DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 7(3), 359-365 (1981)

HIGH-PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF INDOMETHACIN IN PLASMA, AFTER OINTMENT APPLICATION AND ORAL ADMINISTRATION

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ABSTRACT

A sensitive, simple and rapid method for the quantitation of indomethacin in plasma using 1-(p-fluorobenzoyl)-5-methoxy-2-methylindole acetic acid as the internal standard was developed. The method is based on reversed phase high-pressure liquid chromatography using a mobile phase continuing acetonitrile - 0.1M acetic acid (55/45 v/v). Indomethacin quantities as low as 0.1 mcg/ml can be assayed with a relative standard deviation of ± 0.07. Sensitivity can be increased by using lower sensitivity settings.

INTRODUCTION

A number of methods are available for the determination of Indomethacin in plasma (1-5). Though the gas chromatographic method is specific, it is time consuming since it requires a number of steps to prepare derivatives before chromatography and requires relatively large amounts of plasma sample.

The use of paired-ion, reverse-phase, high-pressure liquid chromatography (HPLC) for quantitative drug analysis has been demonstrated (6-8). This paper reports a simple, rapid and highly sensitive HPLC method for the determination of indomethacin in plasma, after oral and topical administration.

EXPERIMENTAL

Materials - Indomethacin[1] and 1-(p-flurobenzoyl)-5-methoxy-2-methylindole acetic acid[1] were the reference and internal standard respectively. Chromatographic grade acetonitrile[2], acetic acid[2] and methanol[3] were used. All other reagents, analytical grade or better were used as received.

Dosage forms - The oral suspension was prepared by suspending 1 Gm indomethacin in 100 ml of water. The ointments were prepared by incorporating enough indomethacin in aquaphor and hydrophylic base to prepare 1% ointment.

Animals - Adult, male New Zealand type rabbits, 2-3 kg were used.

Mobile phase - A mixture of acetonitrile/0.1M acetic acid (55:45 v/v) was used.

Internal standard solution - Accurately weighed 10 mg of internal standard in a 10 ml volumetric flask was dissolved in, and diluted to volume with methanol. 1 ml of this solution was diluted to 20 ml with methanol and used as internal standard.

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1 MSD, West Point, Pa.

2 Waters Associates, Milford, Mass.

3 Fisher Scientific Co, Fair Lawn, N.J.

Standard Solution - Accurately weighed indomethacin (100 mg) in a 100 ml volumetric flask was dissolved in, and diluted to volume with methanol. Blood samples of approximately 3 ml is removed from rabbits by cardiac puncture and the plasma was separated. 0.5 ml of plasma is pipetted in a microtest tube, 0.1 ml of the internal and known quantities of indomethacin was added and the mixture was shaken for 30 seconds. The volume of the mixture was q.s. to 2.5 ml with methanol and centrifuged. for 15 minutes at 1000 rpm. The organic phase was separated and filtered through an organic filter[4]. Ten µl of filtered organic phase is injected into the chromatogram, and by utilizing peak heights, a standard curve was constructed.

Plasma Level Study - To demonstrate the applicability of this method to the determination of plasma indomethacin levels from a bioavailability study, ointment containing 1% of indomethacin was applied to three rabbits. Blood samples of approximately 3 ml were removed by cardiac puncture at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 24 hours. To 0.5 ml of the separated plasma, 0.1 ml of the internal standard was added shaken for 30 seconds, q.s. to 25 ml with methanol, and centrifuged for 5 min. at 1000 rpn. The organic phase is separated, filtered through an organic filter and 10 µl is injected into the chromatogram and the indomethacin concentration is calculated from a previously constructed standard curve.

HPLC - Samples were chromatographed on a high pressure liquid chromatograph[5](model 440) equipped with a universal liquid chromatographic injector with a UV (254 n) absorbance detector and a strip-chart

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4 Waters Associates, Milford, Mass.

5 Waters Associates, Milford, Mass.

detector. The deproteinated plasma samples were chromatographed at room temperature on a microparticulate[6] reverse-phase HPLC column, 4mm x 30 cm, with an eluting mobile phase of acetonitrile/0.1M acetic acid (55:45 v/v). The mobile phase flow rate was adjusted to 1 ml/min with an inlet pressure of about 2000 psi and sensitivity of 0.1 mm. The chart speed was 0.2 inches/min. The ratio of the peak height of indomethacin to that of the internal standard was used to calculate the acetaminophen concentration, based on a calibration curve prepared from spiked plasma samples.

RESULTS AND DISCUSSION

Typical chromatograms of blank rabbit plasma (A), blank rabbit plasma spiked with indomethacin (B), blank rabbit plasma spiked with indomethacin and internal standard (C), and plasma collected 30 minutes after application of indomethacin ointment base and spiked with internal standard are shown in Figure 1. Indomethacin and the internal standard were well resolved and eluted with retention times of 7.5 and 6.5 min. respectively.

Table I illustrates recovery studies of indomethacin from spiked plasma from 0.1 mcg/ml to 10 mcg/ml with a standard deviation range from 0.01 to 0.92. The standard curve is the average of three determinations and the regration line slope was calculated to be 1.3 with a standard correlation matrix of 0.9986 indicating excellent linearity. The 1-way ANOVA analysis gave F=0.0008 and probability 0.9992, indicating no significant difference among the data.

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[6] Bondapak C-18, Waters Associates, Milford, Mass.

