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Short Communication

High-performance liquid chromatography of ergometrine and preliminary pharmacokinetics in plasma of men

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ABSTRACT

An isocratic high-performance liquid chromatographic (HPLC) method with fluorescence detection has been developed for the measurement of ergometrine in human plasma. The quantitation limit in plasma was 75 pg/ml. An example of the plasma concentration–time curves obtained after both oral and intravenous administration of ergometrine in one volunteer is shown. This HPLC method makes it possible to describe the pharmacokinetic parameters of oral ergometrine.

INTRODUCTION

Post-partum haemorrhage (PPH) is one of the most common causes of maternal death [1]. In such cases death invariably occurs within a few hours after birth. Management and prevention of this condition should take place at all levels of obstetric care as emergency referral is often difficult to arrange, especially in the circumstances prevailing in many Third-World countries.

The prophylactic use of oxytocic drugs such as ergometrine, methylergometrine and oxytocin reduces the risk of PPH plus the need for further oxytocic therapy in the puerperium [2]. The wide use of injectable ergometrine has become a point of discussion as the ampoule has proved to be unstable under tropical conditions [3–5]. Therefore, supported by the World Health Organization (WHO), a project has been started to assess the place of *oral* ergometrine in the prevention of PPH in Third-World countries.

Pharmacokinetic data regarding pharmacologically similar ergot alkaloids, ergometrine and methylergometrine are scarce [6,7] and refer to

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publications in the late 1970s [8,9]. In these studies measurement of the compounds took place using a radioimmunoassay method. The few studies dealing with the determination of ergot alkaloids in plasma by HPLC methods do not include ergometrine [10–12], or were developed for the analysis of pharmaceutical formulations [13,14].

The aim of this investigation was to develop a sensitive and selective HPLC analysis that would enable a pharmacokinetic analysis of ergometrine.

EXPERIMENTAL

Drugs

Pure ergometrine (reference substance 086) was obtained from Sandoz (Basle, Switzerland). Ergometrine maleate (0.2-mg tablet, equivalent to 0.147 mg free base) was obtained from Wolfs (Antwerpen, Belgium; batch No. 91102). Ergometrine maleate 0.15 mg/ml FNA (injectable solution) was obtained from Medisch Spectrum Twente (Enschede, Netherlands; batch No. 93125020). The ergometrine batches fulfilled the requirements of a content uniformity test according to standard quality control criteria.

Analytical-grade potassium dihydrogenphosphate and diethylamine were obtained from Merck (Darmstadt, Germany). Acetonitrile was obtained from FSA Laboratory Supplies (Loughborough, UK).

HPLC analysis

The HPLC system consisted of a Spectra Physics SP 8775 autosampler (Spectra Physics, Eindhoven, Netherlands), an SP 8800 ternary HPLC pump (Spectra Physics), a Hitachi F-150 fluorescence spectrophotometer (Merck, Amsterdam, Netherlands) and an SP 4290 integrator (Spectra Physics). The column (25 cm × 4.6 mm I.D.) was packed with Spherisorb 5-ODS (particle size 5 μm) (Chrompack, Middelburg, Netherlands) with a guard column (75 mm × 2.1 mm I.D.) packed with 10-μm pellicular reversed phase (Chrompack, catalogue No. 028653). An injection loop of 100 μl was used.

The mobile phase consisted of a mixture of 0.067 *M* potassium dihydrogenphosphate and 0.5 ml of diethylamine in water (1:1) as solvent A, and acetonitrile as solvent B. The mixture consisted of 65% A and 35% B. All reagents were of analytical grade. The flow-rate was 1.2 ml/min. Fluorescence detection was achieved at 315 nm excitation and 430 nm emission. The retention time was 5.26 min, capacity factor was 4.54 and the analysis was carried out at room temperature.

Sample preparation

Plasma samples of 300 μl were deproteinated by 300 μl of acetonitrile, and centrifuged at 11 000 *g* for 4 min. Of the clear supernatant, 100 μl were injected into the HPLC system.

Calibration curves

Standard solutions were freshly made before each new set of runs. Standard solutions were stable for fourteen days when kept at 4°C in the refrigerator. Calibration curves were constructed by adding variable quantities of the standard solution to blank plasma. The correlation coefficient was 0.99949 for ergometrine and the equation $y = 33.32x + 0.408$ in the concentration range 0.075–5.0 ng/ml (x = concentration and y is peak height).

Concentration

The concentration of ergometrine was measured using a calibration curve in which the peak heights of the compound (y) were expressed *versus* spiked concentrations in plasma (x).

Subjects

One male (45 years) volunteered for a pilot pharmacokinetic study of both oral and intravenous administration of ergometrine. The volunteer was screened for possible contraindications (cardiovascular disease and chronic obstructive lung disease). Body weight, height, blood pressure, haemoglobin level, liver and renal functions were recorded. During the experiments the blood pressure was monitored. This study was approved by the Committee for Experimental Research Involving Human Subjects (CEOM) of

the University Hospital, Sint Radboud (Nijmegen, Netherlands).

Dosage

A single oral dose of ergometrine maleate (0.200 mg, =0.147 mg base; Wolfs) was given after a standard breakfast. One month later 0.075 mg of ergometrine maleate (=0.055 mg base; Medisch Spectrum Twente) was injected intravenously over 1 min in the same volunteer, again after a standard breakfast.

