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Note

Determination of guaiphenesin and its metabolite, β-(2-methoxyphenoxy)-lactic acid, in plasma by high-performance liquid chromatography

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Guaiphenesin [3-(2-methoxyphenoxy)propane-1,2-diol; I (Fig. 1)] is a widely used expectorant known to be a centrally acting muscle relaxant in high doses. Especially in equine anaesthesiology, guaiphenesin is accepted as a casting agent because of its negligible effect on respiratory function, cardiac action and haematological parameters [1]. In order to study the fate of guaiphenesin in the horse we needed a method for the quantitative determination of this drug in plasma. Because it is known from studies in man [2] that β-(2-methoxyphenoxy)lactic acid (II) is an important metabolite of guaiphenesin, the method should be capable of differentiating between these two compounds. Several methods have been described in the literature

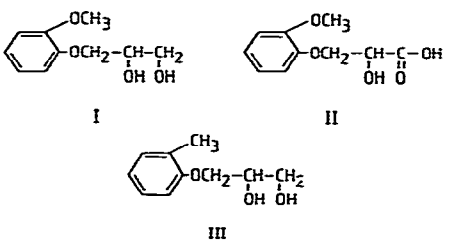

Fig. 1. Structural formulae of guaiphenesin (I), its metabolite β-(2-methoxyphenoxy)lactic acid (II), and the internal standard mephenesin (III).

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for the determination of guaiphenesin in biological fluids. Most of them [3, 4] are based on periodate oxidation of the number one atom of the propyl chain to formaldehyde and colorimetric determination with chromotropic acid. However, these methods are not specific. Gas—liquid chromatography has been used but derivatization is required [5].

This paper describes a high-performance liquid chromatographic (HPLC) method with a reversed-phase column (RP-8) for the separation and quantification of both guaiphenesin and β-(2-methoxyphenoxy)lactic acid using mephenesin (III) as internal standard.

MATERIALS AND METHODS

Materials

Guaiphenesin and mephenesin were commercially obtained from OPG (Utrecht, The Netherlands). β-(2-Methoxyphenoxy)lactic acid was isolated by ether extraction from acidified horse urine (pH 2—3) after intravenous injection of 100 mg/kg guaiphenesin. Its identity was verified by mass spectrometry, nuclear magnetic resonance and gas chromatography—mass spectrometry after derivatization with bis-(trimethylsilyl)-trifluoroacetamide (Pierce, Rockford, IL, U.S.A.).

LiChrosorb RP-8 (5 μm) was obtained from E. Merck (Darmstadt, G.F.R.). All other chemicals were of analytical reagent grade.

High-performance liquid chromatography

A Hewlett-Packard 1084B high-pressure liquid chromatograph equipped with a microprocessor and an automatic sampling system was used. The variable-wavelength UV detector was used and operated at 275 nm. The column was a 15 cm × 4.6 mm I.D. stainless-steel tube packed by the slurry technique with LiChrosorb RP-8 (5 μm) and operated at 30°C. The elution solvent consisted of a mixture (40:60) of methanol and citrate buffer (pH 6.5) (ultimate concentration 0.01 *M*). Elution was performed at a flow-rate of 1.0 ml/min. Under these conditions the retention times of I, II and III are about 2.8, 4.7 and 8.8 min, respectively.

EXPERIMENTAL

Sample preparation

Into a centrifuge tube with Teflon-lined screw cap are pipetted 0.5 ml of the plasma sample, 50 μl of the internal standard solution (1 mg/ml in water) and 60 μl of 1 *N* HCl. After homogenization the mixture is extracted once with 5 ml of freshly distilled diethyl ether by shaking for 30 min in an automatic shaker, and centrifuged for 15 min at 1000 *g*. The diethyl ether layer is transferred to another tube and evaporated to dryness under a gentle stream of dry air at 30°C. The residue is taken up in 0.5 ml of the elution solvent and transferred to a sample vial of the automatic sampling system of the HPLC system.

Calibration and recovery

Known quantities of I and II were added to blank plasma. The samples were then treated according to the sample preparation procedure described above. UV detection was carried out at a wavelength of 275 nm. Linear calibration curves could be constructed by plotting the absorbance of I or II in the samples as related to the absorbance of the internal standard (III) against the amount of I or II added to the plasma. In practice, the variable amounts of I and/or II added were calculated by the microprocessor according to Lambert—Beer's law. By plotting these calculated amounts against the added amounts linearity was confirmed unambiguously.

