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High-Performance Liquid Chromatographic Determination of Organic Substances by Metal Chelate Derivatization. II.[1] Microdetermination of Methamphetamine and Amphetamine

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The reaction of Ni(II) dithiocarbamate chelate formation was applied as a color reaction in high-performance liquid chromatography (HPLC) analysis of aliphatic primary and secondary amines. The reaction proceeded quantitatively and almost instantaneously at room temperature in alkaline media. The deep yellow ($\log \varepsilon \fallingdotseq 4.5$ at 325 nm) reaction products were extractable with organic solvents and gave sharp peaks on both normal-phase and reverse-phase chromatography. This method was applied to the microdetermination of stimulant drugs, *e.g.* methamphetamine and amphetamine, in urine, and as little as 1 ng of these drugs could be detected.

Keywords——methamphetamine; amphetamine; aliphatic primary amine; aliphatic secondary amine; Ni(II) dithiocarbamate; psychotropic drug; high-performance liquid chromatography

Methamphetamine and amphetamine are well known stimulant drugs, and the abuse of these drugs is a serious social problem. For this reason, microdetermination of these drugs is of forensic interest, and gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) have been utilized as routine techniques.[2] Other methods such as thin-layer chromatography (TLC),[3] immunoassay,[4] and fluorimetry[5] have also been reported. High-performance liquid chromatography (HPLC) seems to be a reliable method and several reports on this approach have already appeared.[6] However, microdetermination of these stimulant drugs faces the problem of their weak absorption in the ultraviolet (UV) range. The development of a rapid and convenient color reaction is therefore desirable, and several attempts[7] to do this have been made, including our previous study based on 1,2-naphthoquinone-4-sulfonic acid derivatives.[8] In the present report, we describe the derivatization of various aliphatic primary and secondary amines including methamphetamine and amphetamine, into Ni(II) dithiocarbamate chelates, and HPLC analysis of the reaction products. The formation of dithiocarbamate is an important color reaction for aliphatic primary and secondary amines,[9] and has already been applied as a color reaction in a screening test for methamphetamine in urine.[10]

The quantitative formation of dithiocarbamate chelates and their extraction with organic solvent over a wide pH range[11] have been examined in detail by many workers.

$$2R_1R_2NH + 2CS_2 + 2OH^- + M^{2+} \rightleftharpoons (R_1R_2NCSS)_2M + 2H_2O \quad (1)$$

Our previous reports[1,12] have elucidated the characteristic features of chromatograms of metal dithiocarbamates. When M is a divalent metal ion and two kinds of dithiocarbamates, A and B, are present, three metal chelates, MA_2, MB_2 and MAB, are formed in the equilibrium state as follows.

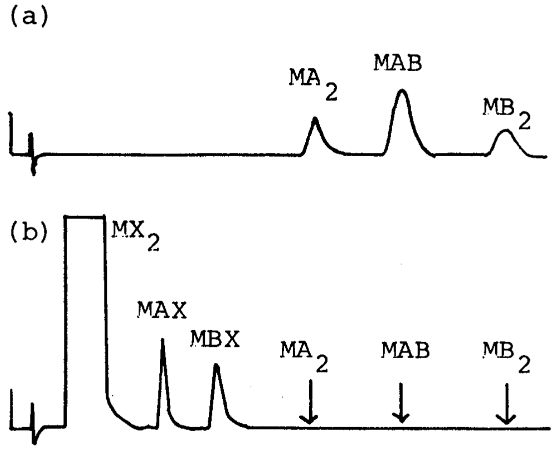

Fig. 1. Chromatogram Patterns of Ni(II) Dithiocarbamates of Aliphatic Amines in the Presence of Foreign Amine

Two aliphatic amines (A_0 and B_0) were derived into the corresponding dithiocarbamates (A and B) and extracted as Ni(II) chelates. To this mixture was added a solution of Ni(II) dithiocarbamate derived from a foreign amine (MX_2). After standing for some time to equilibrate ternary complex formation ($MA_2+MX_2 \rightleftharpoons 2MAX$, *etc.*), the solution was subjected to HPLC. The chromatogram pattern exemplified in this figure for a silica-gel column, hexane–isopropyl acetate and dibutylamine as the column, eluent and foreign amine, respectively.
(a) $[X]=0$ and $[A]\fallingdotseq[B]$. (b) $[X]\gg([A]+[B])$.

