Journal of Pharmaceutical & Biomedical Analysis
Vol. 4, No. 4, pp. 541–543, 1986
Printed in Great Britain

0731–7085/86 $3.00 + 0.00
Pergamon Journals Ltd.

Short Communication

Liquid-chromatographic analysis of a novel antitussive agent, 2′,4′-dimethyl-6′-methoxy-3-(2-methyl-piperidyl)propionanilide in human urine

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Keywords: *Liquid chromatography; antitussive agents.*

Introduction

The antitussive action of a novel cough remedy 2′,4′-dimethyl-6′-methoxy-3-(2-methyl-piperidyl)-propionanilide (OR K-242) has been previously studied using different animal species [1]. The chemical structure of OR K-242 is shown in Fig. 1. It has been shown in several animal experiments that this anilide derivative has a positive influence on cough symptoms, very much like the opium alkaloid codeine phosphate, which is one of the most effective antitussive agents today. In addition, OR K-242 is a local anaesthetic. The detection and quantification of OR K-242 in plasma and in urine are of relevance in the study of pharmacokinetics of this new drug. Recently OR K-242 has been determined in human plasma [2] using liquid chromatography (LC). At that time this drug could not be resolved from interfering peaks in urine samples. In this paper a simple liquid chromatographic (LC) method for the determination of OR K-242 in human urine is presented.

Experimental

Reagents

OR K-242 hydrochloride was obtained from Orion Pharmaceutical Research Center (Espoo, Finland). Acetonitrile, HPLC grade S, was from Rathburn Chemicals Ltd

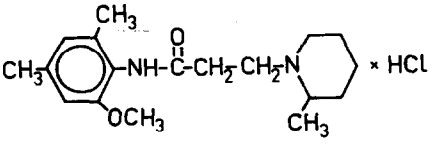

Figure 1
The chemical structure of 2′,4′-dimethyl-6′-methoxy-3-(2-methyl-piperidyl)-propionanilide (OR K-242).

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(Walkerburn, Scotland) and dichloromethane, Uvasol grade, from E. Merck (Darmstadt, FRG). All other chemicals were of analytical reagent quality.

Apparatus

The LC analyses were carried out using a combination of a Waters Model 6000A pump, and a Waters Intelligent Sample Processor (WISP) Model 710B (Waters Associates, Milford, MA, USA) equipped with a Beckman 150 × 4.6 mm 5 μm Ultrasphere-Cyano column (Beckman Instruments, Fullerton, CA, USA). A Kratos Model 773 variable wavelength detector at 214 nm (Kratos Analytical Instruments, Ramsey, NJ, USA) was used for the detection of the components.

Specimen material

The urine samples were collected at 2-h intervals during 24 h from healthy adults who had obtained oral doses (5–15 mg) of the antitussive OR K-242. The urine collections were stored at −20°C until analysed.

Preparation of urine samples

Urine samples (0.5 ml) were diluted with 0.9% w/v NaCl (0.5 ml) and made alkaline by an addition of 1 N NaOH (0.1 ml). The antitussive agent was extracted from the samples with dichloromethane (6 ml) by shaking in stoppered tubes for 10 min at room temperature. After centrifugation (5 min at 1100 ***g***) 4 ml of the organic layer was evaporated to dryness in nitrogen atmosphere at 37°C. The residue was dissolved in 0.5 ml of the mobile phase. Fifty microlitres was injected into the liquid chromatograph.

Six dilutions in duplicate of an OR K-242 standard stock solution were prepared for a standard curve ranging from 31 to 1000 ng ml^{-1}. The standard solutions (0.1 ml) were added to blank urine (0.5 ml) with no detectable interfering compounds followed by an additional dilution with 0.9% NaCl (0.5 ml). The extraction was carried out as described for the urine samples.

