Jikeikai Med J 2020 ; 67 : 1-6

Examining the Effect of Liquid-phase Fraction in the Dual Test Procedure with Simon's Reagent

Toshiaki IRII[1,2], Kyoko MAEBASHI[1], Kenji FUKUI[1], Sari MATSUMOTO[1], Shojiro TAKASU[1], and Kimiharu IWADATE[1]

[1]*Department of Forensic Medicine, The Jikei University School of Medicine*
[2]*Forensic Science Laboratory, Metropolitan Police Department*

ABSTRACT

We have previously reported a simple method of simultaneously testing syringes for DNA typing and methamphetamine detection in drug-related crimes. We reported that the liquid-phase fraction separated with our method can be analyzed with gas chromatography/mass spectrometry (GC/MS). The present study examined the effect of components contained in the liquid-phase fraction on a qualitative test using Simon's reagent. We examined 100 μL of the liquid-phase fraction separated with our method and tested it with Simon's reagent. The result of each section can be visually judged as positive with a color change to deep blue. A nonspecific positive reaction was not observed in comparison with distilled water and a negative control. However, in a positive reaction with the liquid-phase fraction, the color change to deep blue was less red, and the reaction speed was slower than that in control samples. The advantage of the method of using Simon's reaction was the simplicity of its steps, but it was less sensitive and accurate than GC/MS. We highly recommend this method as a primary test and suggest that if a negative result is obtained that GC/MS be performed for greater sensitivity and accuracy.

(Jikeikai Med J 2020 ; 67 : 1-6)

Key words : forensic, dual test, methamphetamine detection, Simon's reagent, syringe

INTRODUCTION

Each year in Japan, more than 10,000 people are arrested for violating the Stimulant Drug Control Law. In 2017, these arrests constituted more than 70% of all domestic drug crime[1]. The intravenous injection of stimulant drugs is common in Japan, and the sharing of syringes is a serious problem throughout the world. Additionally, injection drug users have a severe risk of infectious disease, including infections with human immunodeficiency virus, hepatitis B virus, and hepatitis C virus[2-5]. In Japan, the relationship of injection drug users with a high prevalence of hepatitis C virus infection is particularly severe[6].

To investigate the intravenous injection of stimulant drugs, independent tests are required for DNA typing and methamphetamine detection. When the amount of blood obtained with a syringe is small, a single test might require use of the entire sample. In addition, if the syringe contains a solid blood clot, collecting a test sample only by washing the syringe with distilled water might be difficult. For analysis, a clot should be dissolved. We have previously reported a method for performing tests simply and simultaneously on the basis of alcohol's biopolymer-precipitating ability by using washing solution in a syringe[7].

As noted in our previous study[7], the liquid-phase fraction separated by our method can be analyzed after extrac-

Received : February 26, 2020 / Accepted : September 2, 2020
入井　俊昭，前橋　恭子，福井　謙二，柗本　紗里，髙須翔志郎，岩楯　公晴
Mailing address : Toshiaki IRII, Department of Forensic Medicine, The Jikei University School of Medicine, Tokyo 105-8461, Japan.
E-mail : irii@jikei.ac.jp

tion with gas chromatography/mass spectrometry (GC/MS), which has high sensitivity and accuracy. However, the liquid-phase fraction separated with our method contains a solution of proteinase K, components of phosphate-buffered saline (PBS), and methanol. We are concerned whether these components affect other qualitative tests for methamphetamine using the liquid-phase fraction.

On the basis of this background, the present study examined the effect of the liquid-phase fraction's components on qualitative testing. We tried using Simon's reagent for methamphetamine testing because it provides a qualitative reaction for a secondary amine, i.e., reacting to deep blue in the presence of methamphetamine[8]. In Japan, the primary ingredient of an illegally used drug is methamphetamine hydrochloride (MA)[9]. Simon's reagent has been used in previous studies as a qualitative methamphetamine test[10-14] and for other secondary amines without methamphetamine[15,16]. Additionally, secondary amines have been identified by using the difference in reactions between acetaldehyde and acetone[17].

In the present study, we examined the effects of using components of the liquid-phase fraction on qualitative methamphetamine testing with Simon's reagent. Additionally, we used this method with syringe samples as a forensic application.

