Journal of Chromatography, 530 (1990) 154–159
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5335

Note

High-performance liquid chromatography–chemiluminescence determination of methamphetamine in human serum using N-(4-aminobutyl)-N-ethylisoluminol as a chemiluminogen

KENICHIRO NAKASHIMA*, KEIKO SUETSUGU and SHUZO AKIYAMA

Faculty of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852 (Japan)

and

MASANORI YOSHIDA

Forensic Science Laboratory, Nagasaki Prefectural Police Headquarters, 4-8 Manzai-machi, Nagasaki 852 (Japan)

(First received January 23rd, 1990; revised manuscript received March 26th, 1990)

Methamphetamine (MP) has been used as an excellent sympathomimetic agent for a long time. However, the abuse of this drug has caused serious social problems. Therefore, for pharmaceutical studies on its metabolism, distribution and excretion, and for its identification in illegal circumstances, a sensitive and selective determination method of MP is necessary. Many methods have been developed: gas chromatography (GC) [1–3], gas chromatography–mass spectrometry (GC–MS) [4–6], thin-layer chromatography [7], polarization fluoroimmunoassay [8,9] and high-performance liquid chromatography (HPLC) [10,11]. The GC method is the most popular, but its sensitivity is not so high. The GC–MS method is very sensitive and able to detect femtomole levels of MP, but it needs a special and expensive instrument.

Recently, as the alternative to GC–MS, Hayakawa *et al.* [12] developed an HPLC method with chemiluminescence (CL) determination for amphetamine-related compounds. They used dansyl chloride as a precolumn labelling reagent and bis-(2,4,6-trichlorophenyl) oxalate and hydrogen peroxide as post-column chemiluminogenic reagents. The sensitivity of the method is superior to that of GC–MS.

Luminol derivatives are known as excellent chemiluminogenic reagents for many compounds. Among them, N-(4-aminobutyl)-N-ethylisoluminol (ABEI) was used as a precolumn labelling reagent for the sensitive determination of amines or carboxylic acids [13] and eicosapentaenoic acid or some fatty acids [14].

This paper describes a sensitive HPLC–CL method for MP using ABEI as a

0378-4347/90/$03.50

precolumn labelling reagent. The method was applied to the determination of MP in human serum.

EXPERIMENTAL

Materials and reagents

Acetonitrile and methanol (HPLC grade) were purchased from Wako (Osaka, Japan). ABEI, sodium 1-octanesulphonate, β-cyclodextrin and imidazole (reagent grade) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Hydrogen peroxide, potassium ferricyanide, N,N′-disuccinimidyl carbonate (DSC), sodium hydroxide and triethylamine (TEA) were of reagent grade (Wako).

MP·HCl was obtained from Dainippon Pharmacy (Osaka, Japan). MP·HCl was dissolved in methanol or water to give a 10^{-4} *M* solution. The solution was diluted to appropriate concentrations with methanol or water prior to use.

Normal human serum specimens were obtained from healthy volunteers in our department. A serum sample from an MP addict was kindly provided for this investigation by Forensic Science Laboratory of Nagasaki Prefectural Police Headquarters.

Apparatus

The HPLC system consisted of three HPLC pumps (LC-6A, Shimadzu, Kyoto, Japan), a 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.), a guard column (30 mm x 4.6 mm I.D.) packed with TSKm Guardgel ODS-80TM (Toyo Soda, Tokyo, Japan), a Shimpack CLC-C_{18} separation column (150 mm × 6 mm I.D., particle size 5 μm, Shimadzu), an R-01 recorder (Rikadenki, Tokyo, Japan) and a luminomonitor (AL-2220, Atto, Tokyo, Japan) with a spiral flow-cell of 60 μl.

Stainless-steel tubing (0.5 mm I.D.) was used in all flow lines. To obtain an optimum CL intensity, the lengths of mixing coils for MC_1 and MC_2 were set a 200 and 100 mm, respectively.

A schematic diagram of the system is shown in Fig. 1.

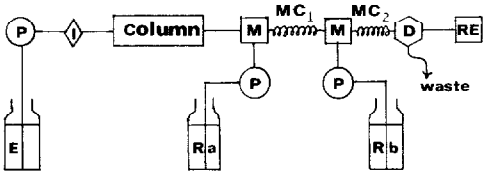

Fig. 1. Schematic flow diagram of the HPLC–CL system. P = pump; I = injector; M = mixing tee; MC = mixing coil; D = detector; RE = recorder; E = eluent (methanol–water, 54:46, containing 30 m*M* sodium 1-octanesulphonate); Ra = $1.5 \cdot 10^{-2}$ *M* $K_3Fe(CN)_6$ in 2.5 *M* NaOH (aqueous); Rb = 0.3 *M* hydrogen peroxide containing 10 m*M* β-cyclodextrin; Column, reversed-phase Shimpack LCL-C_{18} (Shimadzu, Japan).