$$MA_2+MB_2 \rightleftharpoons 2MAB \tag{2}$$

$$K=[MAB]^2/[MA_2][MB_2] \tag{3}$$

In the case of Ni (II), $K=4.0$ and three peaks appear on the chromatograms. This phenomenon results in complicated chromatograms, and Liska *et al.*[13] claimed that it was difficult to determine individual amines by application of the conversion into dithiocarbamate chelates. However, we considered that this difficulty might be overcome as follows. When a mixture of two amines A_0 and B_0 is converted into the corresponding dithiocarbamates, A and B, three peaks appear on chromatograms (Fig. 1(a)). However, when a large excess amount of a foreign amine X_0 coexists and is converted into the dithiocarbamate X, the chromatogram patterns should be different. For example, when more than 100-fold excess of X_0 coexists, more than 99% of A_0 and B_0 should be converted into ternary complexes MAX and MBX, and the presence of binary complexes MA_2 and MB_2 is then negligible (Fig. 1(b)). Thus determination of individual amines should be possible by this procedure.

Experimental

Materials——Standard solution of Ni(II) (0.1 M) was prepared by dissolving $NiCl_2 \cdot 6H_2O$ in 25% aqueous ammonia. The content of Ni(II) was determined by colorimetry as diethyldithiocarbamate.[14] In addition to *d*-methamphetamine and *d*-amphetamine, the following aliphatic primary and secondary amines, including psychotropic drugs, were used: 2,5-dimethoxy-4-methylamphetamine (STP), mescaline, methylphenidate, phenmetrazine, *l*-ephedrine, *d*-pseudoephedrine, *dl*-norephedrine, methylamine, ethylamine, propylamine, isopropylamine, butylamine, isobutylamine, *sec*-butylamine, *tert*-butylamine, isopentylamine, hexylamine, octylamine, benzylamine, phenethylamine, ethanolamine, dimethylamine, diethylamine, dipropylamine, diisopropylamine, dibutylamine, diisobutylamine, pyrrolidine, piperidine, *dl*-2-methylpiperidine, perhydroazepine, dibenzylamine, morpholine, *N*-methylbenzylamine, *dl*-1-benzylmethylamine and diethanolamine. Other related compounds including several aliphatic tertiary amines, quaternary ammonium salts, aromatic amines, amino acids, catecholamines and amides were tested for comparison. Sodium salts of *N*,*N*-diethyldithiocarbamic acid and pyrrolidinedithiocarbamic acid were obtained commercially. Sodium (or ammonium) salts of various dithiocarbamic acids were synthesized by the usual procedure[15] from corresponding amine, carbon disulfide and sodium hydroxide (or aqueous ammonia) in hexane. These salts were recrystallized from either chloroform–methanol or chloroform–hexane. Standard solutions of various Ni(II) dithiocarbamate chelates were prepared as follows. A slight excess of the appropriate dithiocarbamate salt was added to an aliquot of Ni(II) standard solution. The mixture was buffered at pH 6 with KH_2PO_4–Na_2HPO_4, and chelate formed was extracted twice with chloroform. The chloroform layer was made up to a definite volume. Measurements of the absorption spectra of these solutions revealed that each dithiocarbamate has a similar absorption maximum at 325 nm ($\log \varepsilon \fallingdotseq 4.5$).

Apparatus——An HPLC apparatus of our own construction[16] equipped with a variable-wavelength UV detector (model SPD-1, Shimadzu Co., Ltd.) and a minicomputer (Chromatopac E-1A, Shimadzu Co., Ltd.) was used. Silica gel packings (LiChrosorb SI 60 (5 μm), LiChrosorb SI 100 (5 μm), Polygosil 60-5 (5 μm) and Wakogel LC-10H (10 μm)) and reverse phase packings (Nucleosil 5C18 (5 μm), Nucleosil 5C8 (5 μm), LiChrosorb RP 18 (5 μm), LiChrosorb RP 8 (5 μm) and Hypersil ODS (5 μm)) were slurry-packed into stainless steel columns. Since the retention time on silica gel packings was very sensitive to the water content in the eluent, the water content was controlled as follows. First, 50 cm^3 of 3% water–containing acetone was pumped through the column, then water–saturated hexane–isopropyl acetate was passed through the column until base line drift was no longer observed. Reproducible results were thus obtained when water-saturated eluent was used.