Materials and Methods

1. Test samples and sample separation

Blood samples were provided, with written informed consent, by an adult volunteer who was not taking any medications. The stimulant used was MA (Sumitomo Dainippon Pharma Co., Ltd., Tokyo, Japan). All test samples were prepared by adding MA water solution to 1 μL of blood. This study was approved by the Ethics Committee of The Jikei University School of Medicine for Biomedical Research (receipt number : 25-112(7247)).

The separation method used was described in our previous article (Fig. 1)[7]. Samples were lysed with proteinase K in PBS at 56°C for 20 minutes, cooled at −20°C after methanol was added, and then centrifuged at 15,000 rpm. The liquid-phase fraction was used to detect methamphetamine.

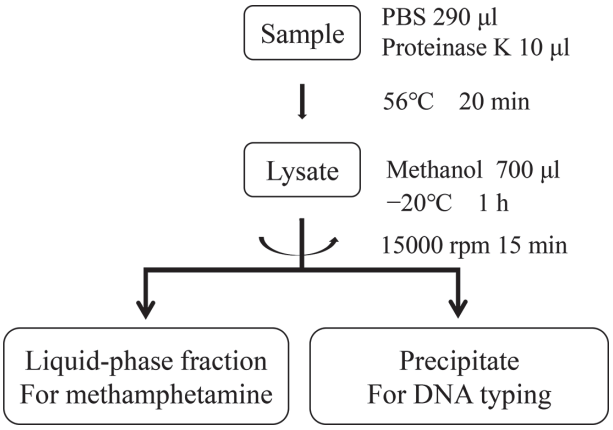

Fig. 1. Sample separation method. With our method, the lysate was separated into a blood cell-derived fraction as a precipitate for DNA typing and a liquid-phase fraction for methamphetamine detection.

2. Simon's reagent

Simon's reagent was purchased from Kanto Chemical (Tokyo, Japan). Simon's reaction was performed according to the manufacturer's protocol[18] by adding, in turn, 1 drop (50 μL) each of 3 solutions (A : 20% sodium carbonate ; B : 1% sodium nitroprusside ; and C : acetaldehyde-ethanol mixed solution) to control the sample or the liquid-phase fraction by the separating method. A positive reaction was judged visually with the solution's color change to deep blue.

3. Liquid-phase components' effect on the qualitative test for methamphetamine

In a qualitative methamphetamine test, to examine the effect of liquid-phase components containing a solution of proteinase K, a component of PBS, and methanol with the separating method, we prepared a liquid-phase fraction in samples with 1 μL of blood without MA. We examined the effect on the qualitative methamphetamine test and nonspecific reactions by adding the liquid-phase fraction to MA.

We tested samples with Simon's reagent by adding 200 μg or 50 μg of MA to 100 μL or 300 μL of liquid-phase fraction without MA. To compare colors, samples were prepared by adding each volume of MA to each volume of distilled water. Simon's reagent is normally used for samples in the crystal or powder form. In the present study, positive control samples were used in a 1-μL solution containing 200 μg or 50 μg MA in a crystal-like condition. The positive control was prepared only with MA, and the negative control was prepared only with Simon's reagent.

4. Test with Simon's reagent for the liquid-phase fraction

We examined the 100-μL liquid-phase fraction in the sample containing 1 μL of blood and 2,000 μg or 500 μg of MA, separated with our method and tested with Simon's reagent. The control sample was 100 μL from each sample dissolved in 1 mL of distilled water and tested without separation. The positive control was prepared with MA in 100 μL of distilled water, and the negative control was prepared with only 100 μL of distilled water.

5. Sensitivity of methamphetamine detection with Simon's reagent for the liquid-phase fraction

Test samples were prepared by mixing 1 μL of blood and MA (at weight of 400, 300, 200, 100, and 50 μg). Simon's reagent was used to test 100 μL of the liquid-phase fraction of each sample separated with our method. The control sample used 100 μL from each sample dissolved in 1 mL of distilled water without separation. The positive control sample was prepared with each volume (above) of only MA in 100 μL of distilled water.

