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A Simple Device of the Dry Tetrabromophenolphthalein Ethyl Ester Reagent Strip for the Detection of Methamphetamine

Myung Ja Choi[1], Eun Young Song[2], Seungki Kim[1], Jeongeun Choi[1], Dong-Seok Lho[1] and Jongsei Park[1]

[1]*Doping Control Center,* [2]*Genetic Engineering Research Institute, Korea Institute of Science and Technology, Seoul 136-791, Korea*

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A new device to detect methamphetamine (MA), amphetamine(A) and its metabolites in urine was developed using the paper strip method and the test tube method of dry chemical reagents. The reagent containing tetrabromophenolphthalein ethyl ester (TBPE) and borax. For the TBPE paper strip method, a device was prepared with a window at each end of the reagent paper strip; one window is for the sample application, and the other window is for the methylene chloride. The diffused sample from one window reacts with reagent in the paper and produces color at the point where it meets with methylene chloride which has diffused form the other side. A positive sample produces as red-purple color and the negative sample a greenish color, with a detection limit of 5-10 ppm. The result can be obtained within one minute. For the TBPE test tube method which contains dry reagents, the detection limit is 5 ppm and the result can be obtained within 30 seconds, however the carry-on is not as convenient as the paper strip method. The performance of both methods were evlauated by comparing with the results of gas chromatography (GC) and fluorescence polarization immunoassay (FPIA). The results were proven that both methods were useful as primary screening reagents to detect MA in urine and in dry powder.

Key words: Methamphetamine detection, Dry color method, On-spot test, Strip device

INTRODUCTION

Methamphetamine, also known as hiroppon, is one of the most potent sympathomimetic amines with respect to stimulation of the CNS. The psychic effects of MA/A adminis tration involve wakefulness, a decreased sense of fatigue, elevation of mood, confidence and euphoria. However, the prologed usage or large doses are followed by mental depression, fatigue, paliptation, dysphoria and delirium. Recently, hard core drug abuse has escalated in the Far East and the relationship between the spread of drug abuse and crime is a serious social concern. Therefore, there is an increasing demand for a simple and easy-to-use screening tool to maintain the safety of society. The immunoassay and instrumental analysis methods presently utilized for the specific analysis of MA/A, are not only expensive and time-consuming but also inadequate for on-site screening. For this reason, chemical color tests utilizing Troipaeolin 00, Ad-Tip method, or TBPE were popular despite their low specificities (Anal. Forensic Sci. Conf., 1979; Mitsui, 1980; Nakahara and Sekine, 1984; Nakahara, 1988). However, most of these chemical methods were also solely used in the laboratory mainly due to the reagent unstability in wet chemicals.

The present study was undertaken to produce an MA/A screening method which provides instant, on-site urine analysis results. Thus, a ready-to-use device to screen for MA/A or its metabolites was developed using TBPE in conjunction with the paper strip method and the test tube method. The performance of the new method was compared with the results of fluorescence polarization immunoassay (FPIA), gas chromatography. The false positive chemicals by the TBPE methods were confinmed by gas chromatography with nitrogen phosphorous detector (GC/NPD) (Gupta *et al.*, 1974; Dugal *et al.*, 1980; Morita *et al.*, 1983; Lho *et al.*, 1990) and gas chromatography with mass selective detector (GC/MSD) (Lho *et al.*, 1990).

MATERIALS AND METHODS

Reagents and instruments

Methamphetamine was obtained from the Korean

Correspondence to: Myung Ja Choi, Doping Control Center, Korea Institute of Science Technology, P.O. Box 131 Cheongryang, Seoul 136-791, Korea

National Institute of Health. For the preparation of MA/A screening reagent, tetrabromophenolphthalein ethyl ester potasium salt (Sigma Chemical, USA.) Borax (Kento Chemical Co., Japan), methylene chloride were used. For derivatization, trifluoroacetic acid (TFA), trimethylchlorosilane (TMCS), *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), *N*-methyl bis (trifluoroacetamide) (MBTFA) were purchased from Sigma Chemical Co. (USA). Acetonitrile, methanol, and hydrochloric acid were from Merck Chemical Co. (Germany). Anhydrous sodium sulfate, *n*-heptane were from Kanto Chemical Co. (Japan). Potassium hydroxide, sodium bicarbonate were from TCI (Japan). Diethylether (Junsei Chemical Co., Japan) and anhydrous potassium carbonate (J.T. Baker, USA) were also used. Methyl orange-CH_3CN/CF_3CO_2H solution was prepared by dissolving methyl orange in a mixed solvent of acetonitrile and trifluoroacetic acid (V/V: 60/40) to a final concentration of 200 ppm. 10^{-4} M methyl orange acid solution was prepared from 10^{-2} M methyl orange and 6 N HCl. Silanized glass beads (125-200 μm, 80-120 mesh) (Serva, Germany) were used for the GC insert liner. Helium (99.999%), hydrogen (99.999%) (Union Carbide, USA) and compressed air were used for GC. For the FPIA, amphetamine/methamphetamine (A/MA) reagents, controls and calibrators were purchased from Abbott Laboratories (USA). The gas chromatographic identifcation was performed using a Hewlett-Packard (HP) 5890A GC equipped with a nitrogen-phosphorous detector (NPD) and an HP 3392A integrator. The mass spectrometric confirmation was performed using GC interfaced with an HP5970B series mass selective detector and an HP5970B mass chemstation including an HP7946 disc drive and an HP 2934A printer. FPIA was performed using a TDx analyzer (Abbott, USA) equipped with software revision 11.2.