Extraction of Methamphetamine and Amphetamine in Urine——An aliquot of urine (100 cm^3) of a methamphetamine addict was mixed with 1 cm^3 of conc. aqueous ammonia, and free bases were extracted with three 50 cm^3 portions of hexane. Then the combined hexane layer was back-extracted with two 20 cm^3 portions of 1% hydrochloric acid. An aliquot of the aqueous layer was used for the color reaction. Addition of methamphetamine or amphetamine to blank human urine (1 mg to 100 cm^3 of urine) resulted in satisfactory recovery by this extraction procedure (91.1%, $n=4$, C.V. 4.8% for methamphetamine and 88.9%, $n=4$, C.V. 4.3% for amphetamine). Large proportions of other hydrophilic amines such as methylamine and ethanolamine were removed by the extraction with hexane.

Urine from rats which had received *d*-methamphetamine was also analyzed. Male rats of the Wistarstrain weighing 130—150 g were used. *d*-Methamphetamine (10 mg/kg) was intraperitoneally injected into five animals, and 24 h urine was collected for a week.

Results and Discussion

Optimum Conditions for Color Reaction

Experiments were carried out to determine the optimum conditions for the formation and extraction of Ni(II) dithiocarbamate derived from various amines. Of several procedures tested, the following gave the best results. Ni(II) standard solution and chloroform containing 5% carbon disulfide were added to a dilute amine solution. The mixture was shaken vigorously in a test tube equipped with a stopper for 0.5—2.0 min to complete the reaction and extraction. The progress of the reaction and extraction was slow when less polar solvents such as hexane and benzene were used instead of chloroform. The reaction rates of *dl*-norephedrine and mescaline were slower than those of other amines. The reactions of these amines were accelerated when the pH of the solution was increased to 12 or more by adding sodium hydroxide. The reaction yields were examined for various amines by comparing the absorbance of reaction products with those of Ni(II) dithiocarbamate solutions prepared as mentioned in the experimental section. The reaction yields were 99.3, 100.1, 98.0, 99.4, 98.3, 99.0 and 99.2% for diethylamine, dibutylamine, pyrrolidine, piperidine, propylamine, phenethylamine and *l*-ephedrine, respectively (mean values of three measurements). Reaction yields of other amines were estimated by measuring peak areas on normal phase chromatography under the conditions described in the following section. Each peak area was similar to (±5%) that of diethylamine when the same amount (in mol) of amine was used. Thus, most amines seem to give quantitative results, provided that the absorption coefficients of these reaction products at 325 nm are similar.

Of various aliphatic primary and secondary amines, only ethanolamine, diethanolamine and methylamine failed to give quantitative results. The incomplete extraction of methylamine can be attributed to the relatively large solubility of the formed chelate in water. Similarly, since dithiocarbamate chelates derived from ethanolamine and diethanolamine are highly soluble in water because of the OH group, these chelates were not extracted with organic solvents such as chloroform and hexane. The reaction did not proceed with amino acids under the present conditions, though they were reported to give dithiocarbamates under conditions of prolonged reaction time and elevated temperature.17) Catecholamines did not give any reaction products in the chloroform layer. This seems to be attributable either to the formation of water-soluble chelates or to the decomposition of chemically rather unstable catecholamines. Aliphatic tertiary amines, quaternary ammonium salts, amides and aromatic amines did not react at all.

Normal Phase HPLC

Ni(II) standard solution and 5% carbon disulfide in chloroform were added to a dilute solution of a mixture of several aliphatic amines (A_0, B_0, C_0 *etc.*), and then the color reaction was carried out. Each amine was converted to the corresponding dithiocarbamate (A, B, C, *etc.*). A large excess amount of Ni(II) dibutyldithiocarbamate (MX_2) solution was added to an aliquot of the chloroform solution, and the mixtures was left standing for at least 1 h. Ternary complexes (MAX, MBX, MCX, *etc.*) were gradually formed and equilibrium was attained. The solution was then subjected to HPLC. Examples of chromatograms of mixtures of several amine samples and psychotropic drugs are shown in Figs. 2 and 3, respectively; more than 100-fold excess of MX_2 was added in these cases. Following the large peak of MX_2, which was eluted first, peaks of ternary complexes appeared on the chromatograms. Peaks of binary complexes (MA_2, MB_2, MC_2, *etc.*) did not appear on the chromatograms. Thus, individual amines could be determined by the present method. The amount of MX_2 required was investigated. A solution of dithiocarbamate chelate derived from *d*-methamphetamine was run with 100-, 200-, 500- and 1000-fold excess of MX_2. On statistical grounds, 99, 99.5, 99.8 and 99.9%, respectively, of methamphetamine should be converted into the ternary complex.[12] Peak heights of methamphetamine of these solutions were identical within 1%.