6. Syringe samples

Syringe samples were prepared by mixing 1 μL of blood with the MA water solution in a syringe (n = 5). Syringe samples were prepared with 1-mL syringes (sterile single-use syringes with insulin needle, MYJECTOR® 29G × 1/2" [0.33 × 13 mm], Terumo Corp., Tokyo, Japan). Syringe samples were prepared once they had absorbed 300 μL of a 100-μg/μL MA water solution and the researchers having pushed it out, after they absorbed 1 μL of blood from a needle. Syringe samples were processed 2 weeks later.

In a 1.5-mL tube previously warmed to 56°C, each sample was washed by absorbing a mixture of 290 μL of PBS and 10 μL of proteinase K. After being held at 56°C for 20 minutes, each syringe was washed and filled with washing solution ; each washing solution was then separated to the liquid-phase fraction and tested with Simon's reagent. Control samples were washed with 1 mL of distilled water, and 100 μL of 1-mL washing solution was tested with Simon's reagent.

Results

1. Effect of liquid-phase components on the qualitative test for methamphetamine

In the 100-μL liquid-phase fraction, at added weights of 200 μg or 50 μg MA, a positive reaction could be judged similarly to that of the control sample with distilled water (Fig. 2). In the 300-μL liquid-phase fraction, a positive reaction could be judged ; however, the positive reaction's color was lighter than with 100 μL. Additionally, as a negative contrast, in the only liquid-phase fraction, a nonspecific positive reaction was not shown, compared with samples of only distilled water and the negative control (only Simon's reagent). The liquid-phase fraction's pH was calculated at pH 8.0 to 8.1 in the liquid-phase fraction without MA and at pH 7.8 to 7.9 in the liquid-phase fraction with 2,000 μg of MA.

On the basis of these results, we decided that the appropriate volume of the liquid-phase fraction was 100 μL, considering that the addition of Simon's solutions A-C was 150 μL total. As the used volume of the liquid-phase fraction increased, the color change became lighter. Additionally, the used volume of the liquid-phase fraction was as low as the used volume of MA, and the detection limit was larger.

The 100-μL liquid-phase fraction in the sample containing blood and MA with the separating method could be tested with Simon's reagent (Fig. 3).

As unusual findings, in all reactions of the liquid-phase fraction, the reaction was slower than in control samples, and the blue color change in a positive reaction was less red than in distilled water.

2. Sensitivity of methamphetamine detection

Compared with control samples and the positive control, the volume limit for MA detection, judged visually with the solution's change in color to deep blue, was 200 μg.

3. Syringe samples

Results for syringe samples with the separating method were upper row, and those without the separating method were lower (Fig. 4). Although the blue varied from deep to light among samples, all samples were judged to have had a positive reaction compared with the negative control.

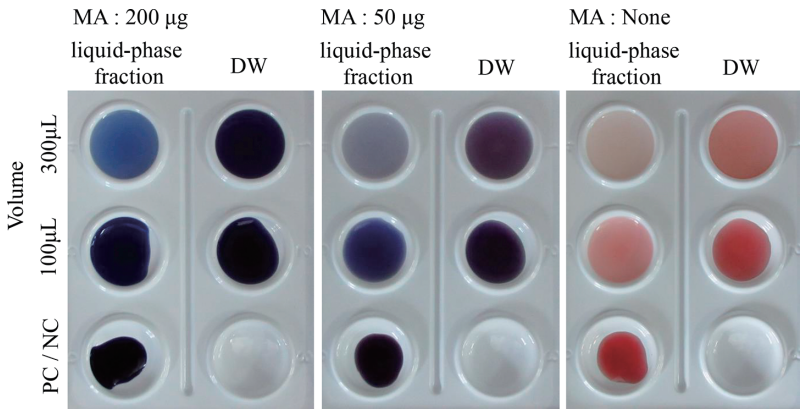

Fig. 2. Color change in the liquid-phase fraction versus distilled water. Added volumes of methamphetamine hydrochloride (MA) were 200 μg on the left, 50 μg in the center, and none on the right. (DW = distilled water, PC = positive control, and NC = negative control)

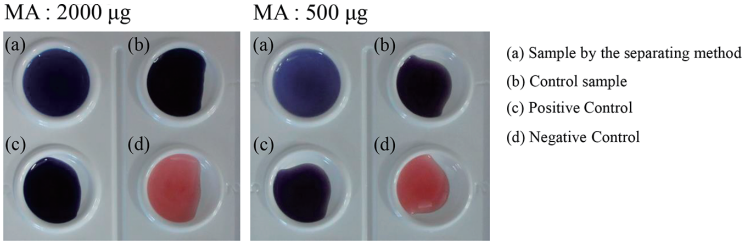

Fig. 3. Simon's reaction in liquid-phase fractions of blood containing methamphetamine hydrochloride (MA) ; the volumes were 2,000 μg on the left and 500 μg on the right.

