Journal of Chromatography, 174 (1979) 379–384
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11,791

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF 2,6- AND 2,4-DIAMINOTOLUENE, AND ITS APPLICATION TO THE DETERMINATION OF 2,4-DIAMINOTOLUENE IN URINE AND PLASMA

PETER D. UNGER and MARVIN A. FRIEDMAN
Allied Chemical Corporation, Corporate Medical Affairs, Morristown, N.J. 07960 (U.S.A.)
(Received February 13th, 1979)

SUMMARY

2,4-Diaminotoluene is used for the production of industrial dyes, and along with the 2,6-isomer, as an intermediate in the production of polyurethane foams. 2,6- and 2,4-diaminotoluene were resolved as sharp peaks by normal-phase high-performance liquid chromatography in 3 min by an acetonitrile–water-saturated chloroform elution solvent (8:2, v/v) with detection by ultraviolet absorbance at 250 nm. The relationship between peak height and amount injected was linear over a range of 0.025–2 µg for both compounds. Retention times and peak heights were highly reproducible. Detection was very sensitive, allowing quantitation of 1–2 ng of either compound. Quantitative recovery of 2,4-diaminotoluene from spiked urine and plasma samples was obtained by extraction with methylene chloride.

INTRODUCTION

2,4-Diaminotoluene (2,4-TDA), an industrial intermediate, is used in the production of polyurethane, industrial dyes and, until 1971, in hair-dye formulations. Most of the diaminotoluene that is produced in the U.S.A. is in the form of a mixture of unisolated isomers, primarily 2,6-TDA (*ca.* 20%) and 2,4-TDA (80%). In the production of polyurethane, the mixture of isomers is first phosgenated to the corresponding toluene diisocyanate (TDI). In 1975, an estimated 265–290 million kg of 2,4-TDA was produced in the U.S.A. and Western Europe. Figures for the production of 2,6- and 2,4-TDA as an unisolated mixture are not available, but in 1975 seven U.S. companies produced 217.4 million kg of a mixture of TDI isomers (20% 2,6-isomer and 80% 2,4-isomer)[1].

2,4-TDA has been reported to be mutagenic in the Ames test after *in vitro* metabolic activation[2], transforming to secondary hamster embryo cells[3], and carcinogenic to rodents[4–7]. Ito *et al.*[7] reported hepatocellular carcinomas in rats fed diets containing 0.06 or 0.1% 2,4-TDA for 30–36 weeks. Umeda[5] reported that weekly subcutaneous injections of 0.5 ml of a 4% solution of 2,4-TDA in propylene glycol produced rhabdosarcomas in all rats surviving more than 8 months (9 of 20).

Several procedures for the detection of 2,4-TDA have been published, including

thin-layer chromatography (TLC)[8–11] paper chromatography[12], and gas chromatography[10]. Gas chromatography has been used to separate the diaminotoluene isomers[13–15]. Guthrie and McKinney[16] have recently reported a sensitive TLC procedure for the determination of 2,6- and 2,4-TDA in urethane foams using fluorimetric detection. No high-performance liquid chromatography (HPLC) procedure for quantitation of diaminotoluenes, however, has been described. The present study, therefore, was undertaken to develop a sensitive and reliable HPLC procedure for the determination of 2,6- and 2,4-TDA, adaptable to biomonitoring and metabolic studies.

EXPERIMENTAL

Standards

2,6- and 2,4-TDA, of greater than 98% purity, were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Crystalline 2,6- and 2,4-TDA were weighed and dissolved in the appropriate volume of the elution solvent to yield standards containing 0.0025, 0.005, 0.01, 0.05, 0.1, and 0.2 μg/μl. Standard solutions were analyzed immediately after preparation, and kept on ice to retard amine oxidation.

Equipment

Normal-phase chromatography was performed using an LDC Constametric IIG HPLC system including an LDC Spectromonitor II variable wavelength absorbance detector (Laboratory Data Control, Riviera Beach, Fla., U.S.A.). Samples were injected onto the column using a Valco N60 fixed-volume (10 μl) loop injector (Valco Instruments, Houston, Texas, U.S.A.). Separations were achieved with a Lion Technology (Dover, N.J., U.S.A.) Silarex SRI silica adsorption column (10 μm particle size) at an elution rate of 3 ml/min. 2,6- and 2,4-TDA were detected at 250 nm, with the absorbance detector at sensitivities of 0.01–1.28 absorbance units full scale (AUFS). Solvent programming (Gradient Master, LDC) was used to establish optimum solvent ratios.

Elution solvent

The elution solvent consisted of glass-distilled acetonitrile and water-saturated chloroform (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.). Water-saturated chloroform was prepared by adding 100 ml of distilled water to 1 l of chloroform, shaking four times (*ca.* 1 min each time), and allowing the mixture to stand overnight. A ratio of 8 parts of acetonitrile to 2 parts of water-saturated chloroform was found to give good resolution of 2,6-TDA from 2,4-TDA, and adequate separation of 2,4-TDA from interfering peaks in plasma and urine extracts.

