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Short Communication

Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography

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Abstract

Indomethacin and mefenamic acid are widely used clinically as non-steroidal anti-inflammatory agents. Both drugs have also been found effective to produce closure of patent ductus arteriosus in premature neonates. A simple, rapid, sensitive and reliable HPLC method is described for the determination of indomethacin and mefenamic acid in human plasma. As these drugs are not applied together, the compounds are alternately used as analyte and internal standard. Plasma was deproteinized with acetonitrile, the supernatant fraction was evaporated to dryness and the resulting residue was reconstituted in the mobile phase and injected into the HPLC system. The chromatographic separation was performed on a C_{18} column (250 × 4.6 mm I.D.) using 10 m*M* phosphoric acid–acetonitrile (40:60, v/v) as the mobile phase and both drugs were detected at 280 nm. The calibration graphs were linear with a correlation coefficient (r) of 0.999 or better from 0.1 to 10 μg/ml and the detection limits were 0.06 μg/ml for indomethacin and 0.08 μg/ml for mefenamic acid, for 50-μl plasma samples. The method was not interfered with by other plasma components and has been found particularly useful for paediatric use. The within-day precision and accuracy of the method were evaluated for three concentrations in spiked plasma samples. The coefficients of variation were less than 5% and the accuracy was nearly 100% for both drugs.

1. Introduction

Indomethacin [1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid] and mefenamic acid [N-(2,3-xylyl)anthranilic acid] are both potent prostaglandin synthetase inhibitors [1,2], widely used clinically as non-steroidal anti-inflammatory and analgesic–antipyretic drugs [3,4]. Recently, both drugs have also been found effective in producing closure of patent ductus arteriosus (PDA) in premature infants [5–7]. Successful PDA closure is particularly difficult in neonates with very large PDA [8]. As the cause of therapeutic failure with indomethacin or mefenamic acid may be inadequate plasma concentrations [9], the availability of a simple, rapid, sensitive, precise and accurate assay for these drugs may be beneficial.

In recent years, a number of high-performance liquid chromatographic (HPLC) methods have been reported for the determination of indomethacin and mefenamic acid in biological fluids [10–23]. However, some of these HPLC methods lack sensitivity [10,11], some require

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time-consuming sample preparation techniques or are expensive [12–16] and others require relatively large plasma samples [17–21] and are not directly applicable to the determination of plasma levels of indomethacin and mefenamic acid in children and infants.

Therefore, the purpose of this investigation was to develop an assay for indomethacin and mefenamic acid, using the same chromatographic conditions, that could be used for pharmacokinetic studies and for routine monitoring of indomethacin and mefenamic acid levels in plasma of children and neonate patients. Indomethacin and mefenamic acid can be determined separately, with the drug not found in the patient's plasma used as an internal standard. In this paper a simple, rapid, relatively inexpensive and reliable reversed-phase HPLC assay is described that is capable for determining low levels of indomethacin and mefenamic acid in 50 μl of plasma, which is an alternative method to those of Brown *et al.* [22] for indomethacin and Sato *et al.* [23] for mefenamic acid.

2. Experimental

2.1. Apparatus

The analyses were performed using a high-performance liquid chromatographic system (Varian, Palo Alto, CA, USA) consisting of two high-pressure solvent delivery pumps (Model 2510), a static high-pressure mixer (Model 2584), a manual injector with a 20-μl fixed loop (Rheodyne, Cotati, CA, USA), a variable-wavelength UV–Vis detector (Varian Model 2550) and an integrator (Varian Model 4290). Separation was performed on a Vydac stainless-steel analytical column (250 × 4.6 mm I.D.) packed with C_{18} bonded-phase silica, particle size 5 μm (Merck, Darmstadt, Germany). A guard cartridge (10 × 4.6 mm I.D.) (Alltech, Deerfield, IL, USA) containing C_{18} bonded-phase silica, particle size 5 μm, was used in conjunction with the analytical column.

2.2. Chemicals

Indomethacin and mefenamic acid were purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile and analytical-reagent grade 85% phosphoric acid were obtained from Merck (Darmstadt, Germany).

2.3. Chromatographic conditions

The mobile phase was acetonitrile–10 m*M* phosphoric acid (60:40, v/v) and was filtered through a 0.45-μm pore size nylon filter (Alltech) and degassed by ultrasonic treatment before use. The pH of the mobile phase was 2.6. The HPLC system was operated isocratically at a flow-rate of 0.9 ml/min at ambient temperature and the detector was set at 280 nm, close to the absorption maximum of mefenamic acid (285 nm).

2.4. Standard solutions

Stock standard solutions of indomethacin and mefenamic acid (0.1 mg/ml) were prepared in acetonitrile and stored at −20°C. From each stock standard solution, fresh working standard solutions of indomethacin and mefenamic acid (0.01 mg/ml) were prepared in acetonitrile. Internal standard solutions consisting of 0.6 μg/ml of indomethacin and of 0.75 μg/ml of mefenamic acid were prepared in acetonitrile and stored at −20°C.

Calibration samples were prepared using 1.5-ml Eppendorf polypropylene tubes (Eppendorf, Hamburg, Germany) in 50 μl of drug-free plasma spiked with 250 μl of internal standard solution and appropriate volumes of working solutions to formulate indomethacin and mefenamic acid concentrations of 0.1–10 μg/ml. Aliquots of acetonitrile were added to each tube to make the final volumes equal (350 μl).