The retention behavior of various amines was examined on different silica gel packings. The following order of increasing retention times was found: LiChrosorb SI 100 < Wakogel LC-10H < Polygosil 60-5 < LiChrosorb SI 60. The elution sequence of amines did not differ when different packings were used. The retention times of amines on Polygosil 60-5 and LiChrosorb SI 60 columns are summarized in Table I. The retention times of amines were found to decrease with increase in the carbon number of the alkyl chain. This can probably be interpreted in terms of the solubility of the metal chelate in the eluent, because the solubility

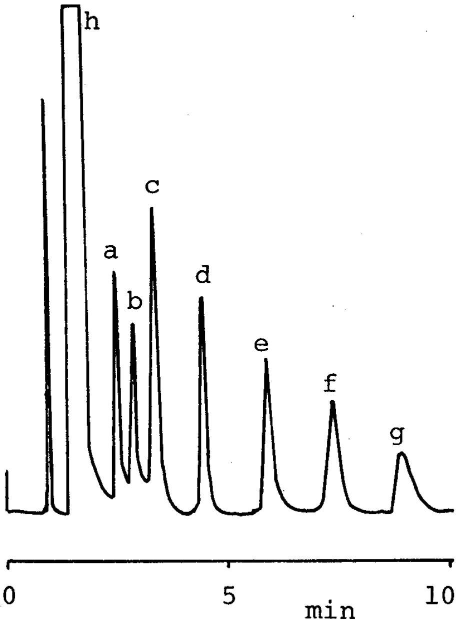

Fig. 2. Chromatogram of Simple Amines on Silica-gel Packings

Column: Polygosil 60-5 (5 μm), 4 mm × 25 cm.
Eluent: hexane–isopropyl acetate = 100 : 5 (water-saturated) at 2.0 ml/min. Detection: UV 325 nm.
a, diethylamine; b, perhydroazepine; c, piperidine; d, pyrrolidine; e, butylamine; f, propylamine; g, ethylamine; h, dibutylamine (peak of excess binary complex MX_2).

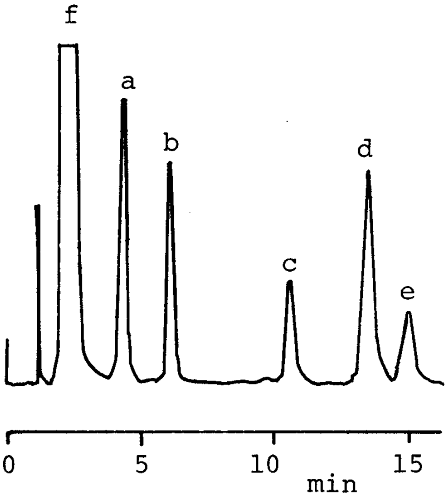

Fig. 3. Chromatogram of Psychotropic Amines on Silica-gel Packings

Column: LiChrosorb SI 100 (5 μm), 4 mm × 25 cm.
Eluent: hexane–isopropyl acetate = 100 : 3 (water-saturated) at 2.0 ml/min. Detection: UV 325 nm.
a, *d*-methamphetamine; b, phenmetrazine; c, *d*-amphetamine; d, phenethylamine; e, methylphenidate; f, dibutylamine (peak of excess binary complex MX_2).

Table I. Retention Times of Ni(II) Dithiocarbamates of Aliphatic Amines on Silicagel Packings

