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Note

Analysis of impurities in illicit amphetamine by high-performance liquid chromatography

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Amphetamine produced illicitly often contains impurities arising from side reactions and incomplete reactions[1]. Gas chromatography (GC) is usually employed for the analysis of these impurities. Capillary columns are used for the resolution of complex impurity patterns and the identification of compounds is carried out by GC–mass spectrometry (MS)[2,3]. The impurity pattern may give valuable information by comparison of samples from different seizures and the detection of characteristic by-products may provide information about the method of synthesis.

The Leuckart method is frequently employed in illicit amphetamine production and an investigation of samples taken in Norway showed that illicit amphetamine was most frequently synthesized by this method[4]. The purpose of the present study was to develop a high-performance liquid chromatographic (HPLC) method for the separation of impurities, special attention being paid to compounds arising from the Leukart synthesis of amphetamine. A typical Leuckart impurity pattern has been evaluated and the HPLC method has been compared with the more commonly used GC method.

EXPERIMENTAL

Chemicals

Analytical grade methanol and benzene were obtained from E. Merck (Darmstadt, F.R.G.). The benzene was redistilled before use. HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, U.K.). Standard N-formylamphetamine was synthesized according to the method of LeBelle *et al.*[5].

Apparatus

High-performance liquid chromatography. A Series 2 liquid chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.) equipped with a LC-85 UV detector was used. The eluent was monitored at 254 and 280 nm. The injector was a Rheodyne Model 7125 with a 6-μl sample loop. Samples were chromatographed on a Perkin-Elmer C_{18} column HS-5 (125 × 4.6 mm). The mobile phase was a linear gradient with 25% methanol in water as eluent A and methanol as eluent B. The gradient was programmed from 40 to 100% B in 20 min. The flow-rate was 1 ml/min and the analysis was carried out at ambient temperature.

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Gas chromatography. A Fractovap 2900 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame ionization detector and a fused-silica capillary column (25 m × 0.33 mm I.D.) wall-coated with SE-30 was used. The injector and detector temperatures were 275°C. The oven temperature was programmed from 130 to 145°C at 4°C/min, then isothermal 145°C for 5 min and from 145 to 250°C at 4°C/min. Helium was used as carrier gas and the flow-rate was adjusted to 3.0 ml/min through the capillary column, 30 ml/min at the outlet of the splitter and 5 ml/min as septum flush. Samples of 1 μl were injected.

Gas chromatography–mass spectrometry. A Micromass 7070 F mass spectrometer (VG-Micromass, Altrincham, U.K.) combined with a Fractovap 4200 gas chromatograph (Carlo Erba) was used. The electron energy was 70 eV.

Extraction of impurities

Amphetamine (300–500 mg) was dissolved in 5 ml distilled water and 2 ml redistilled benzene were added. The aqueous phase was made weakly acidic (pH 5–6) and trace components were extracted into benzene by vigorous shaking for 10 min. The benzene extract was transferred to a glass tube and evaporated to dryness under a stream of nitrogen at 35°C. The residue was dissolved in 50 μl methanol.

Identification of impurities

The identification of impurities eluted from the HPLC column was performed by GC–MS. The eluate from 5–10 injections was collected and extracted with redistilled benzene. The benzene extracts were transferred to a glass tube and concentrated to 50 μl. Samples of 1 μl were injected into the gas chromatograph connected to the mass spectrometer.

RESULTS AND DISCUSSION

Fig. 1 shows a typical impurity chromatogram of a sample of a Leuckart-synthesized amphetamine after gradient elution on the C_{18} column. Separation of the impurities by isocratic elution was not possible because of the too large polarity difference. Peaks 1 and 2 were identified by GC–MS as the Leukart-specific impurities N-formylamphetamine and 4-methyl-5-phenylpyrimidine respectively. The high boiling pyridines described by Van der Ark *et al.*[6] were eluted after 16 min. N-Formylamphetamine, 4-methyl-5-phenylpyrimidine and the pyridines are usually the major contaminants in a Leukart-synthesized product monitored with UV detection at 254 nm. These compounds had a low UV absorption at 280 nm. The response ratio, 254 nm/280 nm, may, however, be useful for the identification of compounds. Acetonitrile–water gradients were also examined as mobile phases. A linear gradient starting from 40 to 100% acetonitrile in 20 min was the most successful. Excellent resolution of early eluting peaks was observed, contrary to the more retained compounds that showed peak broadening and tailing. Since this gradient eluted N-formylamphetamine completely and specifically, it is well suited for determination of this impurity in samples of crude amphetamine seizures.

