JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 413–416

Received June 1, 2000
Accepted October 30, 2000
ISSN 0022-3573

A rapid and sensitive method for the determination of the amount of theophylline in blood spots

D. G. Watson, E. J. Oliveira, A. C. Boyter and K. D. Dagg

Abstract

Monitoring of drugs (such as theophylline) with a narrow therapeutic window could be simplified if patients were able to submit blood spots for analysis. This could reduce clinic attendance for venous blood sampling and save staff time. A rapid sensitive method utilizing liquid chromatography-mass spectrometry has been developed to determine the amount of theophylline in blood spots. The lowest level of theophylline analysed in a blood spot was 15 ng extracted into 250 μL and this was still considerably above the limit of quantification (3 ng in 250 μL). The levels of theophylline in blood spots correlated well with theophylline levels in plasma samples obtained from the same patients. The assay might be of use in therapeutic drug monitoring of theophylline and blood spot sampling could be applied to other drugs where therapeutic monitoring is required.

Introduction

Theophylline, in common with all drugs with a narrow therapeutic window, requires frequent monitoring (Young & Koda-Kimble 1995). There would be distinct advantages in being able to conduct this type of monitoring without patients having to attend clinics for venous blood samples to be taken. The aim of this study was to develop a rapid and simple method, which would enable precise determination of theophylline in samples of blood spotted onto sampling cards. The method should require minimal sample preparation and short analysis times. There are numerous methods for the analysis of theophylline by HPLC. Typical limits of detection are approximately 0.5 μg (mL plasma)$^{-1}$ when UV monitoring is used (Schrieber-Deturmeny & Bruguerolle 1996; Kamberi et al 1999), however lower limits of detection have been reported (Radawan et al 1995). A highly sensitive method based on liquid chromatography-frit-fast atom bombardment mass spectrometry (LC-frit-FAB-MS) with a limit of detection of 5 ng mL^{-1} for theophylline in plasma has been reported but this required solid phase extraction before analysis (Hieda et al 1995). In this study we report a rapid liquid chromatography-mass spectrometry (LC-MS) method with minimal sample preparation for the determination of theophylline in blood spots and plasma.

Department of Pharmaceutical Sciences, SIBS, University of Strathclyde, Glasgow G4 0NR, UK

D. G. Watson, E. J. Oliveira, A. C. Boyter

Department of Respiratory Medicine, Glasgow Royal Infirmary, Glasgow G31 2ER

K. D. Dagg

Pharmacy Practice Unit, Greater Glasgow Health Board and Glasgow Royal Infirmary

A. C. Boyter

Correspondence: D. G. Watson, Department of Pharmaceutical Sciences, SIBS, University of Strathclyde, Glasgow G4 0NR, UK. E-mail: D. G. Watson@strath.ac.uk

Funding: We thank the Wellcome Trust for support.

Materials and Methods

Chemicals and reagents

Chemicals and reagents were obtained as follows: theophylline, dimethylformamide, chloromethylpivalate, anhydrous potassium carbonate and deuteromethyliodide (Sigma-Aldrich Chemical Co., Dorset, UK); methanol (BDH Chemical Co., Poole, Dorset, UK); sampling cards (Whatman, Maidstone, Kent, UK).

