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RAPID SCREENING METHOD FOR METHAMPHETAMINE IN URINE BY COLOUR REACTION IN A SEP-PAK C_{18} CARTRIDGE

SHINICHI SUZUKI, TAKAKO INOUE and TETSUKICHI NIWAGUCHI*
National Research Institute of Police Science, Sanban-cho, Chiyoda-ku, Tokyo 102 (Japan)
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SUMMARY

A simple screening method for methamphetamine in urine by colour reaction was developed. Methamphetamine, which is quantitatively retained in a Sep-Pak C_{18} cartridge, is (after a clean-up procedure) coloured by Simon's reagent (consisting of sodium nitroprusside solution, sodium carbonate solution and acetaldehyde gas). The detection limit was 0.5 μg/ml using 5 ml of urine sample. The results of the screening method agreed with those of thin-layer chromatography and gas chromatography–mass spectrometry.

INTRODUCTION

Abuse of methamphetamine is spreading throughout the world and is a serious social problem, especially in Japan. Therefore, a rapid and specific screening procedure for methamphetamine in urine is required.

Until recently, the most common methods for the detection of methamphetamine, which is the major compound excreted in human urine after administration of methamphetamine[1,2], have been based on thin-layer (TLC) and gas chromatography (GC). These methods require a large volume of organic solvent for the extraction of methamphetamine and metabolites, and the procedures take many hours. With the development of immunoassay screening methods for the drugs of abuse, radioimmunoassay[3–6], enzyme multiple immunoassay[7–10] and the haemagglutination inhibition test[11] for methamphetamine have been investigated. Although these procedures have high sensitivity and specificity, they require special instruments, antisera and sensitized blood cells.

We have attempted to develop a more simple screening method for methamphetamine in urine by a colour reaction using a Sep-Pak C_{18} cartridge. In this procedure methamphetamine, retained selectively on the resin, is coloured with Simon's reagent[12], consisting of sodium carbonate, sodium nitroprusside and acetaldehyde.

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EXPERIMENTAL

Materials

Methamphetamine hydrochloride and amphetamine sulphate were purchased from Dainippon Pharmaceutical (Osaka, Japan) and Takeda Chemical Industries (Osaka, Japan), respectively. *p*-Hydroxymethamphetamine hydrochloride was synthesized essentially by the method of Buzas and Dufour[13], and the purity of the product (m.p. 142–143°C) was checked by TLC, GC, mass spectrometry (MS) and IR spectrometry. Trifluoroacetic anhydride was purchased from Wako (Osaka, Japan) and the other chemicals used were of analytical-reagent grade. The Sep-Pak C_{18} cartidge was obtained from Waters Assoc. (Milford, MA, U.S.A.).

Screening test for methamphetamine in urine

After connecting a 10-ml glass syringe to the Sep-Pak C_{18} cartridge, the resin in the cartridge was activated by passing 5 ml of methanol and 10 ml of water through the glass syringe. Unless indicated otherwise, urine samples, aqueous solutions, water and methanol were passed through the cartridge at a flow-rate of 5 ml/min.

A urine sample (5 ml) was adjusted to pH 9.0 with sodium carbonate–sodium hydrogen carbonate buffer and poured into an activated Sep-Pak C_{18} cartridge; subsequently 2 ml of 20% (w/v) sodium carbonate solution and 5 ml of water–methanol (1:1, v/v) were poured into the cartridge to eliminate selectively coloured components of urine. After an additional 1 ml of water, 0.2 ml of the colour-producing reagent solution, consisting of 1.2% (w/v) sodium nitroprusside solution–20% (w/v) sodium carbonate solution (5:1, v/v) was poured in, then 20 ml of acetaldehyde gas, which was obtained with a syringe from the headspace of a 5-ml vial containing 1 ml of acetaldehyde, was introduced into the cartridge. Finally, 1 ml of water was poured into the cartridge at a flow-rate 0.5 ml/min and the colour developed was observed.