TBPE test tube method

The TBPE was prepared using methylene chloride according to the modified method of Anal. Forensic Sci. Conf. (1979). 0.1 g of TBPE potassium salt was dissolved in 100 ml of water and 10-13 drops of 10% HCl was added to make a weak acidic solution, and made to a final concentration of 0.033%. 60 mg of borax and 0.5 ml of TBPE in methylene chloride was added to a test and the solvent was evaporated until the tube was dry. For the urine sample test, 1 ml of urine and 1 ml of methylene chloride were mixed in the test tube. The color of the bottom layer was observed after standing approximately 30 seconds (negative for green and positive for red-purple).

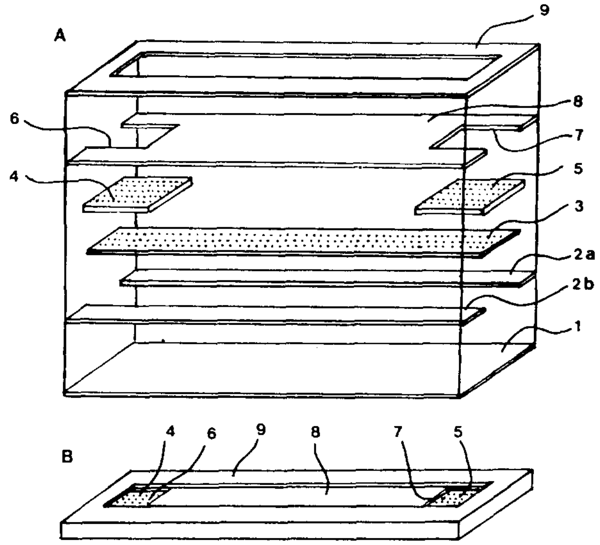

Fig. 1. TBPE strip method device for the detection of MA/A in urine.

A: Parts of the device: 1, back cover; 2a & 2b supporting strips; 3, reagent strip; 4, sample receiving pad; 5, reagent receiving pad; 6, sample application spot; 7, reagent application spot; 8, clear film cover; 9, front cover; B: View of the assembled device

Table I. Identification of drugs in urine samples by GC/NPD

Relative retention time (*RRT± SD)	Drugs
0.323± 0	Amphetamine
0.367± 0	4-methyl-4-formylpentanoic acid
0.374± 0	Methamphetamine
0.490± 0	Indole
0.551± 0.002	Nicotine
0.579± 0	Ephedrine
0.627± 0	Methylephedrine
0.683± 0.004	Piperidine
0.709± 0.002	Ionol
0.945± 0.002	Cotinine
1.06 ± 0	Methyl-3-indolacetate
1.09 ± 0	Caffeine
1.26 ± 0	Chlorophenylamine

Internal standard: *N*,*N*-diisopropyl-amino-*n*-dodecane

$$^{*}\mathrm{RRT} = \frac{\text{retention time of drug}}{\text{retention time of internal standard}}$$

SD = standard deviation

TBPE paper strip method

TBPE solution was prepared to a final concentration of 0.015% in methylene chloride same as described in test tube method above. The filter paper (Toyo No. 2, Japan) was saturated with TBPE and air-dried. The second saturation was made with 1% borax solution and air-dried. This reagent paper was cut into a size of 1×5 cm and assembled as shown in Fig. 1A. Fig. 1B is the finished product of the device which has a window at each and of the reagent strip. For the sample test, the urine sample was applied at one window and the methylene chloride at the other window. The diffused sampel reacted with the reagent in the paper strip and produced color at the point where in met with the methylene chloride which had diffused from the other side. The positive sample produced a red-purple color and the negative sample a greenish color.

FPIA, GC/NPD and GC/MS

A/MA reagents for FPIA were used in accordance with the manufacture's instructions. An A/MA concentration higher than 0.3 μg/ml was evaluated as positive. Extraction of drugs from urine, gas chromatographic identification, and mass spectrometric confirmation were performed according to the methods as described in Lho *et al*., 1980. 25 mg of *N,N*-diisopropyl-1-amino-*n*-dodecane was used as the internal standard. Identification was based on GC relative retention time (Table I) and the characteristic ions in the full scan electron impact MS after selective derivatization of amino and hydroxyl groups to *N*-trifluoroacetyl (*N*-TFA) and *O*-trimethylsilyl (*O*-TMS) groups, respectively.