Preparation of 3-C^2H_3-theophylline

Deuterated theophylline was prepared by slight modification of a previous method (Regal et al 1998). Briefly, 1-methylxanthine (60 mg; 0.36 mmol) was dissolved in 10 mL dimethylformamide and 1.5 molar equivalent of dry K_2CO_3 was added to the stirring mixture. The flask was flushed with nitrogen and 1.1 molar equiv. chloromethylpivalate was added. After 9 h deuteromethylation was started by adding 1 molar equiv. CD_3I. After reaction, the solvent was evaporated and deprotection was carried out by adding 10 mL 2M NaOH. The NaOH was then neutralized with conc. HCl and ethanol (30 mL) was added to the sample, precipitating out NaCl which was filtered off. The supernatant was then evaporated to dryness and dissolved in 2 mL methanol and analysed by TLC, revealing a spot for theophylline and an additional close running spot of a similar size. Chloroform was then added to the methanol solution of the product and the reaction mixture was chromatographed on a silica gel column eluting stepwise with mixtures of methanol/chloroform (3% to 80% methanol). Separation was monitored by TLC of individual fractions (25 mL). Theophylline-containing fractions were combined and further purified by semi-preparative HPLC. Purification was carried out using a Gilson gradient preparative HPLC system. Separation was carried out using a Phenomenex Luna C_{18} column (10 μm, 250 × 10 mm). The mobile phase consisted of phosphoric acid 0.05% and MeOH. A gradient of 20% MeOH to 80% MeOH in 20 min was used at a flow rate of 5 mL min^{-1}. UV monitoring was carried out at 273 nm. The chromatogram contained two peaks of a similar size, the earlier running peak corresponding to 3-C^2H_3-theophylline. Fractions from HPLC were combined and the solvent removed in a rotary evaporator. The overall yield was 23.7%.

Standard solutions

Standard solutions of theophylline (0.562 mg mL^{-1}) and 3-C^2H_3-theophylline (0.509 mg mL^{-1}) were prepared in methanol and diluted as required.

Clinical samples

Patients admitted to the Respiratory ward at Glasgow Royal Infirmary with acute exacerbation of underlying airways disease, who were receiving regular oral theophylline (6.5 mg kg^{-1}) (range 3.2–13.4 mg kg^{-1}), were deemed eligible for the study. Twenty-one patients (10 male) median age 66 years (range 40–76) who had been receiving oral theophylline for at least 48 h as an in-patient were recruited into the study. A 10-mL sample of venous blood was obtained. The venous blood was centrifuged at 1000 *g* for 15 min and the plasma was removed and stored at –20°C until analysis (within six months of sampling). A capillary blood sample was obtained at the same time using a single stab from a Unilet lancet and blood was spotted onto a prepared Guthrie card and stored with the plasma sample until analysis. All patients gave written informed consent and the study had the approval of the Glasgow Royal Infirmary University NHS Trust Ethics Committee.

LC-MS analysis

Two circles (approximately 10 μL blood) were punched from each blood spot and were extracted with 50 μL methanol containing 5 ng μL^{-1} deuterated theophylline and 200 μL water. The samples were shaken for a few seconds and then allowed to settle, and approximately 100-μL extract was transferred to an autosampler vial. Analysis was carried out using an Automass LC/GC-MS system (Thermoquest Ltd). HPLC was carried out using a TSP4000 HPLC system fitted with a Luna ODS2 column (75 × 4.6 mm with 3 μm particle size); a 20-μL sample was injected. The mobile phase consisted of methanol/water (20:80) at a flow rate of 0.5 mL min^{-1}. The mass spectrometer was operated in the negative ion electrospray mode with a cone voltage of −26 V, the source temperature was 500°C. Selected ion monitoring was carried out for the M-1 ions at m/z 179 and 182 for theophylline and 3-C^2H_3-theophylline, respectively.

Samples of plasma (100 μL) were diluted with 1000 μL methanol containing 2 $\mu g\ \mu L^{-1}$ 3-C^2H_3-theophylline. The samples were centrifuged to remove the precipitated protein and 200 μL of the supernatant were diluted to 0.4 mL with water. The samples were then analysed as described for the blood spot samples.

Calibration was carried out using standard solutions containing 1000 ng mL^{-1} 3-C^2H_3-theophylline and theophylline concentrations between 0 and 1000 ng mL^{-1}.