Extraction of methamphetamine and metabolites in urine

A 5-ml urine sample was adjusted to pH 9.0 with sodium carbonate and extracted four times with 10 ml of chloroform–isopropanol (3:1, v/v). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo* after adding a drop of acetic acid to prevent evaporation of amines. The residue obtained was subjected to TLC, GC and GC–MS.

Analytical methods

TLC was carried out on 0.25 mm thick silica gel GF_{254} plates (E. Merck, Darmstadt, G.F.R.); the solvent systems used for development were (I) isopropanol–28% aqueous ammonia (95:5, v/v) and (II) methyl ethyl ketone–dimethylformamide–28% aqueous ammonia (13:1.9:0.1, v/v/v). After development, the plates were examined under UV light (254 nm) and sprayed with either (A) 1% (w/v) iodine–methanol solution or (B) 20% (w/v) sodium carbonate solution and 1% (w/v) sodium nitroprusside solution as detection reagent, and the plate was exposed to acetaldehyde gas for 1 min.

For GC, a Shimadzu GC-4CM gas chromatograph equipped with a flame-ionization detector was used. The glass column (1 m × 3 mm I.D.) was packed with 3% OV-17 on Chromosorb W AW DMCS (100–120 mesh). The carrier gas was

nitrogen (50 ml/min). The column, injection and detector temperatures were 125, 160 and 160°C, respectively. The extract obtained from urine was dissolved in 200 μl of ethyl acetate and 200 μl of trifluoroacetic anhydride were added. The vessel containing the mixture was sealed tightly and heated at 55°C for 20 min. The solvent was evaporated *in vacuo*, the residue obtained was dissolved in 100 μl of ethyl acetate and 1 μl was injected into the gas chromatograph. For the determination of methamphetamine, amphetamine and *p*-hydroxymethamphetamine, 0.25 μg/μl of fluorene in ethyl acetate was used as an internal standard.

GC–MS was carried out with a Hitachi Model M-80 double focusing mass spectrometer by the chemical ionization method using isobutane as the reactant gas. A 1 m × 3 mm I.D. glass column packed with 3% OV-17 on Chromosorb W AW DMCS (100–120 mesh) was used. The column, injector and separator temperatures were 125, 165 and 180°C, respectively. The flow-rate of the helium carrier gas was 50 ml/min. The ionization voltage was 100 eV and the ionization current was 110 μA.

RESULTS AND DISCUSSION

Effect of pH of urine on retention of methamphetamine and metabolites in a Sep-Pak C_{18} cartridge

To 5 ml of control urine obtained from a healthy man, 50 μg of methamphetamine hydrochloride, amphetamine sulphate or *p*-hydroxymethamphetamine hydrochloride were added and the pH was adjusted to 3, 5, 7, 9 or 11 by adding 4.5 ml of hydrochloric acid–sodium acetate buffer (pH 3.0), acetic acid–sodium acetate buffer (pH 5.0), disodium hydrogen phosphate–potassium dihydrogen phosphate buffer (pH 7.0) or sodium carbonate–sodium hydrogen carbonate buffer (pH 9.0 and 11.0), respectively. The treated urine sample was poured into the activated Sep-Pak C_{18} cartridge. The effluent solution from the cartridge was adjusted to pH 9 and extracted four times with chloroform–isopropanol (3:1, v/v). The combined organic extracts were treated to determine methamphetamine, amphetamine and *p*-hydroxymethamphetamine. Table I shows that methamphetamine and amphetamine are completely retained in the cartridge in the pH range 5–11. On the basis of the results urine samples were adjusted to pH 9 in subsequent experiments.

TABLE I

EFFECT OF pH OF URINE ON RETENTION OF METHAMPHETAMINE AND METABOLITES IN A SEP-PAK C_{18} CARTRIDGE

50 μg of methamphetamine hydrochloride, amphetamine sulphate or *p*-hydroxymethamphetamine hydrochloride were added to 5 ml of control urine. Results are averages ± standard deviation (n = 5).

