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HIGH-PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF INDOMETHACIN IN CAPSULES

by

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ABSTRACT

A high-pressure liquid chromatography method was developed for the quantitative determination of indomethacin, a nonsteroidal anti-inflammatory agent, in capsules. The method was based on the utilization of 1-(p-fluorobenzoyl)-5-methoxy-2-methylindol acetic acid as the internal standard of the acetonitrile - 0.1M acetic acid (55:45 vv/v) as the mobile phase. Indomethacin and the internal standard were well resolved and eluted with retention times of 7.5 and 6.5 min. respectively. The applicability of the method is demonstrated.

INTRODUCTION

Indomethacin has been determined by several methods (1-5). The conventional analytical method described is NF 14 (6) is based on the spectrophotometric determination of indomethacin. However, this method is time consuming since it requires dissolution, centrifugation, extraction, etc.

This report describes a specific, simple, rapid and reliable method for the determination of indomethacin in capsule dosage forms.

EXPERIMENTAL

Materials and Reagents -- The following were used (all the analytical grade unless otherwise indicated). Indomethacin capsules 25mg (lot D0398) and 50mg (lot D0308), indomethacin powder[1], 1-(p-fluorobenzoyl)-5-methoxy-2-methylindole acetic acid[1] (internal standard), chromatographic grade acetonitrile[2], acetic acid[2], methanol[3]. The mobile phase was acetonitrile - 0.1M acetic acid (55:45 v/v).

Internal Standard -- 10mg of internal standard was dissolved in 10ml of methanol, 0.1ml of this solution was diluted to 20ml and used as internal standard.

Standard Solution -- Standard indomethacin solution was prepared by dissolving 50mg of pure indomethacin in adequate methanol, so that final concentration of 1mg per ml was obtained. To this solution 2ml of the internal standard was added. From this stock solution aliquots were taken and diluted so that solutions with concentrations from 0.1 to 10mcg/ml was obtained. 10 ul of each of these aliquots were injected into the chromatograph to get a standard curve.

Capsules -- Empty and weigh the contents of twenty capsules in a porcelain mortar and mix well to form powder. The aliquots of 25mg of powder (equivalent to one indomethacin capsule) was dissolved in 250mg of methanol. Pippet out 5ml of this solution, add 0.1ml of the internal standard, and filter through an organic filter. Inject 10 ul of the filtrate into the chromatograph.

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1 MSD, West Point, Penn.
2 Waters Associated. Milford Mass.
3 J.T. Baker Chemical. W. Rochester, N.Y.

HPLC -- A liquid chromatograph equipped with a UV-visible detector (254λ) with a septum injector and a strip-chart recorder was used. The samples were chromatographed at 200°C temperature on a microparticulate (μ Bondapack C-18)[2] 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 1ml/min. with an inlet pressure of about 2500psi and sensitivity of 0.1. The chart speed was 1/5" min. The ratio peak height of indomethacin to that of the internal standard was used to calculate the indomethacin concentration based on a calibration curve previously prepared.

RESULTS AND DISCUSSION

Linearity was shown by injecting into the column various amounts of indomethacin over a concentration range of 0.1 to 10mcg/ml with a constant amount of the internal standard (Fig. 1). The standard curve is a range of five determinations. To determine the accuracy and precision of the method, recovery studies of indomethacin from capsule was performed and the data are shown in Table I. As can be seen from Table I, the average recovery range from 98 to 103.6%. The average recovery at the 1mcg/ml level was 100.6 ± 0.06 (SD). Recoveries at other potency levels were comparable. Analysis of the commercial capsules gave results (Table II) that were consistent with the potency and the amount of indomethacin contained in each capsule. Typical correlation coefficients for the standard curve were usually $\geq$ 0.999. The amount and concentration of the acetic acid and acetonitrile in the mobile phase were critical to the retention time and to the accuracy of the method. Prior to introduction into the high-pressure liquid chrom-

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2 Waters Associated. Milford Mass.