Chromatographic procedure

Analytical separations were performed under the following conditions: sample volume 50 μl; column temperature, ambient; mobile phase, acetonitrile–15 mM NaH_2PO_4 (pH 3.2) (30:70% v/v); elution mode, isocratic; flow rate, 1.5 ml min^{-1}.

Results and Discussion

A typical chromatogram of a blank urine is shown in Fig. 2 A. The low background signal was achieved by using small sample volumes of urine in the sample preparation procedure. Figure 2 B represents a chromatogram of a urine sample from a 2–4 h collection after an oral dose of 10 mg of OR K-242. The concentration of OR K-242 in the sample was 1180 ng ml^{-1}. The compound eluting just before OR K-242 was proposed to be a metabolite of OR K-242, hitherto unidentified. The results were calculated in concentration units (ng OR K-242 excreted per millilitre urine) from a linear standard curve with a regression equation: $y = 0.585\,x - 7.831$; $r = 0.9998$ (y = peak height, x = concentration) over the range from 31 to 1000 ng ml^{-1} ($n = 6$).

The detection limit of OR K-242, defined as 2.5 times the background signal, was found to be as low as 5 ng ml^{-1} in urine, regardless of the low UV detection wavelength at 214 nm. The mean recovery of added OR K-242 from human urine was about 90% in

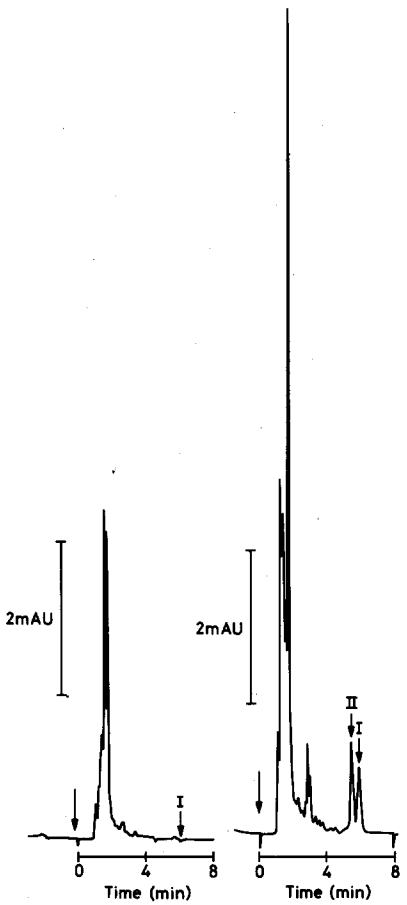

Figure 2
Representative chromatograms of OR K-242 in human urine. (A) Blank urine, (B) Urine containing 1180 ng ml^{-1} OR K-242 determined from a 2–4 h urine collection after an oral dose of 10 mg OR K-242. (I) OR K-242; (II) unidentified metabolite of OR K-242.

the range of 50–1000 ng ml^{-1}. The within-assay and between-assay precisions were determined by measuring a control sample containing 1000 ng ml^{-1} OR K-242 ten times during ten days. The within-assay relative standard deviation (R.S.D.) was 4.4% (n = 10) and the between-assay R.S.D. was 6.8% (n = 10).

In an earlier communication from the present authors' laboratory determination of OR K-242 in human plasma by LC using Spherisorb-nitrile column was reported [2]. However, the parent drug could not be separated from the unidentified OR K-242 metabolite in urine by changing elution conditions. Several other reversed-phase column types including octyl, phenyl and amino bonded were tested without success. The 5 μm particle size Ultrasphere-cyano column finally produced the required separation.

References

[1] P. Männistö, P. Karttunen, S. Lahovaara, E. Nissinen, J. E. Davies, D. R. Algate and M. W. Baines, *Arzneim.-Forsch.*, in press.
[2] E. Nissinen and P. Männistö, *J. Chromatogr.* **278**, 225–227 (1983).

[*Received for review 9 April 1984; revised manuscript received 9 July 1984*]