Compound	*Compound not retained (%)*				
	pH 3	*pH 5*	*pH 7*	*pH 9*	*pH 11*
Methamphetamine	0.5 ± 0.6	—*	—	—	—
Amphetamine	8.0 ± 1.0	—	—	—	—
p-Hydroxymethamphetamine	33.4 ± 3.6	10.3 ± 1.0	3.2 ± 1.0	—	—

* —, Not detected.

Effect of clean-up procedure on retention of methamphetamine and metabolites in a Sep-Pak C_{18} cartridge

To 5 ml of control urine, 0.5–500 μg of methamphetamine hydrochloride, amphetamine sulphate and *p*-hydroxymethamphetamine hydrochloride were added. The treated urine sample was adjusted to pH 9, poured into the activated cartridge and the effluent solution from the cartridge was collected (effluent 1). Coloured components, which were contained in urine and retained in the cartridge, were eliminated by pouring in 2 ml of 20% (w/v) sodium carbonate solution, 5 ml of water–methanol (1:1, v/v) and 1 ml of water. Each effluent solution (effluents 2, 3 and 4) was combined together with effluent 1. A few drops of acetic acid were added, methanol was evaporated and the aqueous solution remaining was adjusted to pH 9 and treated to determine methamphetamine, amphetamine and *p*-hydroxymethamphetamine by GC. The results showed that methamphetamine was sufficiently retained in the cartridge after the clean-up procedure but amphetamine and *p*-hydroxymethamphetamine were eluted with increasing amounts of the compounds added to urine (Table II).

When 5 ml of urine containing 500 μg of methamphetamine hydrochloride, 50 μg of amphetamine sulphate and *p*-hydroxymethamphetamine hydrochloride were applied to the cartridge and the clean-up procedure was carried out, the compounds eluted in effluents 1, 2, 3 and 4 were determined. The results showed that 7% of the methamphetamine added, 60% of the amphetamine added and 98% of the *p*-hydroxymethamphetamine added were eluted in effluent 3. It was concluded that this clean-up procedure is suitable for the detection of methamphetamine by the colour reaction.

Coloration in Sep-Pak C_{18} cartridge

Urine samples were prepared by adding various amounts of methamphetamine hydrochloride to control urine, and 5 ml of the treated urine sample were poured into the Sep-Pak C_{18} cartridge and treated as described above. For coloration, 0.2 ml of 1.2% (w/v) sodium nitroprusside solution–20% (w/v) sodium carbonate solution (5:1, v/v) was poured into the cartridge and acetaldehyde gas was introduced. When 5 μg/ml of methamphetamine was present in urine sample, a blue colour was

TABLE II

EFFECT OF CLEAN-UP PROCEDURE ON RETENTION OF METHAMPHETAMINE AND METABOLITES IN A SEP-PAK C_{18} CARTRIDGE

Results are averages ± standard deviation (n = 5).

Compound added to 5 ml of control urine (μg)			*Compound eluted in effluents 1+2+3+4 (%)*		
Methamphetamine hydrochloride	*Amphetamine sulphate*	*p-Hydroxymethamphetamine hydrochloride*	*Methamphetamine*	*Amphetamine*	*p-Hydroxymethamphetamine*
5	0.5	0.5	–*	–	27.6 ± 3.1
50	5	5	2.6 ± 1.0	48.2 ± 9.2	90.2 ± 6.1
500	50	50	12.6 ± 1.0	56.9 ± 0.9	99.6 ± 0.7

* – = Not detected.

