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Note

A procedure for the differentiation of the optical isomers of amphetamine and methamphetamine by thin-layer chromatography

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The stimulant drugs amphetamine and methamphetamine are widely abused and readily available in The Netherlands, one of the main reasons for their availability being the relative ease of manufacture. They occur in the illicit market as both the *d*-isomer and the racemic mixture. It is sometimes necessary, for legal reasons, to differentiate between the optical isomers.

The separation and identification of these isomers can be readily achieved by the procedure described here. A suitable optically active reagent is added to the samples spotted on a thin-layer chromatography (TLC) plate and then the prepared TLC plate is developed in the appropriate solvent system with subsequent detection with sulphuric acid–formaldehyde.

EXPERIMENTAL

Materials and methods

dl-Amphetamine sulphate and *d*-methamphetamine hydrochloride were obtained commercially (E. Merck, Darmstadt, G.F.R.). *d*-Amphetamine and *l*-amphetamine were prepared by repeated fractional crystallization of the amphetamine bitartrates from 92% ethanol[1]. *l*-Methamphetamine and *dl*-methamphetamine were prepared by the reduction of *d*-ephedrine and *dl*-ephedrine according to the method of Emde[2].

The following standard solutions were prepared. (a) *d*-Amphetamine, *l*-amphetamine, *dl*-amphetamine, *d*-methamphetamine, *l*-methamphetamine and *dl*-methamphetamine: 10 mg/ml in benzene. The solutions were prepared by extraction of the strongly alkaline aqueous solutions of the salts with benzene. (b) *d*-Amphetamine sulphate, *l*-amphetamine sulphate and *dl*-amphetamine sulphate: 20 mg/ml in 50% ethanol. (c) *d*-Methamphetamine hydrochloride, *l*-methamphetamine hydrochloride and *dl*-methamphetamine hydrochloride: 100 mg/ml in 50% ethanol.

The optically active reagent N-trifluoroacetyl-L-prolyl chloride (TPC) was prepared as described by Bonner[3]. The optically active reagent N-benzyloxycarbonyl-L-prolyl chloride (ZPC) was prepared by the reaction of N-benzyloxycarbonyl-L-proline (Merck) with thionyl chloride[3]. The concentration of TPC and ZPC was 0.2 *M* in methylene chloride. The reagents were stored at −20°.

TLC was carried out on non-activated pre-coated silica gel plates of thickness

0.25 mm (Merck). The developing solvents were: (A) chloroform–methanol (197:3); (B) *n*-hexane–chloroform–methanol (10:9:1); and (C) *n*-hexane–ethyl acetate–acetonitrile–diisopropyl ether (2:2:2:1). The reagent used for detection was 96% sulphuric acid–40% formaldehyde solution (10:1). The reagent was freshly prepared, allowed to cool and then poured over the developed plate.

Procedures

I. Differentiation of the optical isomers of amphetamine base. The *d*- and *l*-amphetamines were isolated as the free bases from strongly alkaline aqueous solutions of the salts by extraction with benzene. The concentration of the benzene solution of each free base was approximately 1%.

The sample solution (1 μl) and 1 μl of the appropriate standard solutions (a) were placed upon distinctly marked positions on the starting line of the plate, followed by 1 μl of either the TPC or the ZPC reagent at exactly the same position as the sample solution and the standard solution spots. When the TPC reagent was added solvent system A was used, and when the ZPC reagent was added solvent system B was used.

The TLC plates were developed in cylindrical glass vessels without prior equilibration. When the solvent front had reached a height of 10 cm, the TLC plate was removed from the developing tank and allowed to air dry at room temperature. The spots were made visible by pouring the detection reagent over the developed and dried TLC plate.

II. Differentiation of the optical isomers of methamphetamine base. The procedure was the same as for amphetamine except that ZPC reagent was used and the resulting derivatives were separated with solvent system C.

III. Differentiation of the optical isomers of amphetamine salts. A 2% solution of the amphetamine salt of the sample in 50% ethanol was used together with the appropriate standard solution (b).

The sample spots and the reference spots were well dried with warm air before adding the reagent. The remainder of the procedure was the same as in procedure I.

IV. Differentiation of the optical isomers of methamphetamine salts. It was necessary to use a 10% solution of the methamphetamine salt in 50% ethanol and the appropriate standard solution (c) to achieve the desired results. The remainder of the procedure was the same as described in procedures II and III.

RESULTS AND DISCUSSION

The excellent results obtained when gas–liquid chromatography (GLC) was used for the differentiation of the optical isomers of amphetamine by means of their TPC derivatives[4,5] prompted the investigation of the possibility of adapting this method for the TLC separation of these optical isomers. However, although the diastereoisomers of the TPC derivatives of amphetamine were satisfactorily separated with several solvent systems, the separation of these derivatives of methamphetamine was unsatisfactory. On preparing and testing some other optically active reagents, it was found that the ZPC reagent was suitable for the separation of both methamphetamine and amphetamine isomers.

The separated diastereoisomers became visible as discrete, brown spots, which

TABLE I

SEPARATION OF DIASTEREOISOMERIC TPC AND ZPC DERIVATIVES OF AMPHETAMINE AND METHAMPHETAMINE BY THIN-LAYER CHROMATOGRAPHY

Compound	*Derivative*	*Solvent system*	R_F
d-Amphetamine	TPC	A	0.49
l-Amphetamine	TPC	A	0.55
dl-Amphetamine	TPC	A	0.49, 0.55
d-Amphetamine	ZPC	B	0.43*
l-Amphetamine	ZPC	B	0.47*
dl-Amphetamine	ZPC	B	0.43, 0.47*
d-Methamphetamine	ZPC	C	0.61
l-Methamphetamine	ZPC	C	0.57
dl-Methamphetamine	ZPC	C	0.57, 0.61

* A weak additional spot, caused by the reagent, was sometimes observed at R_F 0.68.

gradually faded, when the detection reagent was carefully poured over the developed and air-dried TLC plates. The R_F values are listed in Table I.

Accepting a slight loss in sensitivity for amphetamine and a considerable loss in sensitivity for methamphetamine, it was possible to apply the TPC or ZPC reagents directly to the salts of amphetamine and methamphetamine (procedures III and IV).

Before using the described procedures for "street drug" identification, the amphetamine or methamphetamine should first be tentatively identified. This preliminary identification can be accomplished rapidly by one of the existing TLC procedures developed for this purpose by Brown *et al.*[6] and Van Welsum[7].

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