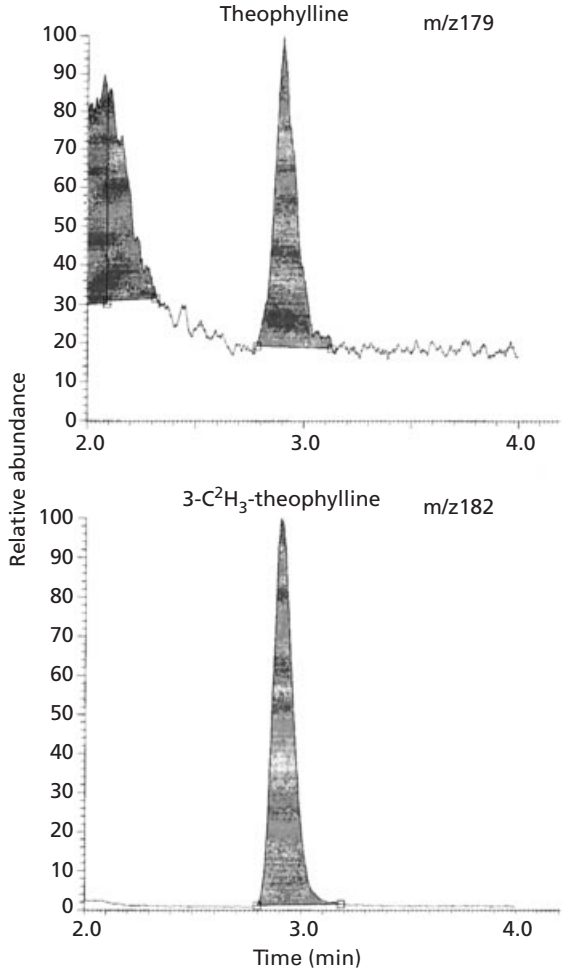

Figure 1 Selected ion trace of theophylline (15.3 ng) extracted from a blood spot in comparison with 3-C^2H_3-theophylline (250 ng).

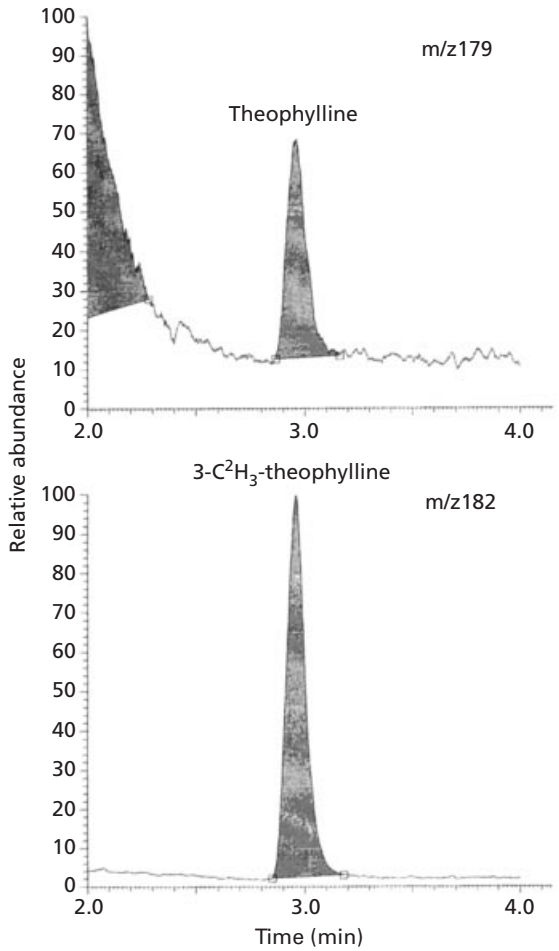

Figure 2 Selected ion trace of theophylline (1882 ng mL^{-1}) in plasma (100 μL plasma diluted × 20) in comparison with 3-C^2H_3-theophylline (1000 ng mL^{-1}).