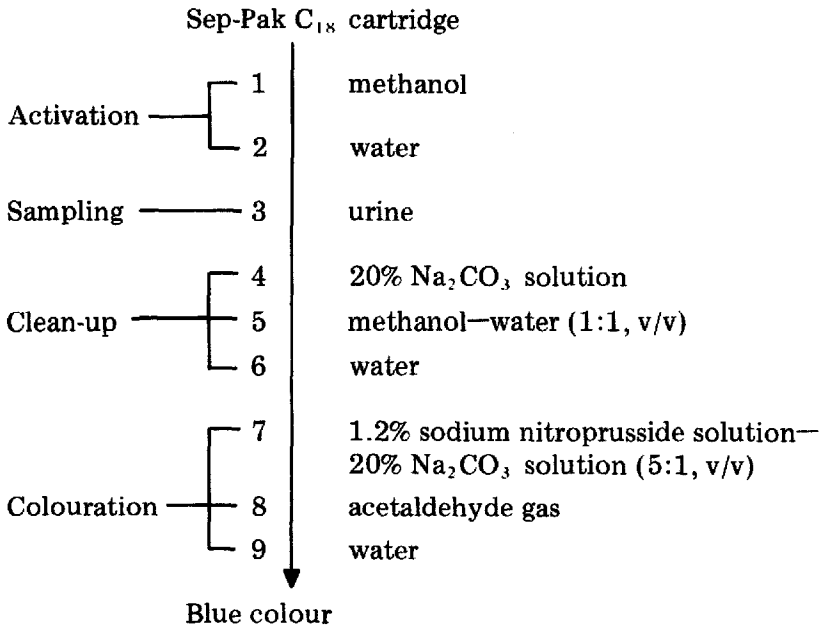

Fig. 1. Screening test for methamphetamine in urine.

clearly observed in the upper part of the resin in the cartridge, and with a control urine a reddish colour was observed in the upper part of the resin. The blue colour developed by less than 2 μg/ml of methamphetamine in urine sample was rendered indistinct by the underlying reddish colour. However, the reddish colour could be selectively eluted by pouring 1 ml of water into the cartridge at a flow-rate of 0.5 ml/min after introducing acetaldehyde gas, and the blue colour developed by methamphetamine could then be clearly observed. The screening test is summarized in Fig. 1. The detection limit of this method is 0.5 μg/ml of methamphetamine in urine when 5 ml of urine is used as the sample.

Screening test for suspect urine

Fifteen urine samples obtained from methamphetamine abuse suspects were examined by the screening method and subjected to TLC, GC and GC–MS, and the amounts of methamphetamine in the same samples were determined by GC. The results of the screening test agreed with those of TLC, GC and GC–MS, as shown in Table III.

Although urine sample 4 in Table III gave a positive coloration with this screening test, methamphetamine was not detected by TLC and GC–MS. It has been found that Simon's reaction is applicable to the detection of secondary aliphatic amines[12] and that methoxyphenamine in drugs possessing a secondary aliphatic amino group gives a positive coloration with similar sensitivity to that of methamphetamine[14,15]. Further, it should be noted that some metabolites, which are produced *in vivo* by N-dealkylation of drugs having a tertiary amino group and excreted in urine, give a positive coloration with Simon's reagent, for example, 1-

TABLE III

COMPARISON OF RESULTS OBTAINED BY THE SCREENING TEST WITH THOSE OBTAINED BY TLC, GC AND GC–MS

− = Negative; + = positive.

No.	*Screening test*	*TLC*	*GC–MS*	*GC: methamphetamine in urine (μg/ml)*
1	−	−	−	0
2	−	−	−	0
3	−	−	−	0
4	+	−	−	0
5	+	+	+	0.1
6	+	+	+	0.2
7	+	+	+	2.5
8	+	+	+	3.3
9	+	+	+	25.0
10	+	+	+	29.5
11	+	+	+	31.7
12	+	+	+	32.6
13	+	+	+	72.8
14	+	+	+	115.4
15	+	+	+	130.2

phenyl-2-(N-benzylamino)propane (benzylamphetamine), an N-demethylated metabolite of 1-phenyl-2-(N-methyl-N-benzylamino)propane (benzphetamine), showed positive coloration[16,17]. Positive coloration of sample 4 is considered to be derived from such compounds.

In order to identify methamphetamine in urine, therefore, urine samples that give a positive coloration by this screening method must be confirmed by GC–MS.

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