Extraction of 2,4-TDA from rat urine and plasma

Rat urine and plasma samples were extracted twice with four volumes of methylene chloride (Burdick & Jackson) by vortexing for 1.5 min each time. The methylene chloride extracts were pooled, evaporated to dryness under a steady stream of helium, and the residue dissolved in the elution solvent for HPLC analysis. To determine the linearity of 2,4-TDA recovery, 5, 10, 20, and 100 μg of 2,4-TDA were added to 0.5 ml urine and plasma samples and extracted as above.

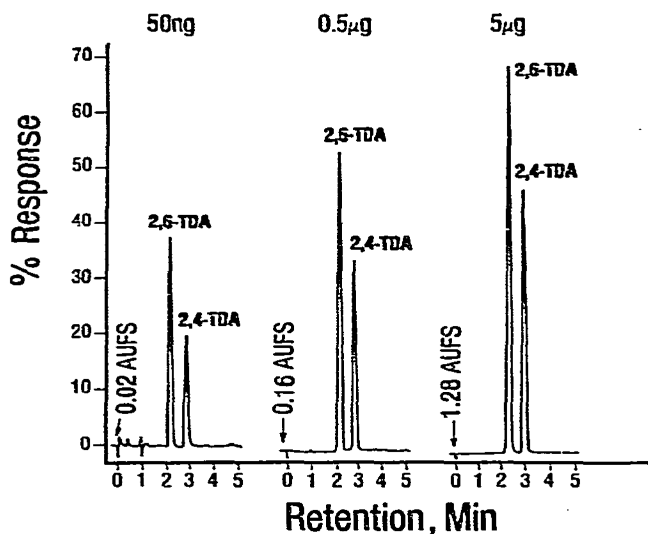

Fig. 1. HPLC resolution of 2,6- and 2,4-TDA on Silarex SRI column; elution solvent acetonitrile–water-saturated chloroform (8:2, v/v); flow-rate 3.0 ml/min; 0.05, 0.5 and 5 μg of each compound injected.

RESULTS AND DISCUSSION

Retention time

At a flow-rate of 3 ml/min, using acetonitrile–water-saturated chloroform (8:2, v/v) as the elution solvent, 2,6- and 2,4-TDA eluted as sharp, symmetrical peaks with baseline resolution (Fig. 1). The retention times were highly reproducible with the elution solvent and column used; ten injections of each compound over a period of several days gave mean retention times of 130 and 180 sec, with coefficients of variation of 0.58 and 1.52% for 2,6- and 2,4-TDA, respectively (Table I).

Linearity

The relationship between peak heights and amount of 2,6- and 2,4-TDA injected was linear over a 0.025–2.0 μg range for both compounds (Fig. 2). The peak heights were converted to a common sensitivity and plotted against the quantity injected.

TABLE I

REPRODUCIBILITY OF RETENTION TIME FOR 2,6- AND 2,4-TDA BY HPLC

Statistic	*2,6-TDA*	*2,4-TDA*
Injections, N*	10	10
Retention time (sec)		
range	129–132	177–184.5
mean	130.6	179.7
standard deviation (sec)	0.76	2.73
coefficient of variation**	0.58	1.52

* Successive injections of a mixture of 0.25 μg 2,6-TDA and 0.375 μg 2,4-TDA.
** Standard deviation/mean × 100.

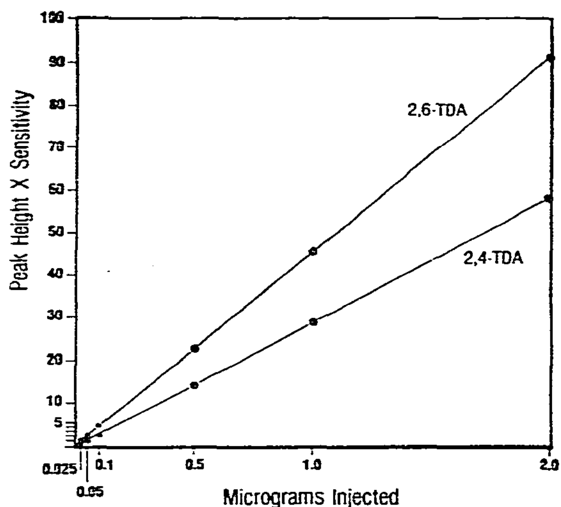

Fig. 2. Linearity, peak height *vs.* amount of 2,6- and 2,4-TDA injected.

Precision and sensitivity

Precision was evaluated by injecting ten 10-μl aliquots of a mixture of 2,6- and 2,4-TDA containing 0.25 and 0.375 μg, respectively. Reproducibility of peak height was good, with coefficients of variation of 1.003–1.18%, representing the combined errors of HPLC resolution, injection and detection (Table II).

