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Note

Simultaneous determination of theophylline and caffeine after extractive alkylation in small volumes of plasma by gas chromatography—mass spectrometry

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Theophylline is widely used as a bronchodilator in the treatment of reversible airway obstruction [1]. In the last few years it has also been used in the treatment of apnéa in premature infants [2]. Several rather specific and sensitive gas chromatographic (GC) and liquid chromatographic methods for its determination have been described [3—7]. These may also be used, after slight modification, for the determination of caffeine. However, in these methods, quantification of both theophylline and caffeine may be difficult to achieve in small sample volumes and may also involve tedious extraction procedures. A GC—mass spectrometric (MS) method with high sensitivity and specificity for the determination of caffeine only has been reported [8]. Sensitive immunotechniques such as radioimmunoassay (RIA) and enzyme multiplied immunotechnique (EMIT®) are also available for the determination of theophylline.

The aim of the present work was to design a method for determining theophylline and caffeine in the plasma of premature infants treated with theophylline for neonatal apnéa. In contrast to adults, such infants have been shown to metabolize theophylline to caffeine to a significant degree [9], producing plasma concentrations of caffeine that probably have pharmacological effects. The method described is capable of handling small sample volumes (50 μl) and involves only one extraction step, which is combined with the derivatization stage. The effect of variations in the extraction yield on the

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precision of the method can be reduced by the use of deuterated analogues of theophylline and caffeine as internal standards.

MATERIALS AND METHODS

Instrumental

A Finnigan 4000 gas chromatograph—mass spectrometer was used for the measurements. It was equipped with a multiple ion monitoring unit (Finnigan, Sunnyvale, CA, U.S.A.). The injector, of the Grob capillary type, was operated at 225°C in a splitless mode, and was equipped with valves which were programmed to vent the injector 60 sec after injection. The glass capillary column was 25 m OV-225. The column temperature was programmed from 170—210°C at a rate of 10°C/min. A pressure of 20—25 kPa of helium was applied to the column which was directly coupled to the ion source. The electron energy was set at 70 eV. For injection, solid sample syringes (SGE, North Melbourne, Australia) were used. They were cleaned in a Hamilton syringe cleaner between injections.

Internal standards

Deuterated theophylline was synthesised by monomethylation of 1-methylxanthine [10] with trideuteromethyl iodide. Deuterated caffeine was synthesised by dimethylation of 1-methylxanthine with the same alkylating agent.

Method

Plasma (50 μl) in a 10-ml screw-capped tube was added to 100 μl of a solution containing trideuterotheophylline (4.2 μg/ml) and hexadeuterocaffeine (5.2 μg/ml), 1 ml of 0.5 *M* pH 10 carbonate buffer and 50 μl 0.1 *M* tetrabutylammonium ion solution (prepared by dissolving tetrabutylammonium hydrogen sulphate in molar amounts of sodium hydroxide solution and adding water to make up the volume). The mixture was shaken with 5 ml of dichloromethane containing 2.5% of ethyl iodide in a water bath at 50°C for 20 min. After centrifugation the organic phase was removed and evaporated (Büchler Vortex evaporator). The residue was dissolved in 20 μl acetone and 1—2 μl were then transferred to a solid sample syringe and injected into the chromatograph.

RESULTS AND DISCUSSION

Theophylline was extracted in an ionized form as an ion pair with tetrabutylammonium into the organic phase where it was ethylated. Since theophylline was consumed by ethylation in the organic phase the extraction was > 95%. Caffeine was extracted to the organic phase in a neutral form (ca. 95%).

Fig. 1 shows chromatograms of plasma samples obtained by monitoring the molecular ions obtained from ethylated theophylline and caffeine respectively.

The ethyl derivatives of 1,7-dimethylxanthine and 3,7-dimethylxanthine (theobromine) were not separated on the column used in the method