	Retention time of MAX (min)					
	Column: Polygosil 60-5 Eluent: hexane–isopropyl acetate			Column: LiChrosorb SI 60 Eluent: hexane–isopropyl acetate		
	100:3.5	100:7	100:15	100:3.5	100:7	100:15
Methylamine		10.0			14.9	
Ethylamine		6.8			9.7	
Propylamine	13.6	5.3			7.4	
Isopropylamine	11.5	4.7			6.4	
Butylamine	11.0	4.3			6.0	
Isobutylamine	10.9	4.3			5.9	
sec-Butylamine	9.9	4.1			5.5	
tert-Butylamine	9.6	4.1			5.6	
Isopentylamine	9.3	3.8		13.6	5.1	
Hexylamine	8.4	3.4		12.1	4.5	
Octylamine	7.1	3.0		10.8	3.9	
Benzylamine	13.9	5.3			7.4	
Phenethylamine	15.8	5.6			8.0	
Dimethylamine	11.1	5.6		15.3	7.8	
Diethylamine	5.8	3.0		7.7	4.2	
Dipropylamine	3.5	2.1		4.5	2.6	
Diisopropylamine	4.3	2.7		5.5	3.2	
Dibutylamine[a)]	2.6	1.8		3.2	2.1	
Dibenzylamine	3.1	2.0		4.0	2.5	
N-Methylbenzylamine	5.7	3.0		7.9	4.1	
dl-1-Benzylmethylamine	11.2	4.4		16.6	6.0	
Pyrrolidine	9.0	4.5		12.7	6.0	
Piperidine	6.4	3.3		8.9	4.5	
dl-2-Methylpiperidine	4.9	2.7		6.5	3.5	
Perhydroazepine	5.3	2.8		7.2	3.8	
Morpholine		8.2			12.0	
d-Amphetamine	12.0	4.6		17.6	6.2	
d-Methamphetamine	6.0	3.2		8.3	4.2	
l-Ephedrine		10.7	3.6		15.7	4.9
d-Pseudoephedrine			7.5			11.1
dl-Norephedrine			6.0			8.7
Phenmetrazine	9.5	4.4		13.8	5.9	
STP		6.2			8.8	
Methylphenidate		6.7			9.6	
Mescaline			19.8			31.2

Flow rate: 2.0 ml/min. Detector: UV 325 nm. *a*) Retention times of binary complex MX_2.

increases with increase in the chain length of the hydrophobic alkyl group. The presence of a polar group seems to increase retention times markedly, as evidenced by *l*-ephedrine. Calibration curves were obtained for methamphetamine, amphetamine, phenethylamine and diethylamine. These calibration curves were linear over a wide range of sample amount (0.5 ng—5 μg). The detection limit at 325 nm was 0.5 ng for methamphetamine under the conditions given in Fig. 3.

Reverse Phase HPLC

The elution behavior of various amines on reverse phase packings was examined. An

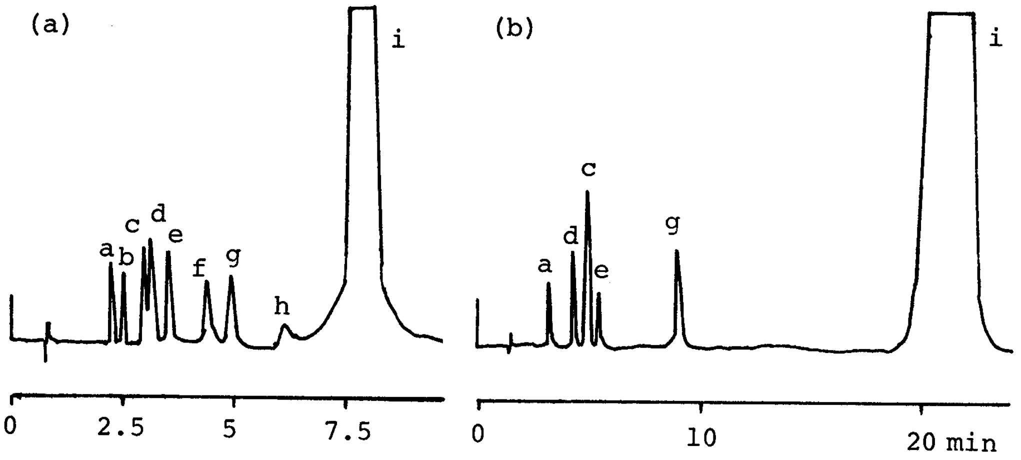

Fig. 4. Chromatogram of Psychotropic Amines on Reverse Phase Packings

(a) Column: Hypersil ODS (5 μm), 4.6 mm × 15 cm. Eluent: acetonitrile–water = 100 : 75 at 2.0 ml/min. Detection: UV 325 nm.

(b) Column: Nucleosil 5C18 (5 μm), 4.6 mm × 15 cm. Eluent: acetonitrile–water = 100 : 45 at 2.0 ml/min.

a, *dl*-norephedrine; b, mescaline; c, *l*-ephedrine; d, *d*-pseudoephedrine; e, *d*-amphetamine; f, STP; g, *d*-methamphetamine; h, methylphenidate; i, dibutylamine (peak of excess binary complex MX_2).

