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Note

Microdetermination of stimulant drugs in urine by high-performance liquid chromatography

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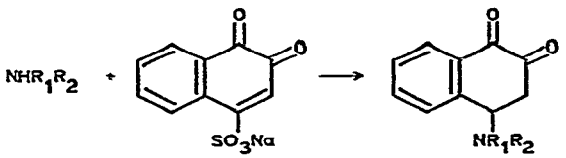
The abuse of stimulant drugs, which had subsided temporarily, has increased again in the last decade. The trace detection of stimulant drugs in urine has been of forensic interest and gas chromatography has been utilized as a routine method. High-performance liquid chromatography (HPLC) is also useful and several reports on the HPLC detection of stimulant drugs based on β-phenethylamine derivatives, such as amphetamine (AP) and methamphetamine (MP), have been published[1–5]. They are, not very sensitive, however, because these amines show only relatively weak absorption bands in UV range (log $\varepsilon = 2.27$ at 257 nm for AP).

In a previous paper[6] we described a colour reaction of these amines with sodium β-naphthoquinone-4-sulphonate (NQS):



The reaction mechanism was elucidated and it was found that the reaction proceeds quantitatively under the optimal conditions. In this paper the application of this colour reaction to the micro-determination of stimulant drugs by HPLC is described.

EXPERIMENTAL

Amphetamine, methamphetamine and other β-phenethylamine derivatives were used. Psychotropic drugs of other types and several simple primary and secondary amines were tested for comparison. They were mescaline, 2,5-dimethoxy-4-methylamphetamine (STP), *l*-ephedrine, *d*-ψ-ephedrine, norephedrine, *dl*-α-phenethylamine, β-phenethylamine, phentermine, piperidine, 2-pipecoline, morpholine, dimethylamine and isopropylamine.

HPLC apparatus of our own construction was used[7]. A single-beam spectrom-

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eter Toshiba Beckman (Model Spectra 20, 210–700 nm) equipped with a flow cell was used as the HPLC detector. Silica gel packings (LiChrosorb SI 100, 10 μm, E. Merck, Darmstadt, G.F.R., and Wakogel LC 5H, 5 μm, Wako, Osaka, Japan) were packed into stainless-steel columns (25 cm × 2 mm I.D.) by a balanced slurry packing technique[8].

A gas chromatograph (Shimazu Model GL-4BMPG) was used to compare HPLC data with gas–liquid chromatographic (GLC) data.

Absorption spectra of the coloured products were examined with a dual-beam spectrometer (Shimazu Model UV 210).

RESULTS AND DISCUSSION

The optimal conditions for the colour reaction reported previously[6] were as follows. To each amine sample were added 1 cm^3 of 8% sodium hydrogen carbonate solution and 1 cm^3 of 0.5% NQS solution. The mixture was heated at 70°C for 20 min. After cooling, the reaction product was extracted with 2 cm^3 of chloroform. Under these conditions, reaction and extraction of AP and MP proceeded quantitatively.

In order to establish the optimal HPLC conditions, the coloured products were separated by thin-layer chromatography (TLC) on silica gel using various solvent systems. Chloroform–ethyl acetate containing a small amount of ethanol gave the best results, with good separations and no tailing, and was adopted used for HPLC. To adjust the adsorption activity of the silica gel column, each solvent except ethanol was saturated with water. Water-saturated *n*-hexane was added to the solvent to control the retention times of each product. The reproducibility was then very good.

Fig. 1a shows chromatograms of the mixture of coloured products from AP and MP, their retention times being 3.2 and 1.8 min, respectively. The retention times of the reaction products of other amines under these conditions were 6.7 min (mescaline), 2.8 min (STP), 9.0 min (*l*-ephedrine), 14.3 min (*d*-φ-ephedrine), 9.9 min

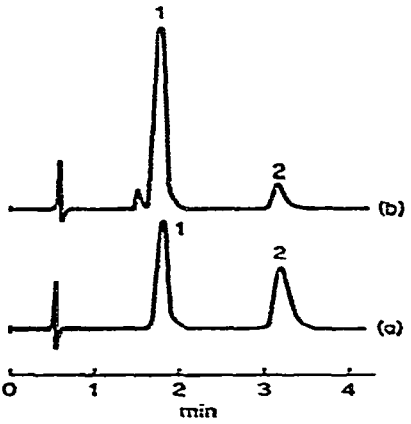

Fig. 1. Chromatograms of amphetamine and methamphetamine derivatives. (a) Standard sample (containing 1 μg of amphetamine and methamphetamine); (b) sample of urine of a methamphetamine addict. Peaks: 1 = methamphetamine; 2 = amphetamine. Column, Wakogel LC 5H (25 cm × 2 mm I.D.); eluent, chloroform–ethyl acetate–ethanol–*n*-hexane (25:10:1:50); flow-rate, 1.2 cm^3/min; detection, 450 nm; sample size, 100 μl.

(norephedrine), 2.3 min (*dl*-α-phenethylamine), 5.0 min (β-phenethylamine), 1.7 min (phentermine), 1.3 min (piperidine), 1.2 min (2-pipecoline), 2.7 min (morpholine), 6.7 min (dimethylamine) and 7.2 min (isopropylamine). The peaks of these amine derivatives did not overlap the peaks of the AP and MP derivatives. The absorption maxima for the AP and MP derivatives were 451 nm ($\log \varepsilon = 3.62$) and 464 nm ($\log \varepsilon = 3.56$), respectively, which suggests that the derivatization resulted in an increase in sensitivity in the visible range of about 25-fold. As the coloured products have stronger absorption bands in the UV range, with two absorption maxima at 280 and 248 nm, UV detection was about 2.4-fold (280 nm) and 3.7-fold (248 nm) more sensitive. Detection in UV range, however, suffered from interferences from coexisting foreign substances in a few instances when urine samples were tested. No such interference was observed when detection was carried out in the visible range.

Calibration graphs for AP and MP in the visible range were linear from 0.25 to 2 μg. They deviated from linearity with smaller amounts and the peak heights were slightly smaller than the values predicted by a linear plot. The detection limit with the present apparatus was 5 ng (at 464 nm) and 2 ng (at 280 nm) for MP under the conditions specified in Fig. 1a.

The proposed method was applied to the micro-determination of stimulant drugs in the urine of drug addicts. The urine (50 cm^3) was made alkaline with 1 cm^3 of concentrated ammonia solution and the free bases were extracted with three 50-cm^3 volumes of *n*-hexane. A small amount (0.5 cm^3) of concentrated hydrochloric acid–ethanol (1:6) was added to the combined *n*-hexane extracts to convert the free amines into the hydrochlorides. After the solvent had been removed by distillation on a steam-bath, the colour reaction was carried out as described above.

Fig. 1b shows an example of the chromatogram from an MP addict. AP is one of the metabolites of MP, two peaks (due to MP and AP) always appeared on the chromatogram when samples of MP addicts were tested. For comparison, the GLC determination of AP and MP as the acetate derivatives was carried out[9]. The HPLC and GLC results agreed well. The sensitivities of the two methods were comparable when HPLC detection was carried out in the visible range.

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