Fig. 2 shows a gas chromatogram of the same sample that was analysed by HPLC (Fig. 1). Both the retention behaviour and response differ greatly in the HPLC and GC methods. Approximately the same number of components can be detected

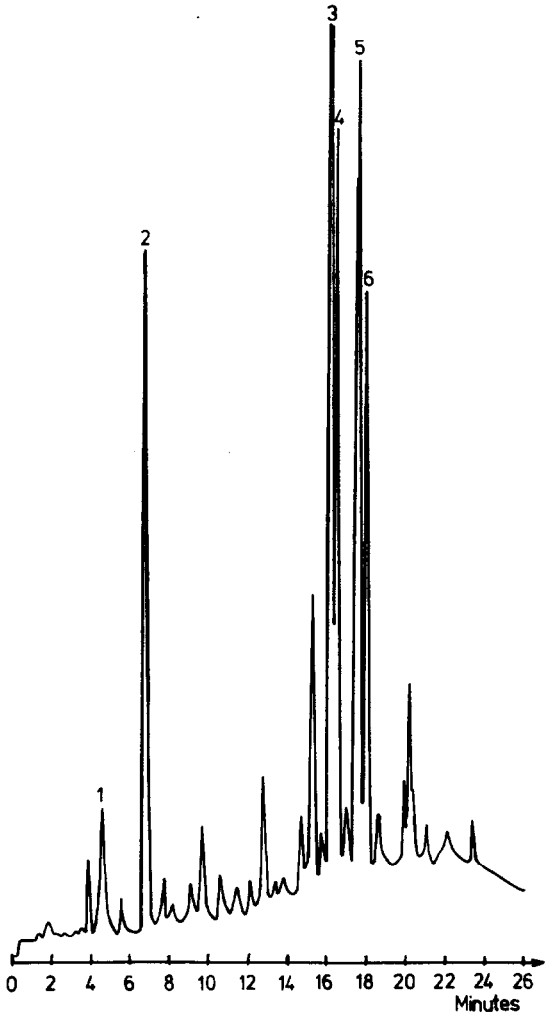

Fig. 1. Liquid chromatogram of impurities in amphetamine. Detection by UV absorption at 254 nm. Peaks: 1 = N-formylamphetamine; 2 = 4-methyl-5-phenylpyrimidine; 3–6 = high boiling pyridines.

by the two methods. With the 12.5 cm high-speed C_{18} column used, HPLC analysis can be carried out in 25 min while 40 min were required for temperature programming of the capillary GC column. The absence of a solvent peak which may mask other early eluting peaks is an adventage of the HPLC method.

The HPLC method has been tested by analysing a variety of seized amphetamine samples with amphetamine contents ranging from 20 to 90%. The diluents were mostly sugar and glucose. All samples gave a detailed fingerprint of the contaminants and the chromatograms were suitable for the comparison of samples from different seizures of amphetamine. The Leukart-specific contaminants were easily detected.