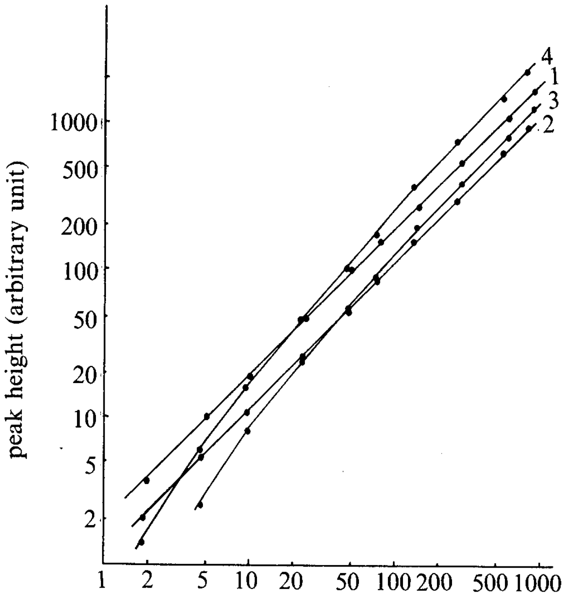

Fig. 5. Calibration Curves of *d*-Methamphetamine and *d*-Amphetamine on Normal and Reverse Phase Chroamtography

1,3, *d*-methamphetamine; 2,4 *d*-amphetamine.
Column: 1,2, LiChrosorb SI 100 (5 μm), 4 mm × 25 cm; 3,4, Nucleosil 5C18 (5 μm), 4.6 mm × 15 cm.
Chromatographic conditions for normal and reverse phase were as described in Figs. 6 and 7, respectively.

example of chromatograms of psychotropic amines is shown in Fig. 4. The large peak of MX_2 was eluted last in the reverse phase system. Retention times on Nucleosil 5C18, LiChrosorb RP 18, LiChrosorb RP 8 and Hypersil ODS are summarized in Table II. High-carbon-content C18 group chemically bonded packings (LiChrosorb RP 18) gave longer retention times than low-carbon-content packings (Hypersil ODS) or C8 group chemically bonded packings (LiChrosorb RP 8). The retention times were little affected by addition of salts (*e.g.* sodium chloride) or by pH change (3.0—7.5), as expected from the fact that these dithiocarbamates are neutral substances. The elution order on reverse phase packings as a whole was opposite to that on normal phase packings, though some discrepancies exist. The elution sequence of amines on different reverse phase packings showed some slight anomalies (*e.g.* *l*-ephedrine and *d*-pseudoephedrine in Fig. 4). The calibration curves were obtained for methamphetamine

Table II. Retention Times of Ni(II) Dithiocarbamates of Aliphatic Amines on Reverse Phase Packings

	Retention time of MAX (min)			
	Column			
	Nucleosil 5C18	Hypersil ODS	LiChrosorb RP 18	LiChrosorb RP 8
	Eluent: acetonitrile–water			
	100 : 30	100 : 90	100 : 30	100 : 50
Methylamine	2.0	2.6	2.4	2.7
Ethylamine	2.3	3.0	2.8	3.1
Propylamine	2.6	3.6	3.4	3.6
Isopropylamine	2.5	3.5	3.2	3.5
Butylamine	3.1	4.5	4.4	4.5
Isobutylamine	2.7	4.3	4.1	4.4
sec-Butylamine	2.9	4.2	3.9	4.6
tert-Butylamine	2.9	4.2	4.1	4.6
Isopentylamine	3.6	5.3	5.4	5.4
Hexylamine	4.8	7.0	7.5	7.1
Benzylamine	2.9	4.3	3.9	4.2
Phenethylamine	3.2	4.9	4.5	4.7
Dimethylamine	2.8	3.4	3.5	3.7
Diethylamine	3.9	5.0	5.4	5.2
Dipropylamine	6.0	7.7	9.3	8.2
Diisopropylamine	5.0	6.6	7.6	7.0
Dibutylamine$^{a)}$	10.0	13.0	16.9	13.7
N-Methylbenzylamine	4.9	6.5	7.2	6.4
dl-Benzylmethylamine	3.0	4.7	4.1	4.6
Pyrrolidine	3.5	4.4	4.6	4.6
Piperidine	4.2	5.3	5.8	5.6
dl-2-Methylpiperidine	4.9	6.3	7.2	6.4
Perhydroazepine	5.2	6.2	7.6	6.8
Morpholine	2.7	3.3	3.3	3.5
d-Amphetamine	3.5	5.4	4.9	5.2
d-Methamphetamine	5.1	7.6	6.2	6.3
l-Ephedrine	3.3	4.5	4.6	4.8
d-Pseudoephedrine	3.0	4.8	4.1	4.7
dl-Norephedrine	2.5	3.4	3.3	3.5
Phenmetrazine	5.2	6.9	7.7	6.8
STP	4.1	6.8	7.5	7.0
Methylphenidate	5.7	9.6	9.0	8.4
Mescaline	2.6	3.9	3.2	3.6