2.5. Sample preparation

Venous blood samples were collected in heparinized tubes and centrifuged at 3000 *g* for 4

min, and the plasma fractions were decanted. These plasma samples were stored at −70°C and thawed and vortex mixed before analysis. A 250-μl volume of internal standard solution and 50 μl of acetonitrile were added to a 1.5 ml eppendorf tube containing 50 μl of plasma. After mixing on a vortex mixer, the sample was centrifuged at 9000 *g* for 3 min. An aliquot of the supernatant (250 μl) was transferred into a new Eppendorf tube and evaporated to dryness in a Savant (Farmingdale, NY, USA) SpeedVac concentrator (Model SC110A). The residue was dissolved in 50 μl of the mobile phase and a 20-μl aliquot was injected into the HPLC system.

The concentrations of indomethacin and mefenamic acid in plasma samples were determined by comparing the indomethacin/internal standard and the mefenamic acid/internal standard peak-height ratios with calibration graphs of peak-height ratio *versus* indomethacin or mefenamic acid concentration. With all the plots, we made a straight-line fit of the data by least-squares linear regression analysis.

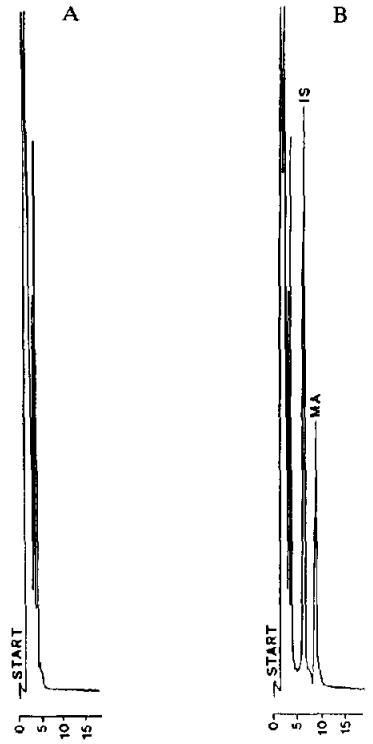

Fig. 1. HPLC of plasma samples: (A) drug-free plasma; (B) sample obtained from a child 2 h after a single 5 mg/kg oral dose of mefenamic acid containing 6.25 μg/ml of the drug. Peaks: IS = indomethacin (internal standard); MA = mefenamic acid.

3. Results and discussion

The method requires minimum pretreatment of the plasma samples from which proteins were removed by acetonitrile precipitation. To improve the performance characteristics of the method, the acetonitrile used in the plasma protein precipitation step has to be removed because of peak broadening effects due to the high concentrations of acetonitrile in the samples [24].

The peaks of the two drugs are well separated from each other, with retention times of 6.3 and 9.2 min for indomethacin and mefenamic acid, respectively. Representative chromatograms obtained from human plasma samples are depicted in Fig. 1. Fig. 1A shows the chromatogram from the analysis of drug-free human plasma. No interfering peaks due to endogenous substances were observed. Fig. 1B shows the chromatogram of a plasma sample obtained from a hospitalized feverish child who had received a 5 mg/kg oral administration of mefenamic acid (syrup). The plasma concentration of this patient 2 h after administration was 6.25 μg/ml.

Calibration graphs were constructed by plotting the peak-height ratio of drug to the internal standard (y) *versus* drug concentration (μg/ml) in spiked plasma samples (x). The calibration graphs were linear for concentrations ranging from 0.1 to 10 μg/ml for each drug and nearly passed through the origin. A typical calibration graph with eight points for indomethacin had the regression equation of $y = 0.019 + 0.403x$ with a correlation coefficient (r) of 0.9997 and the detection limit calculated for a signal-to-noise ratio of 4 was *ca.* 0.06 μg/ml. The corresponding calibration graph for mefenamic acid had a typical regression equation $y = 0.018 + 0.231x$

Table 1
Within-day precision and accuracy of the determination of indomethacin and mefenamic acid in human plasma

Concentration added (μg/ml)	Concentration found (μg/ml)	Coefficient of variation ($n = 6$) (%)
Indomethacin		
0.5	0.51	4.9
5.0	4.98	3.3
10.0	9.98	2.6
Mefenamic acid		
1.0	1.01	4.4
5.0	4.96	3.5
10.0	9.99	2.8

with $r = 0.9998$ and the limit of detection was *ca.* 0.08 μg/ml.

The within-day precision and accuracy of the method were evaluated for three concentrations in spiked plasma samples, 0.5, 5 and 10 μg/ml for indomethacin and 1, 5 and 10 μg/ml for mefenamic acid. The results, expressed as the means of six determinations, are presented in Table 1. The within-day precision of the assay, expressed as the coefficients of variation for the determined concentrations of both drugs, was less than 5% and the accuracy, assessed by calculating the differences between the nominal and estimated concentrations, was nearly 100%, for the same concentrations of the drugs.

In conclusion, the HPLC method developed for the determination of indomethacin and mefenamic acid in plasma is simple, rapid, relatively inexpensive, precise, accurate and sufficiently sensitive for routine clinical monitoring of indomethacin and mefenamic acid in small volumes of plasma, and particularly useful for paediatric use.

4. References

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