RESULTS AND DISCUSSION

The TBPE color test device for on-site MA/A detection was prepared as a drug device by incorporating reagents together in a paper strip or test tube and then drying. The detection limit of the test, which can be distinguished from zero concentration was 5 ppm for both the TBPE test tube and paper strip methods using 1 ml urine sample. When the sample volume was increased to 2 ml, the color distinctions becamek easy and clear. The color development in the strip was not inhibited by acidic urine (pH 4.9-53) or with alkaline urine (pH 7.8-8.2), but its sensitivity decreased with urine whose pH was under 4.0. We tested several strip materials; Korean filter paper (KFP) No. 462 and No. 2 were better than the others. A TBPE concentration of 0.015-0.02 was best for the stripe treatment. The borax treatment was essential to increase the stability of the strip and sensitivity of the MA/A detection. The treatment of 5 mg/ml borax raised the detection limit to 5 ppm. The drying method also affected the sensitivity of the strip; natural air drying (detection limit 5 ppm) was better than the 36℃ drying (detection limit 12.5 ppm). A disadvantage of the TBPE chemical reagent is that it reacts with other amine compounds in addition to A and MA. Thus, its cross-reactivity was studied using the TBPE strip. Table II indicats that heroin, codeine ephedrine and norephedrine caused color inteference in the TBPE strip method, but no interference was seen with benzoylecognine and phenylamine compounds such as *p*-hydroxy amphetamine, epinephrine and isopreterenol.

Table II. Drug interference study by the TBPE color method in urine

Drugs	Concentration (μg/ml)
Methamphetamine HCl	7.8
Amphetamine sulfate	31.3
p-hydroxyamphetamine	n/c
Epinephrine	n/c
Ephedrine	15
dl-Methylephedrine HCl	15.6
Benzoylecognine	n/c
Norephedrine HCl	125
Methoxyphenamine HCl	7.8
Isoproterenol	n/c

n/c: no color interference over a concentration of 1,000 μg/ml

To evaluate the TBPE paper strip and test tube method, we screened 442 urine samples for the on-site detection of MA/A. Urinee samples were obtained from athletes for the drug doping test and from suspects of drug abuse at the police office. All samples were screened by the TBPE test tube and paper strip method, FPIA and GC. These screening methods indicate the possible presence of drugs, but do not provide definite proof. For this reason, all positive urine samples were confirmed by GC/NPD and the samples which included ephedrine, methylephedrine and indole were confirmed by GC/MSD using the corresponding reference compounds. Table III lists the results of the comparison study for the screening of MA and A in urine. Four cases out of 442 samples were genuine MA/A positive. 10-15% were falsely identified as positive by the TBPE color methods whereas 2.0% were false positive by the FPIA immunoassay.

The FPIA immunoassay showed less false positives, but the cost of the color method is much lower than FPIA. The negative samples by the TBPE color methods were also screened by GC/NPD and none of them were found to contain MA/A. Therefore, no false negatives were observed.

In conclusion, we established an on-site screening method of MA/A in urine and in dry powder using the TBPE paper strip or test tube method. The dry reagent devices are simple to use and results can be otbained within 30 seconds by visual detection. Despite the less than 15% false positive reactions, the test shows no false negatives. Thus, the TBPE paper strip or test tube method can be conveniently applied as a primary on-site test for the detection of MA/A.

REFERENCES CITED

Anal, Forensic Sci. Conf. Japan, 1979 (Abstract), Improvement of the tetrabromophenolphthalein ethyl ester method for the screening of stimulant in urine. p10; The tetrabromophenolphthalein ehtyl ester method for the detection of methamphetamine in urine. p16.

Dugal, R., Masse, R., sanchez, G. and Bertrand, M., An integrated methodological approach to the computer-assisted gas chromatographic screening of basic dugs in biological fluids using nitrogen selective detection. *J. Anal. Toxicol.,* 4, 1-12 (1980).

Gupta, R. N., Chittim, B. G. and Keane, P. M., Screening of major methadone metabolite and methamphetamine in urine. *J. Chromatgr. Sci.,* 12, 67-70 (1974).

Lho, D. S., Shin, H. S., Kang, B. K. and Park, J., Systemic analysis of stimulants and narcotic analgesics by gas chromatography with nitrogen specific detection and mass spectrometry. *J. Anal. Toxicol.,* 14, 73-76 (1990).

Mitsui, T., Screening Test for Methamphetamine in Urine. II. Color Reacton Test with Tropaeolin 00. *Eisei Kagaku,* 26, 264-266 (1980).

Morita, M., Ando, H. and Kagaku, E., Analysis of methamphetamine and its metabolites in urine from habitual user of stimulant. *Eisei Kagaku,* 29(5), 318-322 (1983).

Nakahara, Y., Evaluation and improvement of field test kits identification of abused drugs. *Jpn. Natl. Inst. Hyg. Sci. Report,* 106, 111-115 (1988).

Nakaharara, Y. and Sekine, H., A high selective screening test for methamphetamine in human urine. *Forensic Sci. Int.,* 26, 277-282 (1984).