Flow rate: 2.0 ml/min. Detector: UV 325 nm. *a*) Retention time of binary complex MX_2.

and amphetamine. These calibration curves, which were not linear, are shown in Fig. 5 together with calibration curves obtained on normal phase packings. These results suggest that normal phase chromatography gives better results. Identification of methamphetamine and amphetamine can be confirmed by combining normal and reverse phase chromatography.

Analysis of Methamphetamine and Amphetamine in Urine

It has been reported that along with aromatic amines several aliphatic amines such as ethanolamine, methylamine and dimethamine are present in human urine.$^{18)}$ Therefore, these

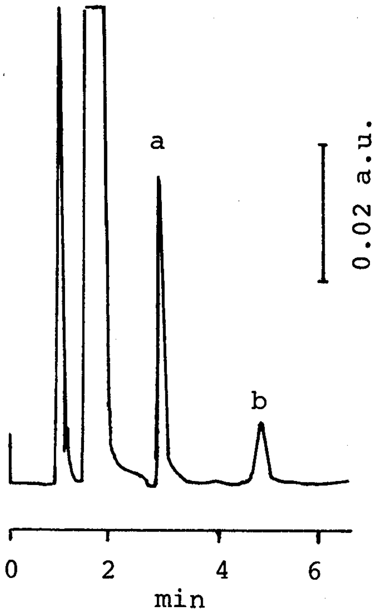

Fig. 6. Chromatogram of Urine of a Methamphetamine Addict

Column: LiChrosorb SI 100 (5 μm), 4 mm × 25 cm. Eluent: hexane–isopropyl acetate = 100 : 4 (water-saturated) at 2.0 ml/min. Detection: UV 325 nm. Sample size: 10 μl.

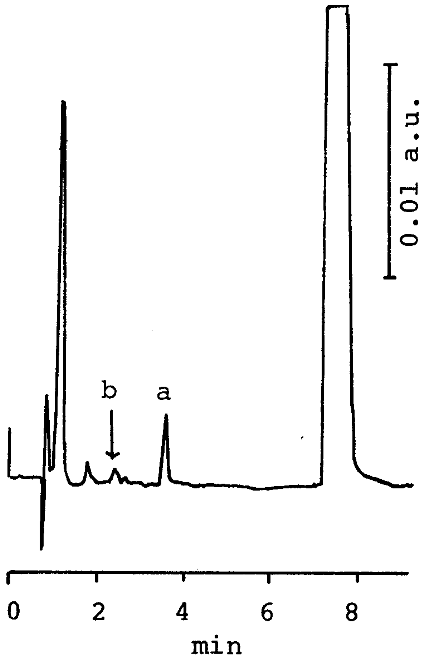

Fig. 7. Chromatogram of Urine of a Rat Given Methamphetamine

Column: Nucleosil 5C18 (5 μm), 4.6 mm × 15 cm. Eluent: acetonitrile–water = 100 : 20 at 1.5 ml/min. Detector: UV 325 nm. Sample size: 10 μl. a, *d*-methamphetamine; b, *d*-amphetamine.

amines might interfere with the detection of methamphetamine and amphetamine in urine. A blank test of human urine revealed that no peak was observed corresponding to the retention times of methamphetamine and amphetamine. It seems that the bulk of these simple amines was removed during extraction. Furthermore, these amines can be separated from methamphetamine and amphetamine under optimum HPLC conditions. Examples of normal phase HPLC chromatograms of urine from a drug addict are shown in Fig. 6. A peak of amphetamine as well as a peak of methamphetamine appeared on the chromatograms because amphetamine is one of the main metabolites of methamphetamine. The amounts of methamphetamine and amphetamine in this sample were calculated to be 135 ng (C.V. 1.5%) and 47 ng (C.V. 1.9%), respectively in five measurements. Figure 7 shows a chromatogram of urine from a rat administered methamphetamine. In this sample, the contents of methamphetamine and amphetamine were calculated from calibration curves (Fig. 5) to be 7.0 ng (C.V. 5.2%) and 2.5 ng (C.V. 15.2%) in four measurements.