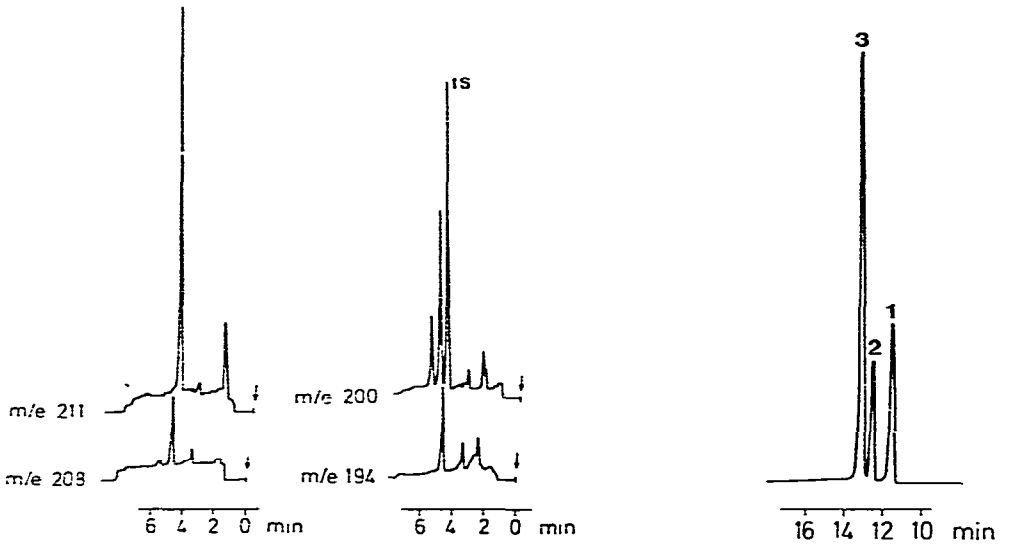

Fig. 1. Chromatograms from a plasma sample treated according to the method described. The sample contained 1.7 μg/ml of theophylline and 2 μg/ml of caffeine. An arrow indicates the injection. *m/z* 194, caffeine; *m/z* 200, hexadeuterocaffeine (internal standard, IS); *m/z* 208, ethyltheophylline and *m/z* 211, trideuteroethyltheophylline.

Fig. 2. Chromatogram of pentafluorobenzyl derivatives of dimethylxanthines on a 25-m OV-17 glass capillary column programmed from 190°C to 230°C at 10°/min. The molecular ions at *m/z* = 362 were monitored on the mass spectrometer. Peaks: 1 = theophylline; 2 = 1,7-dimethylxanthine and 3 = theobromine.

described. Since the molecular ions of these substances have the same *m/z* value as the ethyl derivative of theophylline, they will interfere with the determination of theophylline. This has no importance when determining theophylline and caffeine concentrations in premature infants because they have been reported to lack the ability to demethylate caffeine [11]. To accomplish the separation of these substances a more selective column has to be used. On a 25-m OV-17 capillary column it was possible to separate the ethyl derivative of theophylline from those of 1,7- and 3,7-dimethylxanthine, but the temperature had to be increased rather slowly.

Exchange of the alkylating agent ethyliodide for pentafluorobenzyl bromide in the described method will give the pentafluorobenzyl derivative of the dimethylxanthines. Fig. 2 shows the chromatogram obtained with this derivatization, making it possible to quantify caffeine and all three dimethylxanthines simultaneously.

The calibration graphs were constructed by analysing plasma samples spiked with theophylline (0—15 μg/ml) and caffeine (0—15 μg/ml). The peak height ratios of the ions *m/z* 208/211 and 194/200 were plotted against the concentration of theophylline and caffeine respectively.

The precision of the method was 3.9% for theophylline (0.6 μg/ml plasma, $n = 10$) and 3.2% for caffeine (0.6 μg/ml, $n = 10$). The detection limits were 20 and 40 ng/ml plasma for theophylline and caffeine, respectively. The higher detection limit for caffeine was due to the fact that it was eluted on the slope

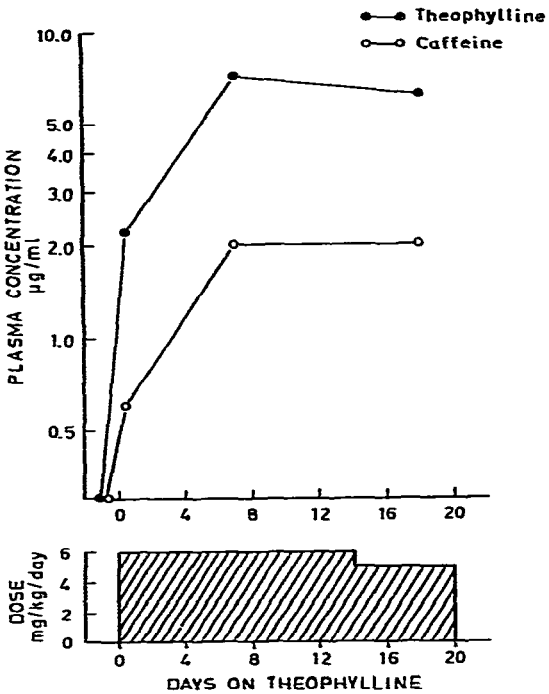

Fig. 3. Plasma concentrations of theophylline and caffeine in a premature newborn infant treated with oral theophylline for neonatal apnéa.

of a small background peak. By using a somewhat slower temperature programme the caffeine peak can be resolved and the detection limit is about the same as that for theophylline.

Fig. 3 shows the plasma concentrations of theophylline and caffeine in a premature newborn infant treated with oral theophylline. The plasma sample obtained before treatment did not contain detectable amounts of the substances. At steady state the caffeine plasma levels were approximately 1/3 of the theophylline levels, and this is in agreement with previous findings [12].

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