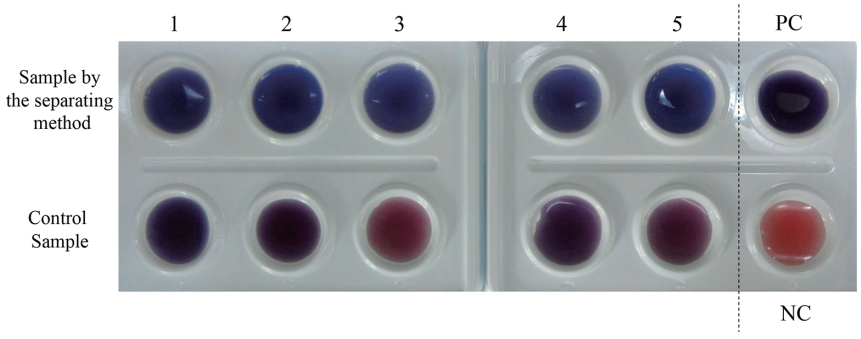

Fig. 4. Results of the syringe samples' washing solution. The sample using the separating method is shown at the top, and the control sample is at the bottom. Each sample was selected at random as numbers 1 through 5. The positive control was 50 μg of methamphetamine hydrochloride in distilled water.

Discussion

With the use of our method, the separated 100-μL liquid phase fraction in the samples containing blood and MA could be tested with Simon's reagent.

From the results of each section, the 100-μL liquid phase fraction was judged to have a positive reaction, as indicated by a color change to deep blue. Furthermore, a non-specific false positive reaction was not shown by the components in the liquid-phase fraction. However, in the reaction with the liquid-phase fraction, the color change to deep blue was less red and was slower than that observed in the control samples. This point should be carefully considered.

The detection limit containing 200 μg of MA assumed the volume of 20 μg of MA contained in 100 μL of 1 mL of

the liquid-phase fraction. The detection limit of Simon's reagent for MA was 10 μg in a previous study[13] and in the manufacturer's protocol[18]. Because 100 μL of the liquid-phase fraction was used in the present study, we assumed that a detection limit of our studywas appropriate. As an application to forensic samples, this method can be used to perform methamphetamine detection in syringe samples.

The method for detecting MA with Simon's reaction is less sensitive and accurate than that with GC/MS. Although Simon's reagent is used for methamphetamine testing in the fields of analytical chemistry and forensic science, it provides a qualitative reaction for secondary amines. Furthermore, although the limit for detection of MA with Simon's reaction is 10 μg, the limit for that with GC/MS is 1 ng. Therefore, in addition to spectrum analysis via MS, Simon's reaction should be used only as a primary screening test for MA before analysis with GC/MS. For high sensitivity and accuracy, MA testing should be performed with GC/MS if a negative result is observed.

The advantages of Simon's reaction are its simple steps—the addition of only 3 drops of reagent and visual detection of color change. Because the MA primary test in the liquid-phase fraction was helpful in judging whether DNA typing was analyzed immediately in an actual case, this method is valuable for criminal investigations. However, it should be noted that the color change to deep blue was less red and was slower than that observed in the control sample. Furthermore, the color change to deep blue caused by Simon's reagent should be objectively judged. In a previous study[14], the color change caused by a reaction with Simon's reagent was judged with RGB data. In the future, we aim to judge a positive reaction via the analysis of the RGB data of the changing color.

Authors have no conflict of interest.

