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Note

Determination of caffeine in serum by straight-phase high-performance liquid chromatography

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Caffeine (1,3,7-trimethylxanthine) and analogous dimethylxanthine derivatives such as theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine), are stimulators of the central nervous system. Caffeine is the most potent stimulator of the central nervous system, especially of the medullary respiratory centre [1]. This property makes caffeine the most useful drug of the three commonly available methylxanthine derivatives in the prevention of apnea in premature infants. Aranda et al. [2] showed the efficacy of treating apnea in low-birth-weight infants with caffeine.

Caffeine is mainly metabolised in the liver, undergoing demethylation and oxidation [3]. The most important metabolite is paraxanthine (1,7-dimethylxanthine), and its physiological activity should not be disregarded. Also the formation of theobromine and theophylline has been described [4]. The oxidised and demethylated metabolites 1-methyluric acid, 1-methylxanthine and 1,3-methyluric acid appear in the urine. Sometimes the biotransformation of the dimethylxanthine derivatives is paradoxical. Bory et al. [5] and Boutroy et al. [6] described the treatment of premature infants suffering from apnea with theophylline, which was biotransformed to caffeine. So even in the use of theophylline one should be aware of the possibility that caffeine might be present.

Unchanged caffeine is excreted in the urine in negligible amounts. The analysis of urinary products has no therapeutic value. The biological half-life ($t_{1/2}$) of caffeine in adults is approximately 3.5 h. The $t_{1/2}$ in premature infants is very much prolonged up to 97 h and the values are spread over a wide range: 40.7—230 h [2]. The reason for this is the incomplete development of the liver functions in the preterm infant. As a consequence of these enormous differ-

ences in rate of metabolism, serum concentrations need to be monitored to optimise the therapy and to avoid toxic or subtherapeutic levels.

In analogy to theophylline, caffeine has no significant efficacy below a serum concentration of 5 mg/l. The optimal level is taken to be 10—15 mg/l. Within this range there is a significant decrease in the incidence and severity of apnea [2]. The serum concentration should not exceed 20 mg/l because of risk of toxicity. Below a level of 20 mg/l no toxic symptoms have been described. In man the fatal oral dose of caffeine is estimated to be about 10 g. Untoward reactions, however, may be observed following the ingestion of 1 g or more.

Several methods for the determination of caffeine in biological fluids have been described [4, 7—10]. When caffeine is used for the indication apnea in premature infants, the serum determination should require very small amounts of serum and be accurate and rapid. We have modified the straight-phase high-performance liquid chromatographic (HPLC) method for the determination of carbamazepine described by Westenberg and De Zeeuw [11].

MATERIALS AND METHODS

Chemicals and reagents

Dichloromethane, p.a. grade, and tetrahydrofuran "rein" were from Merck (Darmstadt, G.F.R.); caffeine was obtained from A.C.F. (Maarssen, The Netherlands), and carbamazepine was from Ciba-Geigy (Basle, Switzerland). Water was double-distilled from glass columns.

Standard solutions

Standard solutions of caffeine were prepared in dichloromethane, water and spiked serum, in the concentration range 5—25 mg/l. The internal standard solution was carbamazepine, 0.5 mg/l in dichloromethane.

Apparatus

A Siemens Model S100 high-performance liquid chromatograph with a Valco UH Pa7 valve with 0.05-ml sample loop was used connected to a UV detector (Zeiss PM_2 DLC) which was operated at 272 nm. The column was stainless steel (10.0 cm × 4.6 mm I.D.) packed with silica gel (Partisil 5, Chrompack, Middelburg, The Netherlands; particle size 5 μm). The eluent was 20% tetrahydrofuran in dichloromethane, the flow-rate 1.5 ml/min (pressure drop 80 bar).

A Finnigan 3200 mass spectrometer connected to a Finnigan 9500 gas chromatograph was operated as follows: column, 3.8% SE-30 on Chromosorb W AW DMCS 80—100 mesh; column oven temperature, 240°; mass spectrometry ionizing voltage, 70 eV; electromultiplier voltage, 1600 V.

Assay

Transfer 0.2 ml of a serum sample into a 10-ml test-tube, and add 5.0 ml of internal standard solution. Rotate for 1 min on a Vortex mixer and centrifuge at 2000 *g* for 5 min. Transfer 4 ml of the organic layer into a test-tube and evaporate to dryness by heating the tube in a water-bath of ca. 37°. Add 0.250 ml of dichloromethane and inject 0.020—0.050 ml into the chromatograph.