Operating conditions

The mobile phase for separation of the ABEI derivative was methanol–water (54:46, v/v) containing 30 m*M* sodium 1-octanesulphonate. The flow-rate was set at 1 ml/min. The CL reaction of the ABEI derivative was performed with $1.5 \cdot 10^{-2}$ *M* potassium ferricyanide in 2.5 *M* sodium hydroxide and 0.3 *M* hydrogen peroxide containing 10 m*M* β-cyclodextrin. The flow-rate for both solutions was 1.0 ml/min.

Derivatization of MP with ABEI

A 5-μmol amount of ABEI in 2 ml of methanol and 5 μmol of DSC in 2 ml of acetonitrile were mixed in a 10-ml screw-capped test-tube and allowed to stand for 2 h. To 500 μl of the resulting solution (ABEI–DSC) were added 500 μl of MP in methanol and 20 μl of 0.5% TEA in methanol in a 5-ml screw-capped vial. After vortex-mixing, the mixture was heated at 80°C for 30 min. After cooling to room temperature, a 20-μl aliquot of the mixture was injected into the HPLC column.

Procedure for determination of MP in serum

To 100 μl of normal serum were added 10 μl of the aqueous solution containing MP·HCl (0.05–0.5 nmol). After vortex-mixing, 1 ml of acetone was added. The solution was further vortex-mixed for 2 min and centrifuged at 2000 *g* for 10 min. A 500-μl aliquot of the supernatant was used for the derivatization procedure.

A serum sample from an MP addict, which contained 3.6 μg/ml MP as determined by GC, was diluted ten-fold with water, and 100 μl of diluted serum was treated as described above.

RESULTS AND DISCUSSION

ABEI has been synthesized by Schroeder *et al.* [15] and used as a label for chemiluminescent immunoassay of steroid or protein. Recently, Kawasaki *et al.* [13] reported that ABEI is an excellent chemiluminogenic labelling reagent for HPLC–CL of primary and secondary amines or carboxylic acids. Although the method developed is very sensitive and can detect femtomole levels of ABEI derivatives, the results reported were achieved by using only authentic samples [13]. The application studies of ABEI as a chemiluminogen seem to be helpful to analytical researchers, since HPLC–CL of ABEI derivatives is very sensitive. More recently, Yuki *et al.* [14] successfully applied this method to the determination of eicosapentaenoic acid in serum. Thus, we applied this sensitive method to the detection of MP in human serum.

Derivatization of MP with ABEI consisted of two steps (Fig. 2). In the first step, the ABEI–DSC intermediate was prepared according to the literature [13]. For the second step, the preparation of MP-ABEI, the reaction temperature,

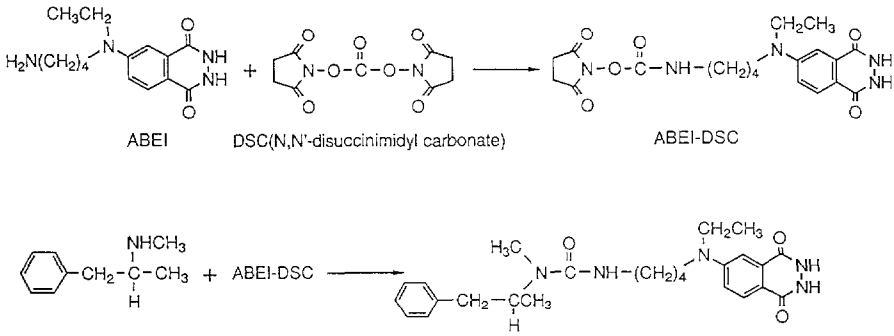

Fig. 2. Derivatization reaction of methamphetamine with ABEI.

time, catalyst and ABEI–DSC concentration were examined. In preliminary experiments, TEA was found to be a suitable catalyst for the condensation of ABEI–DSC with MP. Therefore, the effect of TEA on the condensation was examined and it was found that the use of 10–20 μl of 0.5% TEA in methanol was effective for the esterification of MP with ABEI–DSC. ABEI–DSC was treated with MP at 25, 60 and 80°C for 15–120 min. The highest reaction yield was obtained at 80°C for 30–60 min. The effect of ABEI–DSC concentration was examined for 10^{-7} *M* MP. The maximum and constant reaction yield was obtained over 500 μM of ABEI–DSC. The use of sodium 1-octanesulphonate was effective for the separation of MP-ABEI from the reagent blank. Under the condition described in Experimental, complete separation takes *ca.* 30 min.

The conditions for the chemiluminescence reaction of the ABEI derivative were examined. It was found that $1.5 \cdot 10^{-2}$ *M* potassium ferricyanide in 2.5 *M* sodium hydroxide and 0.3 *M* hydrogen peroxide aqueous solution resulted in the greatest CL intensity. These results were very similar to those in the literature [13]. Although microperoxidase could be used instead of potassium ferricyanide [14], we chose the latter because it is inexpensive. Addition of 10 m*M* β-cyclodextrin to the hydrogen peroxide solution slightly increased the CL intensity and improved the stability of the baseline.