Recoveries of I and II were determined by adding known amounts to blank plasma. After extraction mephenesin was added and the relative peak area ratio was calculated. This value was compared with the ratio obtained by direct analysis of the same amounts of I, II and III in standard solutions.

RESULTS AND DISCUSSION

Typical chromatograms of a reference sample, a blank plasma sample and a spiked plasma sample are shown in Fig. 2. Guaiphenesin is well separated from its metabolite. The behaviour of guaiphenesin in our chromatographic system can be fully characterized by the following parameters: number of theoretical plates, 25,000—30,000/m; the capacity factor, 2.77; and the factor of symmetry (the width of the right half divided by that of the left half of the peak, as measured at 10% of peak height) is 1.5—1.8.

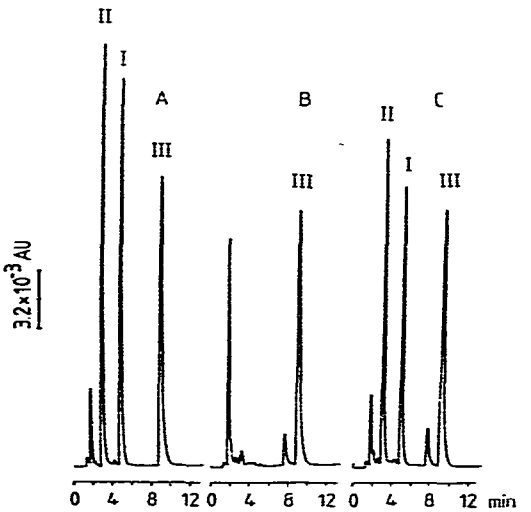

Fig. 2. High-performance liquid chromatograms obtained by the procedure described in the text for (A) a reference sample of standard solutions, (B) blank plasma spiked with internal standard (III) only, and (C) blank plasma spiked with guaiphenesin (I), its metabolite (II) and the internal standard (III). The plasma samples show an extra peak (retention time 7.4 min), the identity of which is unknown.

The plasma extract is clean except for a peak with a retention time of about 7.4 min, the identity of which is as yet unknown, but which is present in all blank samples. In the concentration range studied (5—200 μg/ml) there was no serious interference by endogenous compounds and peak areas were linear with concentration (correlation coefficient 0.997). The recovery was 91 ± 5% (S.D.) for I and 74 ± 2% (S.D.) for II.

The method described was used to study the pharmacokinetics of guaiphenesin in the horse. A typical plasma profile of a horse that received 100 mg/kg guaiphenesin intravenously is shown in Fig. 3. It appears that the decay of guaiphenesin can be adequately described by a two-compartment system [6]. In Fig. 3 also the plasma concentration of the acid metabolite II is plotted as a function of time after administration. As might be expected this concentration rises from zero to a maximum relatively shortly after administration.

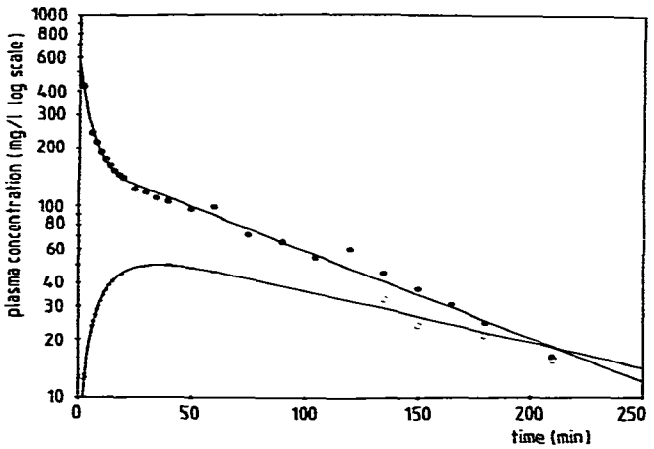

Fig. 3. Typical profile of a plasma curve of guaiphenesin and its lactic acid metabolite after intravenous administration of guaiphenesin to a horse. The closed circles (•) represent guaiphenesin, the open circles (○) the metabolite. See text for further explanation.

Thereafter its disappearance proceeds more slowly than that of the parent compound I. The total elimination of guaiphenesin and its metabolites from the body of the horse occurs largely via renal pathways. Substance I, however, hardly appears in urine as the free form, but almost wholly in the conjugated form, mainly as glucuronides. The analysis of urine samples therefore offers special problems, which will be described and discussed in a forthcoming paper.

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