References

1. Keisatsucho (National Police Agency). Keisatsu Hakusho (The white paper on police). Tokyo : Nikkei Printing Inc. ; 2018. Table 4-8, Yakubutsu Jihan no Kenkyo Jinin (Heisei 29 nen, 2017) (Arrested people for domestic drug crime). p. 140.
2. Degenhardt L, Peacock A, Colledge S, Leung J, Grebely J, Vickerman P, et al. Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs : a multistage systematic review. Lancet Glob Health. 2017 ; 5 : e1192-e1207.
3. Degenhardt L, Charlson F, Stanaway J, Larney S, Alexander LT, Hickman M, et al. Estimating the burden of disease attributable to injecting drug use as a risk factor for HIV, hepatitis C, and hepatitis B : findings from the Global Burden of Disease Study 2013. Lancet Infect Dis. 2016 ; 16 : 1385-98.
4. Aceijas C, Stimson GV, Hickman M, Rhodes T. Global overview of injecting drug use and HIV infection among injecting drug users. AIDS. 2004 ; 18 : 2295-303.
5. Aceijas C, Rhodes T. Global estimates of prevalence of HCV infection among injecting drug users. Int J Drug Policy. 2007 ; 18 : 352-8.
6. Wada K, Greberman SB, Konuma K, Hirai S. HIV and HCV infection among drug users in Japan. Addiction. 1999 ; 94 : 1063-9.
7. Irii T, Maebashi K, Fukui K, Sohma R, Matsumoto S, Takasu S, et al. Development of a dual test procedure for DNA typing and methamphetamine detection using a trace amount of stimulant-containing blood. Leg Med. 2016 ; 20 : 53-60.
8. Kovar K, Laudszum M. Chemistry and reaction mechanisms of rapid tests for drugs of abuse and precursors chemicals. SCITEC/6. Vienna : United Nations ; 1989.
9. Suzuki O, Yashiki M, editors. Yakudokubutsu bunseki jissen handobukku : kuromatogurafi wo chushin toshite (Handbook of Practical Analysis of Drug and Poisons in Human Specimens : chromatographic methods). Tokyo : Jihou, Inc. ; 2002. p. 151. (in Japanese)
10. Suzuki S, Inoue T, Niwaguchi T. Rapid screening method for methamphetamine in urine by colour reaction in a Sep-Pak C18 cartridge. J Chromatogr. 1983 ; 267 : 381-7.
11. Nakahara Y, Sekine H. A high selective screening test for methamphetamine in human urine. Forensic Sci Int. 1984 ; 26 : 277-82.
12. Hayakawa K, Imaizumi N, Ishikura H, Minogawa E, Takayama N, Kobayashi H. Determination of methamphetamine, amphetamine and piperidine in human urine by high-performance liquid chromatography with chemiluminescence detection. J Chromatogr. 1990 ; 515 : 459-66.
13. O'Neal CL, Crouch DJ, Fatah AA. Validation of twelve chemical spot tests for the detection of drugs of abuse. Forensic Sci Int. 2000 ; 109 : 189-201.
14. Choodum A, Parabun K, Klawach N, Daeid NN, Kanatharana P, Wongniramaikul W. Real time quantitative colourimetric test for methamphetamine detection using digital and mobile phone technology. Forensic Sci Int. 2014 ; 235 : 8-13.
15. Uchiyama N, Kawamura M, Kamakura H, Hanajiri R, Goda Y. Analytical data of designated substances (Shitei-Yakubutsu) controlled by the pharmaceutical affairs law in Japan, Part II : color test and TLC (in Japanese). Yakugaku Zasshi (Journal of the Pharmaceutical Society of Japan). 2008 ; 128 : 981-7.
16. Inoue H, Iwata Y, Kanamori T, Miyaguchi H, Tsujikawa K, Kuwayama K, et al. Analysis of benzylpiperazine-like compounds. Nihon Kanshiki Kagaku Gijutsu Gakkai Shi (Japanese Journal of Science and Technology for Identification). 2004 ; 9 : 165-84.
17. Kawakami T, Satoh H, Ohyama N. Identification by the color reaction of methamphetamine and the 2-phenethylamine and

N-isopropylbenzylamine mixture (in Japanese). Nihon Ho Kagaku Gijutsu Gakkai Shi (Japanese Journal of Forensic Science and Technology). 2018 ; 23 : 87–90.

18. Kanto Chemical Co., Inc. manufacturer's protocol. (in Japanese)