RESULTS

The standard solutions of caffeine in dichloromethane, water and spiked serum were determined as described in the assay procedure. Peak height ratios were calculated and plotted against the known concentrations. The slopes and correlation coefficients were calculated using a least-squares procedure. There is a good linear correlation between peak height ratios and serum levels in the range of the calibration curve (correlation coefficient = 0.9997). The standard error of the determination at 12 mg/l (n = 12) is 1.5%. There is no significant difference between the extraction of caffeine from serum and water. With these results in mind, we use an aqueous solution for the caffeine standard for routine determinations since blank serum might be contaminated with caffeine.

The detection limit of the method is 4 ng per injection with a signal-to-noise ratio of 4:1. Thus, with the described procedure it is possible to detect caffeine accurately using 0.1 ml of serum. Fig. 1 shows a chromatogram of the serum from a patient treated with caffeine in a single dose of 5 mg/kg. The peaks numbered 3 and 4 were identified by gas chromatography—mass spectrom-

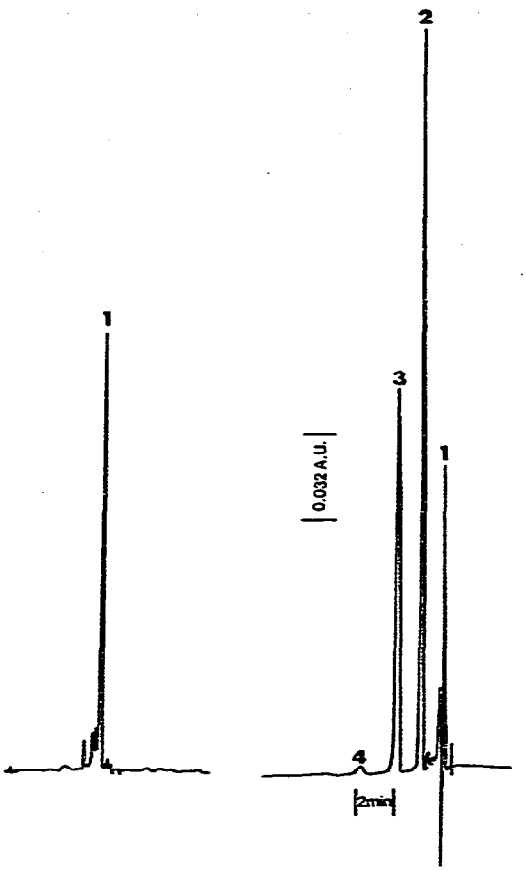

Fig. 1. Chromatogram of blank serum (left) and of a patient's serum sample (right). Peaks: 1, solvent; 2, internal standard (carbamazepine); 3, caffeine; 4, paraxanthine. Peak 3 represents 560 ng of caffeine.

etry, peak 4 being first flash-ethylated using a modified method of Brochman-Hansen and Oke [12] to obtain a better peak shape.

The three possible dimethylxanthine derivatives have the same retention time. Whenever one of these is present in significant amounts its identity can be determined using an other eluent [4] or mass spectrometry.

CONCLUSION

The described method is suitable for the routine determination of therapeutic serum levels of caffeine as well as for toxicological purposes.

REFERENCES

1 J.M. Ritchie, in L.S. Goodman and A. Gilman (Editors), The Pharmacological Basis of Therapeutics, Macmillan, New York, 1975, pp. 367—378.
2 J.V. Aranda, W. Gorman, H. Bergsteinsson and T. Gunn, Pediatrics, 90 (1977) 467—472.
3 H.H. Cornish and A.A. Christman, J. Biol. Chem., 228 (1957) 315—323.
4 M. Danhof, B.M.J. Loomans and D.D. Breimer, Pharm. Weekbl., 113 (1978) 672—675.
5 C. Bory, P. Baltasat, M. Porthault, M. Bethenod, A. Frederich and J.V. Aranda, Lancet, ii (1978) 1204—1205.
6 M.J. Boutroy, P. Vert, P. Monin, R.J. Royer and M.J. Royer-Morrot, Lancet, i (1979) 830.
7 J. Axelrod and J. Reichtenthal, J. Pharmacol, Exp. Ther., 107 (1953) 519—423.
8 J.I. Routh, N.A. Shane, E.G. Arredondo and W.D. Pau, Clin. Chem., 45 (1969) 661—668.
9 J.L. Cohen, C. Cheng, J.P. Henry and Y.L. Chan, J. Pharm. Sci., 67 (1978) 1093—1095.
10 C.E. Cook, C.R. Tallent, E.W. Anderson, M.W. Myers and J.A. Keppler, J. Pharmacol. Exp. Ther., 199 (1976) 679—686.
11 H.G.M. Westenberg and R.A. de Zeeuw, J. Chromatogr., 118 (1976) 217—224.
12 E. Brochman-Hansen and T.O. Oke, J. Pharm. Sci., 58 (1969) 370—371.