Sampling procedures

Oral administration of ergometrine. Blood samples of 5 ml were collected through an intravenous canula (Venflon, 1.0 mm O.D.) at 0, 10, 20, 30, 60, 90, 180, 270, 360, 450 and 540 min after ergometrine administration.

Intravenous administration. In addition to the sampling times as described under *Oral administration of ergotamine*, two extra samples were taken at 3 and 5 min after the start of the injection. After centrifugation of the blood samples, plasma samples were stored at −20°C pending analysis.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of two plasma samples containing ergometrine at concentrations of 1.95 and 0.70 ng/ml and the chromatogram of a blank plasma sample. Ergometrine was separated from endogenous compounds. The detection limit of ergometrine in water was 50 pg/ml and the quantitation limit of ergometrine in plasma was 75 pg/ml, both at a signal-to-noise ratio of 3. The intra- and inter-day variations are given in Table I.

Fig. 2 shows the ergometrine plasma concentrations (ng/ml) *versus* time after oral administration of 0.2 mg of ergometrine maleate and after intravenous administration of 0.075 mg of ergometrine maleate in one male volunteer.

After oral administration the compound is rapidly absorbed with a lag time of 5 min. A maximum plasma concentration of 1.32 ng/ml was reached after 12 min. The half-life of ergometrine is 1.4 h in this volunteer. The half-life after the intravenous administration was similar to that after the oral administration. The relative bioavailability of the oral administration was calculated to be 1.00.

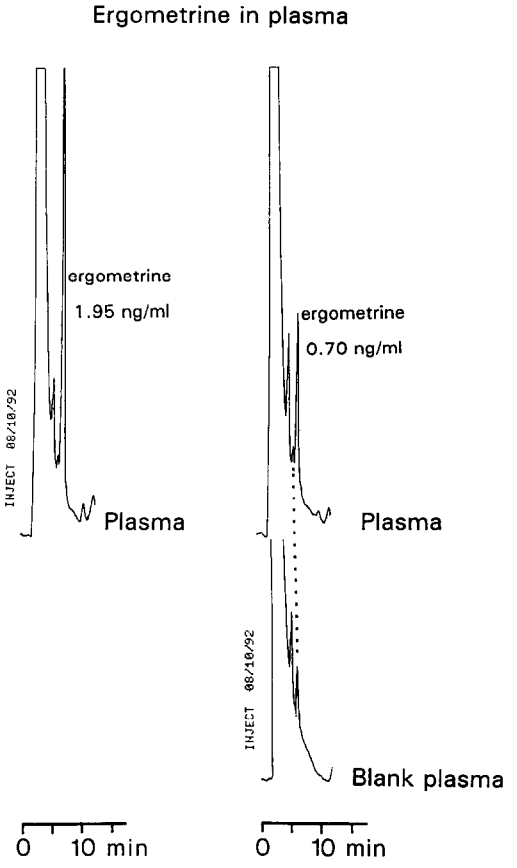

Fig. 1. Chromatograms of ergometrine in human plasma samples.

TABLE I

INTER-DAY AND INTRA-DAY COEFFICIENT OF VARIATION OF ERGOMETRINE IN HUMAN PLASMA.

Concentration (ng/ml)	Coefficient of variation ($n=4$) (%)	
	Inter-day	Intra-day
3.76	1.68	2.99
1.89	2.11	2.68
0.43	5.14	1.96
0.10	6.10	3.05

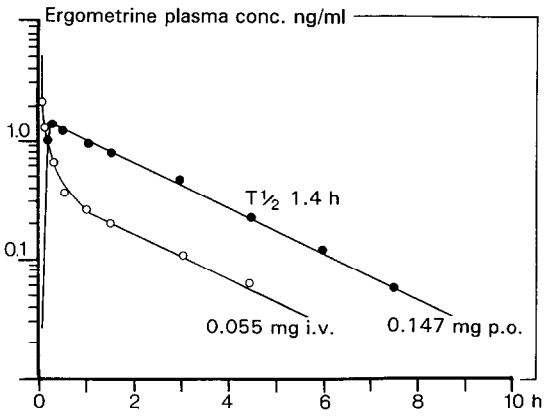

Fig. 2. Plasma concentration *versus* time curves of ergometrine after administration of an intravenous dose of 0.055 mg of ergometrine base and after oral administration of 0.147 mg of ergometrine base to the same male human subject.

Ergometrine concentrations in plasma could be accurately measured by HPLC with fluorescence detection. With this analytical method proper pharmacokinetics of oral and parenteral ergometrine can be described. The method is similar to those reported for methylergometrine, but avoids the extraction step [10]. The limit of quantitation of 75 pg/ml is similar to that of methylergometrine (20 pg/ml [12] and 100 pg/ml [10]) and sufficient to carry out pharmacokinetic studies. The volunteer in this study also took part in the pharmacokinetic study of ergometrine with six male volunteers. The results of this study will be published elsewhere.

Compared with the pharmacokinetic studies of methylergometrine [8,9,12] the bioavailability and absorption time, the lag time after administration and the peak plasma concentration of ergometrine are as rapid as that of methylergometrine. No side-effects of oral ergometrine were recorded in the volunteer.

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