In the present color reaction, only aliphatic primary and secondary amines give deep yellow reaction products (log $\varepsilon \fallingdotseq 4.5$ at 325 nm). Furthermore, since the reaction proceeds quantitatively and almost instantaneously at room temperature, procedures such as heating are unnecessary.

References and Notes

1) Part I: M. Moriyasu, Y. Hashimoto and M. Endo, *Bull. Chem. Soc. Jpn.*, **54**, 3369 (1981).
2) For example, *a*) K. Watanabe, *Gekkan Yakuji*, **21**, 55 (1979); *b*) A. Momose and K. Kamei, *Shinkei Seishin Yakuri*, **2**, 229 (1980); *c*) B. J. Gudzinowicz and M. J. Gudzinowicz, "Analysis of Drugs and Metabolites by Gas-chromatography," Vol. 4, Marcel Dekker Inc., New York, 1978, pp. 1—138.
3) T. Niwaguchi, *Eisei Kagaku*, **18**, 235 (1972).
4) *a*) S. Inayama, Y. Tokunaga, E. Hosoya, T. Nakadate and T. Niwaguchi, *Chem. Pharm. Bull.*, **25**, 838 (1977); *b*)

S. Inazawa, Y. Tokunaga, E. Hosoya, T. Nakadate, T. Niwaguchi, K. Aoki and S. Saito, *Chem. Pharm. Bull.*, **28**, 2779 (1980).

5) *a*) C. R. Nix and A. S. Hume, *J. Forensic Sci.*, **15**, 595 (1970); *b*) J. J. Stewart and D. M. Lotti, *J. Pharm. Sci.*, **60**, 461 (1971).

6) *a*) P. J. Cashman and J. I. Thorton, *J. Chromatogr. Sci.*, **11**, 7 (1973); *b*) M. L. Chan, C. Whetsell and J. D. McChesney, *J. Chromatogr. Sci.*, **12**, 512 (1974); *c*) I. Lurie, *J. Assoc. Off. Anal. Chem.*, **60**, 1035 (1977).

7) *a*) T. H. Jupile, *Am. Lab.*, **8**, 85 (1976); *b*) C. R. Clark and M. M. Wells, *J. Chromatogr. Sci.*, **16**, 332 (1978); *c*) F. T. Noggle, Jr., *J. Assoc. Off. Anal. Chem.*, **63**, 702 (1980).

8) M. Endo, H. Imamichi, M. Moriyasu and Y. Hashimoto, *J. Chromatogr.*, **196**, 334 (1980).

9) For example, F. Feigl, "Spot Tests in Organic Analysis," 6th ed., Elsevier Pub. Co., Amsterdam, 1960, p. 270.

10) T. Matsuoka, T. Mitsui and K. Yamamoto, *Eisei Kagaku*, **25**, 127 (1979).

11) H. Bode and F. Newmann, *Z. Anal. Chem.*, **172**, 1 (1960).

12) M. Moriyasu and Y. Hashimoto, *Bull. Chem. Soc. Jpn.*, **53**, 3590 (1980).

13) O. Liska, G. Guichon and H. Colin, *J. Chromatogr.*, **171**, 145 (1979).

14) I. M. Kolthoff and P. J. Elving, "Treatise on Analytical Chemistry," Part II, Vol. 2, John Wiley and Sons, New York, 1962, p. 419.

15) A. W. Hofmann, *Chem. Ber.*, **1**, 25 (1868).

16) M. Moriyasu and Y. Hashimoto, *Anal. Lett.*, **11**, 593 (1978).

17) R. Zahradnik, *Chem. Listy*, **50**, 808 (1956).

18) *a*) S. Baba and S. Ohgiya, *Yakugaku Zasshi*, **89**, 399 (1969); *b*) S. Baba, *Yakugaku Zasshi*, **89**, 1712 (1969).