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Short Communication

Determination of bamifylline hydrochloride impurities in bulk material and pharmaceutical forms using liquid chromatography with ultraviolet detection*

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Introduction

Bamifylline hydrochloride (**I**), a xanthine derivative with bronchodilatator properties, is used in the treatment of asthma and reversible airway obstruction. The physico-chemical and pharmacokinetic properties of bamifylline differ from those of theophylline [1].

Some HPLC methods for the assay of **I** and its metabolites in biological fluids have been described [2–4].

The aim of this study was to develop a liquid chromatographic assay for the determination of bamifylline hydrochloride impurities, 8-benzyltheophylline (**II**) and 7-chloroethyl-8-benzyltheophylline (**III**), in bulk material and pharmaceutical forms. Chemical structures are shown in Fig. 1.

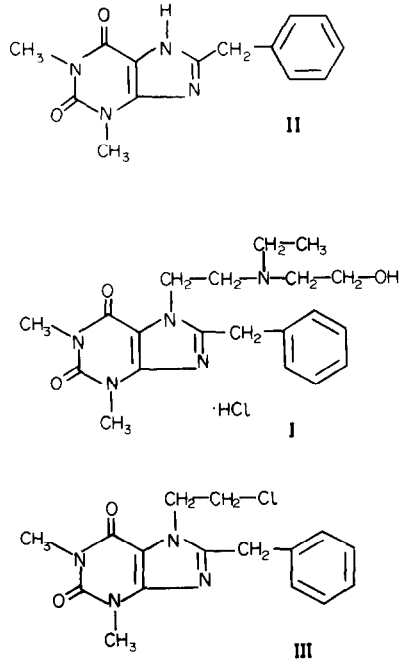

Figure 1
Chemical structures of **I**, **II** and **III**.

Experimental

Chemicals and reagents

Methanol (HPLC-grade) and potassium dihydrogenphosphate were purchased from Farmitalia Carlo Erba (Milan, Italy). Tetrahydrofuran (HPLC-grade) was supplied by Merck (Darmstadt, FRG). Water used in this assay was distilled, treated by a Milli-Q water-purification system (Millipore, Bedford, MA, USA) and subsequently filtered through a 0.22 μm filter. Bamifylline hydrochloride, or 7-[2-(ethyl-2-hydroxyethyl)amino]-3,7-dihydro-1,3-dimethyl-8-(phenylmethyl)-1H-purine-2,6-

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dione hydrochloride, **II** and **III** were a gift from the Department of Internal Medicine of this University.

Apparatus

The Waters liquid chromatographic system comprised a Model 590 pump and a Lambda Max Model LC-481 variable spectrophotometer detector (Waters Assoc., Milford, MA, USA) connected to a Model CC-12 computing integrator (Perkin–Elmer Corp., Norwalk, CT, USA). The injector was a Model 7125 Rheodyne (Rheodyne Inc., Cotati, CA, USA) equipped with a 20 μl sample loop.

Chromatographic conditions

The analysis was performed using a 250 × 4.6 mm i.d. column packed with 5 μm Erbasil ODS (Farmitalia Carlo Erba, Milan, Italy) connected to a disposable 20 × 4.6 mm i.d. Pelliguard pre-column (40 μm) (Supelco, Bellefonte, CA, USA). The mobile phase, methanol–tetrahydrofuran–potassium dihydrogenphosphate (pH 7.5; 0.01 M)–HPLC-grade water (60:4:40, v/v/v), was prepared daily and delivered at a flow-rate of 1.0 ml min^{-1}. The phosphate buffer was filtered through a 0.45 μm HA filter; the methanol and tetrahydrofuran were filtered through a 0.5 μm FA filter (Millipore, Bedford, MA, USA). The mixture was degassed before use for 10 min in an ultrasonic bath. The eluate was monitored at 278 nm. The total time for the chromatographic run was 15 min and the chromatographic system was maintained at room temperature (20 ± 2°C).

Calibration curves

Standard solutions were methanolic solutions of **I** (600 μg ml^{-1}) containing **II** and **III** in concentrations of 10–100 ng ml^{-1}. 20 μl aliquots were used for the analysis; the results are expressed as the mean of five determinations for each sample. Seven concentration values were subjected to regression analysis. The corresponding equations were: for **II**, $y = 3.36 \times 10^4x - 8.99 \times 10^2$ ($r = 0.9999$); for **III**, $y = 1.5 \times 10^4x + 2.35 \times 10^2$ ($r = 0.9996$), where y = peak area and x = concentration of **II** or **III** in μg ml^{-1}.

Analysis of bulk material

The powdered bulk material was dissolved in methanol to obtain a solution containing 600 μg ml^{-1} of bamifylline hydrochloride. After filtration, the solution was analysed by HPLC.

Analysis of pharmaceutical formulations

Tablets. Five tablets were crushed and combined. An amount of material equivalent to about 100 mg of bamifylline hydrochloride was accurately weighed and transferred to a 100 ml calibrated flask; 5 ml of methanol was added and the mixture was sonicated for 5 min and then diluted to 100 ml with methanol. The solution was filtered, diluted with methanol to obtain a concentration of **I** equivalent to 600 μg ml^{-1} and analysed by HPLC.

Suppositories. Five suppositories were crushed and combined. An amount of material equivalent to about 100 mg of **I** was accurately weighed into a screw-capped tube, 10 ml of methanol was added and the mixture was shaken vigorously for 15 min. The methanolic solution was removed, filtered, diluted to obtain a solution containing 600 μg ml^{-1} of **I**

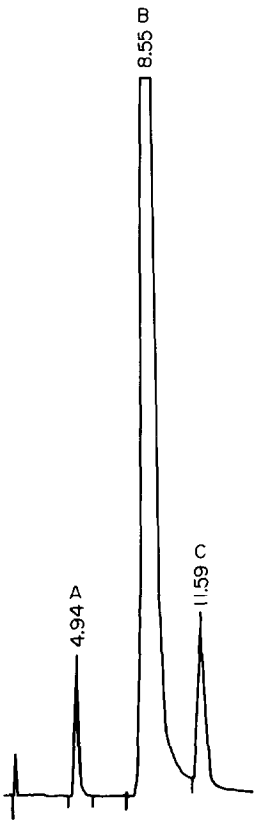

Figure 2
Liquid chromatogram of a methanolic solution of **I** (B) (600 μg ml^{-1}), **II** (A) (20 ng ml^{-1}) and **III** (C) (30 ng ml^{-1}). Vertical axis: UV detector response (278 nm); horizontal axis: retention times (min) for **II**, **I** and **III** were 4.9, 8.5 and 11.6, respectively. Injection volume 20 μl.

and analysed as previously described for the tablets.

Results and Discussion

Figure 2 illustrates a typical chromatogram of a solution containing **I**, **II** and **III**. No components were observed near the retention time corresponding to **I**, **II** or **III**. The minimum concentration of impurities detectable by the described procedure was 10 ng ml^{-1}. The RSD of the results was approximately 2% in the concentration range examined for **II** and **III**.

Table 1 shows the results obtained in the analysis of commercial bulk material and pharmaceutical formulations. The procedure described for the determination of impurities in bamifylline hydrochloride is very simple and rapid and provides accurate and precise results.

Table 1
Results obtained in the analysis of impurities of **I**

	II Found (ppm)*	**III** Found (ppm)*
Bulk material 1	10	—
Bulk material 2	28	15
Bulk material 3	12	10
Tablets	14	10
Suppositories	9	—

*With reference to the bamifylline hydrochloride content.

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