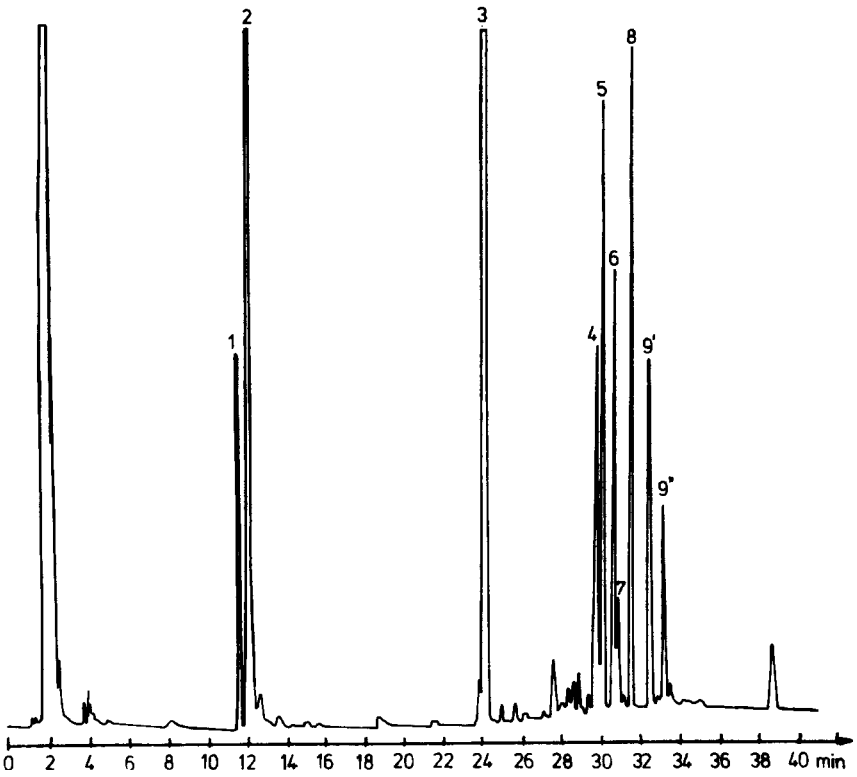

Fig. 2. Gas chromatogram of impurities in amphetamine. Flame ionization detection. Peaks: 1 = 4-methyl-5-phenylpyrimidine; 2 = N-formylamphetamine; 3 = di(β-phenylisopropyl)amine; 4–8 = high boiling pyridines; 9′ and 9″ = N,N-di(β-phenylisopropyl)formamide.

Fig. 3 shows a chromatogram from an HPLC analysis of a sample of seized amphetamine with 4-methyl-5-phenylpyrimidine as the dominant peak and the high boiling pyridines as minor peaks. This pattern is typical for illicit amphetamine purified by steam distillation[2].

CONCLUSION

This investigation has shown that HPLC with gradient elution on a C_{18} column is well suited for the analyses of impurities in illicit amphetamine samples. The method can be used for the identification of impurities and for the comparison of samples from different seizures. It is a valuable supplement to GC which has widely been used for such analyses.

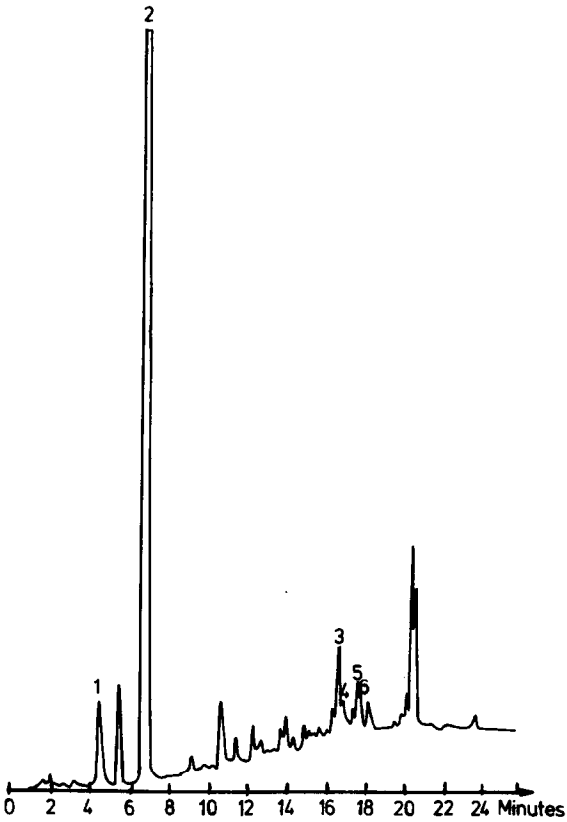

Fig. 3. Liquid chromatogram of impurities in amphetamine. Detection by UV absorption at 254 nm. Peaks as in Fig. 1.

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