Results

The theophylline and 3-C^2H_3-theophylline gave negative ion ESI spectra in which most of the ion current was carried by the M-1 ion at m/z 179 and 182, respectively. The mass spectrometer was set to carry out selected ion monitoring for these ions. Based on the ratio of these two ions, calibration was linear ($R^2 = 0.998$) over the range 0–1000 ng mL^{-1} theophylline. Duplicate analysis of twenty-one blood spots and corresponding plasma samples was carried out. The levels of theophylline in the blood spots ranged from 15.1 to 171.6 ng per circle punched from the spot. Figure 1 shows the chromatogram from a blood spot containing 15.3 ng theophylline extracted into 250 μL methanol/water; the peak for theophylline was still well within the limit of quantification of the method (3 ng/250 μL). Repeat analysis of five circles punched from one patient card on a single gave a level of 95.8 ng/blood spot with a reasonable precision of $\pm$13.2%. The plasma samples ranged in concentration from 1882 to 20 035 ng mL^{-1}. Figure 2 shows the selected ion trace for a sample containing 1882 ng mL^{-1} theophylline prepared from 100 μL plasma (diluted overall × 20). Repeat analysis of five 100-μL plasma samples from one sample of patient plasma gave 10 594 ng mL^{-1} with a precision of $\pm$8.2%.

Figure 3 shows a plot of the plasma levels for theophylline from 21 patients correlated with the amounts of theophylline found in the corresponding blood spots. The correlation between the paired measurements was good ($r^2 = 0.932$).

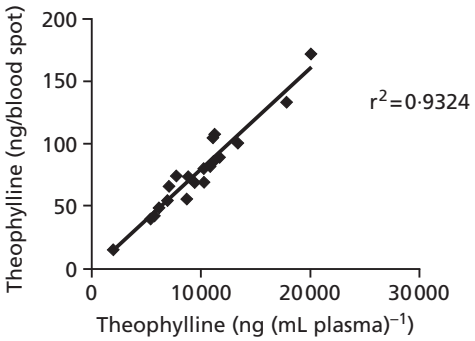

Figure 3 Correlation between theophylline levels in blood spots and the corresponding levels in samples of plasma.

Discussion

The method we have developed is one of the most sensitive reported for the determination of theophylline. The lack of interference from constituents in the sample matrix meant that analysis times could be kept to 4 min per run. The precision for duplicates was not always good and this can be explained by the blood not always saturating the card. This might be remedied by punching smaller circles more towards the centre of the spot or by better sampling by the clinician; the precision with which the card is punched is a major source of variation. Improved correlation between plasma and blood spot levels might be achieved by improved sampling. In preliminary work we developed a method based on GC-MS analysis in the negative ion chemical ionization mode following derivatization of theophylline with pentafluorobenzyl bromide. Although the method was extremely sensitive it had lower precision than the LC-MS method and was much less convenient to use. Electrospray is less frequently used in the negative ion mode, although for electron capturing compounds it can produce very selective detection. In view of the lack of interference from matrix constituents in the current method, analysis times could probably be reduced still further. The use of blood spots for therapeutic drug monitoring has excellent potential and could be applicable to a range of drugs where, for reasons of toxicity and compliance, monitoring is desirable.

References

Hieda, Y., Kashimura, S., Hara, K., Kageura, M. (1995) A highly sensitive and rapid determination of theophylline, theobromine and caffeine in human plasma and urine using gradient capillary high-performance liquid chromatography-frit-fast atom bombardment mass spectrometry. *J. Chromatogr. Biomed. Appl.* **667**: 241–246

Kamberi, M., Hajime, N., Kamberi, P., Uemura, N., Nakamura, K., Nakano, S. (1999) Simultaneous determination of grepafloxacin, ciprofloxacin and theophylline in human plasma and urine by HPLC. *Ther. Drug Monit.* **21**: 335–340

Radawan, M. A. (1995) HPLC assay of theophylline and zidovudine in rat serum. *J. Liquid Chromatogr.* **18**: 3301–3309

Regal, K. A., Howald, W. N., Peter, R. M., Gartner, C. A., Kunze, K. L., Nelson, S. D. (1998) Subnanomolar quantification of caffeine's in vitro metabolites by stable isotope dilution gas chromatography-mass spectrometry. *J. Chromatogr. B* **708**: 75–85

Schrieber-Deturmeny, E., Bruguerolle, B. (1996) Simultaneous high performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma. *J. Chromatogr. Biomed. Apps.* **667**: 305–312

Young, L. L., Koda-Kimble, M. A. (1995) *Applied Therapeutics – The Clinical Use of Drugs*. 6th edn, Applied Therapeutics, Inc., Vancouver