Journal of Chromatography, 182 (1980) 445—447
Biomedical Applications

CHROMBIO. 558

Note

Gas—liquid chromatographic determination of acephylline in urine

J. ZUIDEMA* and H. HILBERS

Department of Biopharmaceutics, University of Amsterdam, Plantage Muidergracht 14, Amsterdam (The Netherlands)

(First received November 29th, 1979; revised manuscript received January 22nd, 1980)

Acephylline piperazine (Acepiphylline, Etaphylline^{R}) has been used for the treatment of asthma for over more than 30 years in many countries. Acephylline is N-7-theophylline acetic acid. Recently the pharmacokinetics, after oral and intravenous (i.v.) administration, have been described [1,2]. A rapid high-performance liquid chromatographic method for the determination of acephylline in human serum has been reported [3]. This method cannot be used for the determination of acephylline in urine since interfering peaks occur. A gas chromatographic method has been developed, which makes use of the OV-17 column. The internal standard used is N-7-theophylline propionic acid which is a structural analogue.

MATERIALS AND METHODS

Synthesis of N-7-theophylline acetic acid (acephylline)

Acephylline was synthesized by reacting sodium chloroacetate and sodium theophyllinate in water, as described by Ride et al. [4]. Identity and purity were confirmed by IR, NMR, HPLC thin-layer (TLC) and gas—liquid chromatography.

Synthesis of N-7-theophylline propionic acid

The internal standard (i.s.) was synthesized in a two-step synthesis. N-7-Theophylline-3-propionitrile was synthesized by reacting theophylline and acrylonitrile in the presence of Triton B. The nitrile is hydrolyzed in concentrated hydrochloric acid, resulting in N-7-theophylline propionic acid. The synthesis is described by Zelnik and Pesson [5]. Identity and purity were confirmed by IR, NMR, TLC, GLC and HPLC.

*To whom correspondence should be addressed.

Procedure

A 1.0-ml sample of urine was pipetted into a tube, containing 60 μl of 8 *M* hydrochloric acid (the pH has to be about 2) and 0.5 ml of the i.s. solution (150 μg/ml). The sample was extracted twice with 5 ml chloroform—isopropanol (20:1). The tubes were centrifuged for 10 min. The organic layers were separated using a glass syringe with a blunt needle, collected and mixed well.

The chloroform—isopropanol mixture was evaporated off at 70°C under nitrogen until a volume of 500 μl was reached. This was transported in a small glass ampoule. The chloroform—isopropanol mixture was evaporated to dryness. Then 100 μl of a 10% ethyl iodide solution in acetonitrile and 5—10 mg potassium carbonate were added. The ampoule was fused after replacement of air by nitrogen and heated for 1 h at 80°C [6]. From this mixture 1—2 μg was injected into the chromatograph (Perkin-Elmer 3920).

GLC conditions: injection temperature 300°C, column temperature 255°C, detection temperature 300°C; column (2 m × 3 mm I.D.), 3% OV-17 on Gas-Chrom Q (100—120 mesh) (Chrompack, Middelburg, The Netherlands); nitrogen flow-rate 40 ml/min; flame ionization detection.

The results were calculated with a Hewlett-Packard integrator, type 3380A.

RESULTS AND DISCUSSION

Chromatograms of urine samples demonstrate that no contamination peaks occur (Figs. 1 and 2). The retention time of acephylline is about 6 min. Dietary xanthines, caffeine, theophylline and theobromine do not interfere with the assay. The standard curve of acephylline added to urine was linear over the ranges 0—5, 0—10, 0—100 and 0—1000 μg/ml and passed through the origin. The correlation coefficients were $r = 0.968$ ($n = 5$, $P < 0.01$); $r = 0.975$ ($n = 12$, $P < 0.01$); $r = 0.991$ ($n = 15$, $P < 0.01$) and $r = 0.986$ ($n = 15$, $P < 0.01$) respectively.

