatograph. Component elution was monitored with a LC-75 variable wavelength detector (160-600 nm) equipped with a LC-75 Autocontrol.The column used was a Hibar--LiChrosorb RP 18 10 µm (Merck,25 x 0.26 cm I.D.).The separations reported were achieved under the following conditions : mobile phase,methanol-water (78:12), adjusted to pH 6.7 with ammonium hydroxide,0.02 M in ammonium acetate;flow rate,2 ml/min;temperature,28 °C;wavelength,290 nm;chart speed,0.5 cm/min. Graphs were generally obtained with an attenuation setting corresponding to 0.04 AUFS on a 10 mV recorder and peak areas were determined by a Perkin-Elmer Sigma 10 integrator.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram obtained on a synthetic mixture of IPPD and DBPD.IPPD and DBPD peaks are symmetrical with baseline resolution.Under the employed conditions the retention times were higly reproducible;20 injections of each compound over a period of two weeks gave mean retention times of 156 and 324 sec,with coefficients of variation of 0.72 and 1.19% for IPPD and DBPD respectively.Calibration curves of peaks area (or height) against the amounts of IPPD and DBPD injected were constructed from triplicate injections of six standard solutions of IPPD and DBPD,they were found to be linear over a 0.05--5.0 µg range for both compounds.The calibration curves obtained with IPPD and DBPD in water and urine showed no significant differences and standard solutions

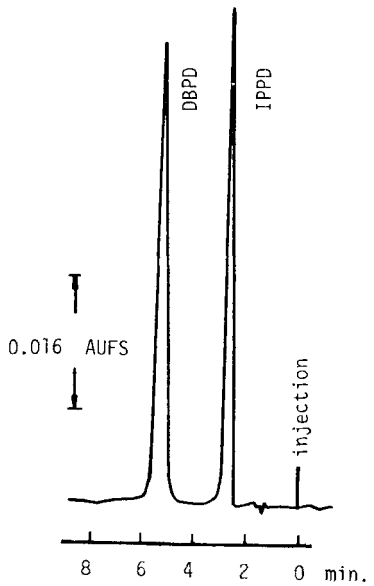

FIGURE 1

HPLC chromatogram of an aqueous solution (3µl) of IPPD and DBPD standard Conditions : column,LiChrosorb RP 18 10 µm (Merck,25 x 0.26 cm I.D.);mobile phase,methanol-water (78:12),adjusted to pH 8.7 with ammonium hydroxide,0.02 M in ammonium acetate;flow rate,2 ml/min;temperature,28 C;wavelength,290 nm;chart speed,0.5 cm/min.

were thus made up in water for convenience.Figure 2 illustrates the HPLC graph of urine containing IPPD,in which it can be seen that the matrix gives no interference at the retention time of the peak of IPPD.

The precision of the outlined method was studied by injecting fifteen 10 µl aliquots of the IPPD and DBPD synthetic mixture containing 0.25 and 0.35 µg respectively.Reproducibility measured as peak height was good,with coefficients of variation of 1.025-1.210% due to the combined errors of HPLC resolution,injection and detection.

With instrument sensitivity of 0.02 AUFS,the minimum detectability was 5--10 ng;below this level baseline detector noise exceeded peak height.

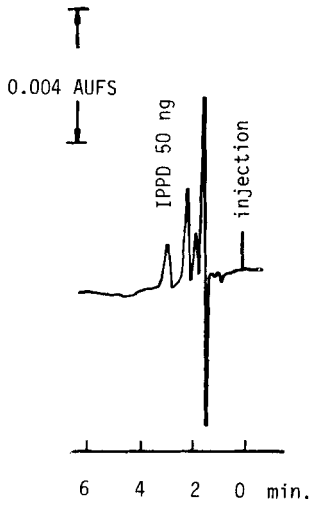

FIGURE 2

HPLC chromatogram of human urine containing IPPD standard (10µl).Conditions same as in FIGURE 1.

Human urine containing IPPD and DBPD showed recovery for IPPD in the range of 93-98.5% with an average recovery of 95.5% and for DBPD in the range of 89--98% with an average recovery of 93.5%.Recoveries were calculated by comparing peak heights from volumetric dilutions of equivalent amounts of IPPD and DBPD.

CONCLUSION

The method described is suitably accurate,rapid,selective and sensitive for determining IPPD and DBPD in water and urine samples.Many other separations of closely related compounds can be performed in a similar way and may be used in routine analysis.The choice of the mobile phase composition (percentage of water in methanol,ammonium acetate concentration and pH) permits the simple regulation

of solute capacity factors,thus providing a rapid and effective solution for specific problems.A one-step extraction procedure was chosen and developed to reduce the amount of sample handling;consequently the potential for loss of material and possible contamination is minimized.No elevated or irregular baselines nor pressure build-ups have been encountered while working with these partially purified samples.

Furtheremore the case under examination,illustrated above (human urines containing exogenous aromatic amines) demonstrates that the method employed is a useful approach to the biomonitoring of IPPD and DBPD in the urines of exposed individuals.

Investigations into the presence of IPPD and DBPD in body fluids of rubber workers are currently in progress in our laboratories.

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