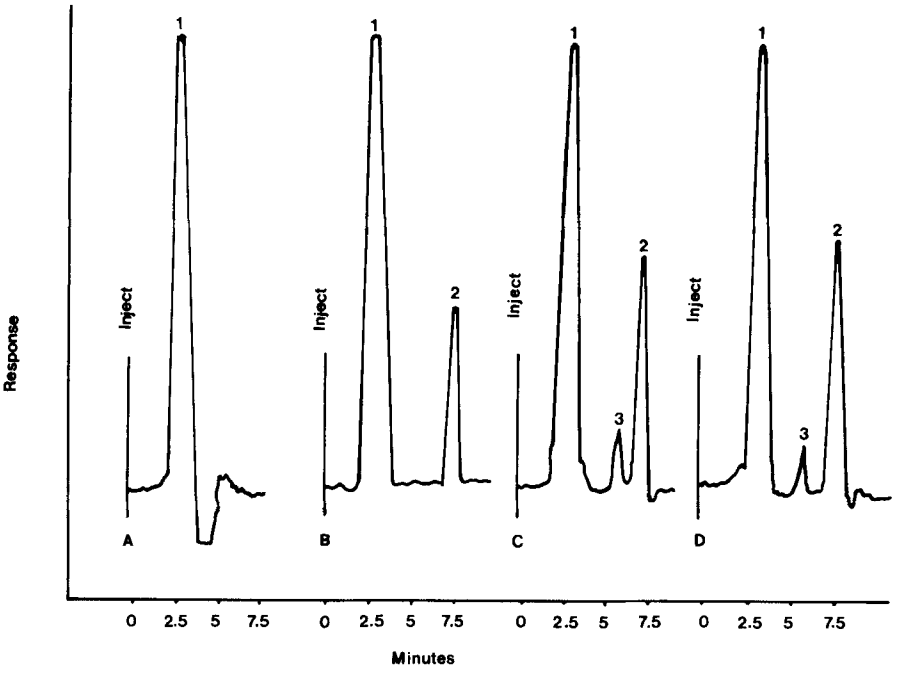

Figure 1 – High pressure liquid chromatograms obtained under assay conditions. Key: 1, Plasma; 2, Indomethocin; 3, Internal Standard.

TABLE I

RECOVERY OF INDOMETHACIN FROM SPIKED PLASMA*

Indomethacin Added (mcg/ml)	Indomethacin mean recovery (mcg/ml)	Standard Deviation
10	10.733	0.92
5	5.051	0.56
2.5	2.577	0.14
1	0.970	0.07
0.5	0.490	0.04
0.2	0.193	0.04
0.1	0.103	0.01

* Each is the average of three determinations

The applicability of the method was tested by preparing two ointments (1% indomethacin in aquaphor base and 1% in hydrophyllic base) and 1% indomethacin suspension. The plasma indomethacin time course for three rabbits in a bioavailability study from the two ointments and the suspension are shown in Figure 2. The AUC for the three preparations are: 68.8 mcg/ml/hr for the oral suspension; 48.3 mcg/ml/hr for the aquaphor base and 44.6 mcg/ml/hr for the hydrophyllic base. From those data it is apparent that the bioavailability of the drug is greater from the oral suspension, whereas the aquaphor base is better than the hydrophyllic base for the preparation of the indomethacin in the ointment form.

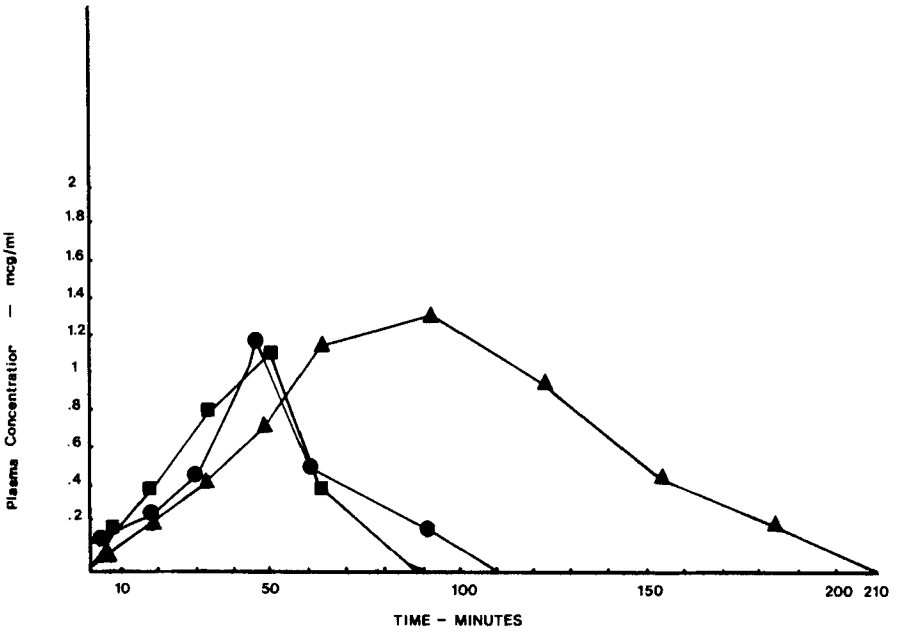

Figure 2 – Serum indomethacin levels after administration of a 1% dose of an ointment of two different bases and one oral preparation.

Key: Aquaphor, ■; Hydrophilic, ●; Suspension, ▲.

ACKNOWLEDGEMENTS

Abstracted from a thesis submitted by Syed Kazmi to the Graduate Faculty, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, in partial fulfillment of the Masters of Science degree requirements.

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