TABLE II

PEAK HEIGHT REPRODUCIBILITY FOR 2,6- AND 2,4-TDA BY HPLC

Statistic	*2,6-TDA*	*2,4-TDA*
Injections, N*	10	10
Peak height (mm)		
range	128.5–132.0	124–128.0
mean	130.4	126.4
standard deviation (mm)	1.31	1.49
coefficient of variation (%)**	1.003	1.18
sensitivity (mm peak height/ng)***	8.35	5.39

* Successive 10-μl injections of 0.25 μg 2,6-TDA and 0.375 μg 2,4-TDA, at a sensitivity of 0.08 a.u.f.s.

** Standard deviation/mean × 100.

*** Calculated to a maximum usable sensitivity of 0.005 a.u.f.s.

The mean sensitivity of detection (millimetres of peak height/nanogram of 2,6- and 2,4-TDA; Table II) and the chromatograms (Fig. 1) indicate that 1–2 ng of either compound can easily be detected.

Recovery of 2,4-TDA from rat urine and plasma

Good recovery of 2,4-TDA was obtained from spiked rat urine and plasma samples (Table III). Chromatogram tracings of methylene chloride extracts of rat

TABLE III

RECOVERY OF 2,4-TDA FROM SPIKED URINE AND PLASMA SAMPLES

2,4-TDA added (μg/ml)	*Recovery (%), mean ± S.E.*	
	Urine	*Plasma*
0	—	—
10	90.31 ± 1.56	89.02 ± 0.83
20	92.36 ± 2.61	90.39 ± 1.02
40	89.53 ± 0.26	90.31 ± 5.27
200	101.64 ± 0.99	87.71 ± 3.52

urine and plasma samples are shown in Fig. 3. Nine different extraction solvents were tested, including hexane, cyclohexane, methylene chloride, ethyl acetate, chloroform and various ratios of a mixture of acetonitrile and ethyl acetate. The poorest recoveries were obtained with hexane and cyclohexane (<13%). Extraction with ethyl acetate yielded much higher recoveries (60–75%). The highest recoveries, however, were obtained by extraction with methylene chloride.

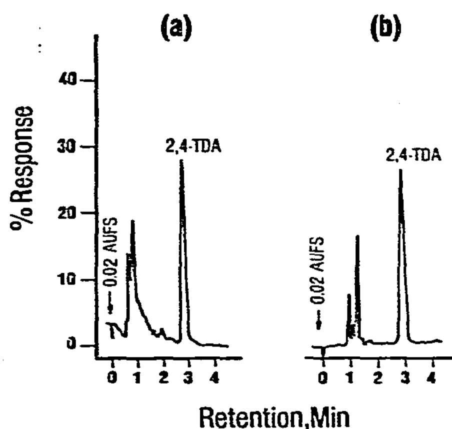

Fig. 3. Chromatogram tracing of extract of urine (a) and plasma (b) spiked with 2,4-TDA to 10 μg/ml. See text for column and conditions.

The procedures described here, with the inclusion of an extraction step for the quantitative determination of known 2,4-TDA metabolites, are currently being used in this laboratory for the study of the metabolism of 2,4-TDA in the rat.

REFERENCES

1 U.S. International Trade Commission, *Synthetic Organic Chemicals, U.S. Production and Sales, 1975*, USITC publication 804 (1977) 22, 36, 42, 49, 60, 62, 65, and 74.
2 B. N. Ames, H. O. Kammen and E. Yamasaki, *Proc. Nat. Acad. Sci. U.S.*, 72 (1975) 2423.
3 R. J. Pienta, M. J. Shah, W. B. Lebherz and A. W. Andrews, *Cancer Lett.*, 3 (1977) 45.
4 S. Watanabe, *Trans. Jap. Pathol., Soc.*, 27 (1937) 421.
5 M. Umeda, *Gann*, 46 (1955) 597.
6 M. Umeda, *Gann*, 47 (1956) 153.
7 N. Ito, Y. Hiasa, Y. Konishi and M. Marugami, *Cancer Res.*, 29 (1969) 1137.

8 C. Kotteman, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 954.
9 G. F. Macke, *J. Chromatogr.*, 36 (1968) 537.
10 T. Glinsukon, T. Benjamin, P. Grantham, E. Weisburger and P. Roller, *Xenobiotica*, 5 (1975) 475.
11 T. Glinsukon, T. Benjamin, P. Grantham, N. Lewis and E. Weisburger, *Biochem. Pharmacol.*, 25 (1976) 95.
12 R. H. Waring and A. E. Pheasant, *Xenobiotica*, 6 (1976) 257.
13 C. E. Boufford, *J. Gas Chromatogr.*, 6 (1968) 438.
14 L. E. Brydia and F. Willeboordse, *Anal. Chem.*, 40 (1968) 110.
15 F. Willeboordse, Q. Quick and E. T. Bishop, *Anal. Chem.*, 40 (1968) 1455.
16 J. L. Guthrie and R. W. McKinney, *Anal. Chem.*, 49 (1977) 1676.