The calibration curve of MP was linear over the range 0.05–5 pmol ($r = 0.998$) per injection. The detection limit for MP was 20 fmol at a signal-to-noise ratio of 3. The reproducibility of the peak height for MP (500 fmol) was 3.32% ($n = 5$). The proposed method was applied to the determination of MP in serum. The serum pretreatment was very simple and needed only deproteinization with acetone. The percentage recovery of MP was calculated from the slopes of calibration curves obtained from standard solutions and spiked serum. By adding 10 μl of an aqueous solution of known concentration of MP to 100 μl of normal serum, a calibration curve for spiked serum was prepared. The recovery for MP was 99.0%. The detection limit for MP spiked was the same as that for a standard solution ($2 \cdot 10^{-11}$ mol/ml of serum). In addition, as a practical exercise, serum

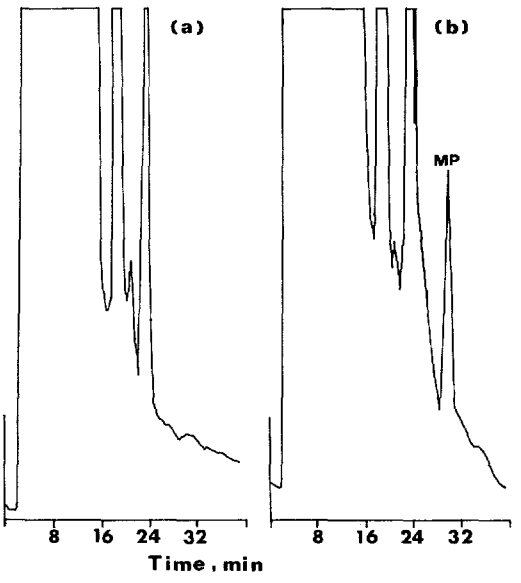

Fig. 3. Chromatograms of MP-ABEI in human serum. (a) Normal human serum; (b) serum of MP addict (MP, 2.8 pmol). Other conditions as in Fig. 1.

from an MP addict was tested for the determination of MP. The net volume of this serum needed was only 10 μl. The amount of MP determined was $5.05 + 0.07$ μg/ml ($n = 5$), which was 1.4 times greater than the value determined by GC. The reason for this difference is not clear, since the GC value came from another institute. More samples should be determined to compare the correlation between the two methods. The chromatogram obtained is shown in Fig. 3.

In conclusion, it is clear that ABEI is an excellent chemiluminogenic labelling reagent for MP. The proposed method is very sensitive and can detect as low as 20 fmol of MP. The sensitivity is comparable with that of the GC–MS method [6] and the HPLC–fluorescence method [12], but slightly lower than that of the HPLC–peroxyoxalate CL method [12]. Furthermore, the method can be successfully applied to the determination of MP in serum. Thus it should be useful for the identification of MP in illegal circumstances and for biomedical studies, in which only small amounts of biological samples should be treated.

REFERENCES

1 R. B. Bruce and W. R. Maynard Jr., *Anal. Chem.*, 41 (1969) 977.
2 M. Terada, T. Yamamoto, T. Yoshida, Y Kuroiwa and S. Yoshimura, *J. Chromatogr.*, 237 (1982) 285.
3 M. Terada, S. Yoshimura, T. Yamamoto. T. Yoshida and Y. Kuroiwa, *Eisei Kagaku*, 29 (1983) 143.
4 C. D. Morgan, F. Cattabeni and E. Costa, *J. Pharmacol. Exp. Ther.*, 180 (1972) 127.
5 K. Kamei, M. Murata, K. Ishii, M. Namekata and A. Momose, *Chem. Pharm. Bull.*, 21 (1973) 1966.

6 T. Yamamoto, M. Terada, S. Yoshimura, T. Sato, H. Kitagawa, T. Yoshida, K. Aoki and Y. Kuroiwa, *Eisei Kagaku*, 27 (1981) 331.
7 F. V. Hoof and A. Heyndrickx, *Anal. Chem.*, 46 (1974) 286.
8 S. A. Eremin, G. Gallacher, H. Lotey, D.S. Smith and J. Landon, *Clin. Chem.*, 33 (1987) 1903.
9 S. A. Eremin, D. E. Schiavetta, H. Lotey, D. S. Smith and J. Landon, *Ther. Drug Monit.*, 10 (1988) 327.
10 B. M. Farrell and T. M. Jefferies, *J. Chromatogr.*, 272 (1983) 111.
11 N. Takayama, H. Kobayashi and A. Tsuji, *Eisei Kagaku*, 30 (1984) 14.
12 K. Hayakawa, K. Hasegawa, N. Imaizumi, O.S. Wong and M. Miyazaki, *J. Chromatogr.*, 464 (1989) 343.
13 T. Kawasaki, M. Maeda and A. Tsuji, *J. Chromatogr.*, 328 (1985) 121.
14 H. Yuki, Y. Azuma, N. Maeda and H. Kawasaki, *Chem. Pharm. Bull.*, 36 (1988) 1905.
15 H. R. Schroeder, R.C. Boguslaski, R. J. Carrico and R. T. Buckler, *Methods Enzymol.*, 57 (1978) 424.