Normal urine concentrations in patients and volunteers vary from 0 to 100 μg/ml after oral administration and to a maximum variation of 1000 μg/ml after i.v. administration. The within-run variation was determined by the repetitive injection of a sample derived from spiked urine with a concentration of 50 μg/ml. The recovery was 100.5% as could be calculated from the mean. The within-run variations were 5.0, 7.4 and 9.9%, expressed as the variation coefficient at 5, 10 and 100 μg/ml respectively, ($n = 10$, $n = 13$ and $n = 11$). The between-run variation was determined by a daily extraction of the 10 μg/ml urine samples and injection onto the gas chromatograph over two weeks. The between-run variation was 6.8%, expressed as the variation coefficient ($n = 10$). This shows that the between-run-variation is not different from the within-run-variation.

We compared a standard curve (0—100 μg/ml), using N-7-theophylline propionic acid as external standard, carrying out only the chromatographic procedure (correlation coefficient $r = 0.599$) with a standard curve, using this substance as internal standard, carrying out the extraction procedure as well (correlation coefficient $r = 0.999$). The results stipulate the important role of the internal standard and the large variation in the extraction efficiency of both acephylline and the internal standard.

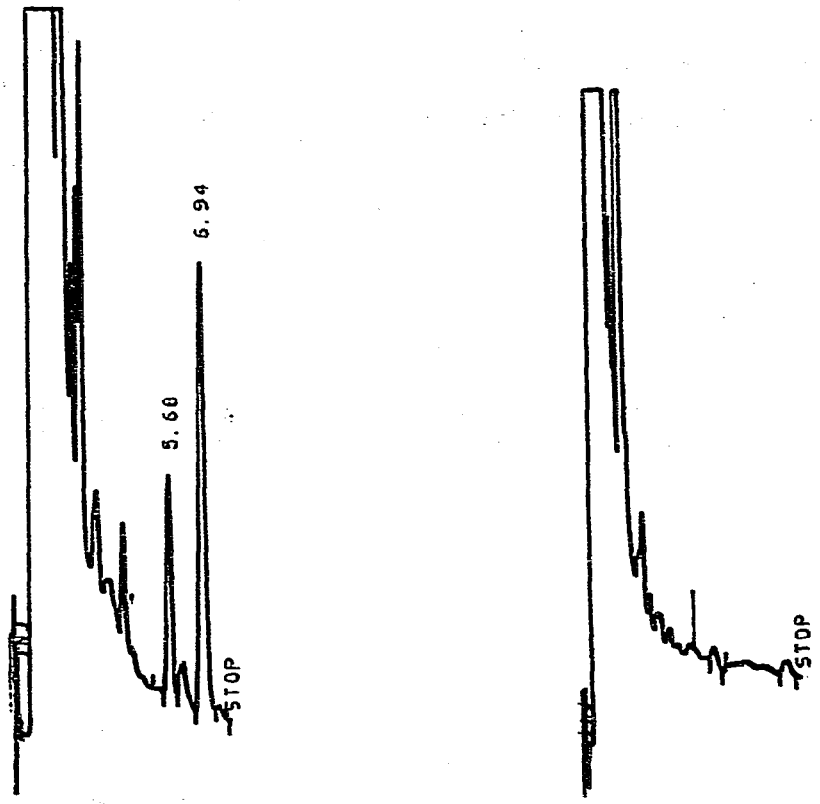

Fig. 1. Chromatogram of a urine sample containing acephylline, 25 μg/ml, retention time = 5.68 and internal standard, 50 μg/ml, retention time = 6.94.

Fig. 2. Chromatogram of a blank urine sample.

We determined urine samples obtained from volunteers and patients with this GLC method. We discovered the low absorption efficiency of acephylline and the rapid complete renal elimination [1,2]. Acephylline appears to be about 50% bound in the urine. These investigations will be continued, focussing on the absorption and elimination mechanisms.

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