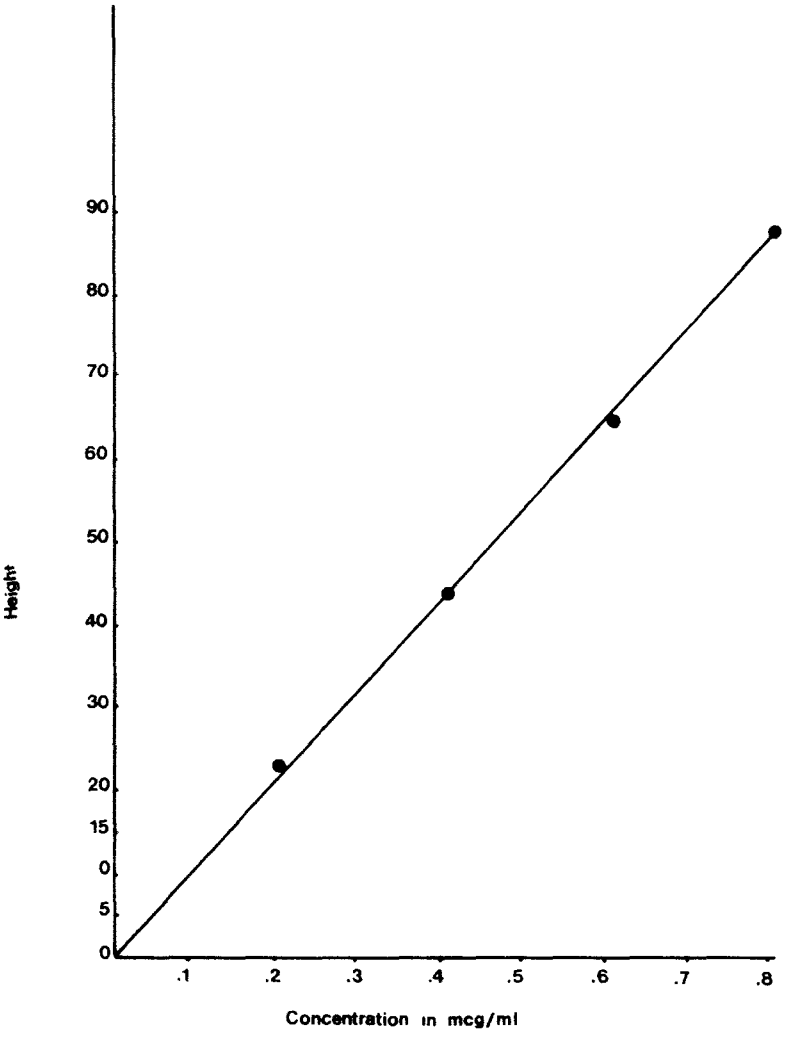

Figure 1 – Peak height ratio of the indomethacin to the internal standard versus the concentration(mcg/ml) of indomethacin in the standard solution

TABLE I - Recovery of Indomethacin Added*

Amount Added, mcg/ml	Amount Recovered, mcg/ml	Recovery, %	SD
10	9.980	99.8	0.89
5.0	5.014	100.2	0.09
2.5	2.522	100.4	0.14
1.0	1.006	100.6	0.06
0.5	0.518	103.6	0.05
0.2	0.195	97.0	0.03
0.1	0.098	98.0	0.02

* Each is the average of 5 determinations

TABLE II - Analysis of Commercial Indomethacin Capsules

Capsule	Found	Mean	SD
25 mg	23, 25, 24, 24, 24	24	1.12
50 mg	50, 49, 49, 52, 51	50.2	1.3

atograph, good chromatographic paractices generally requires filtering the analytical solutions through an organic filter. In this study it was found that filtering improved the accuracy of the method.

A typical chromatogram of an indomethacin capsule is shown in Fig. 2. The retention time for indomethacin is 7.5 min. and for the

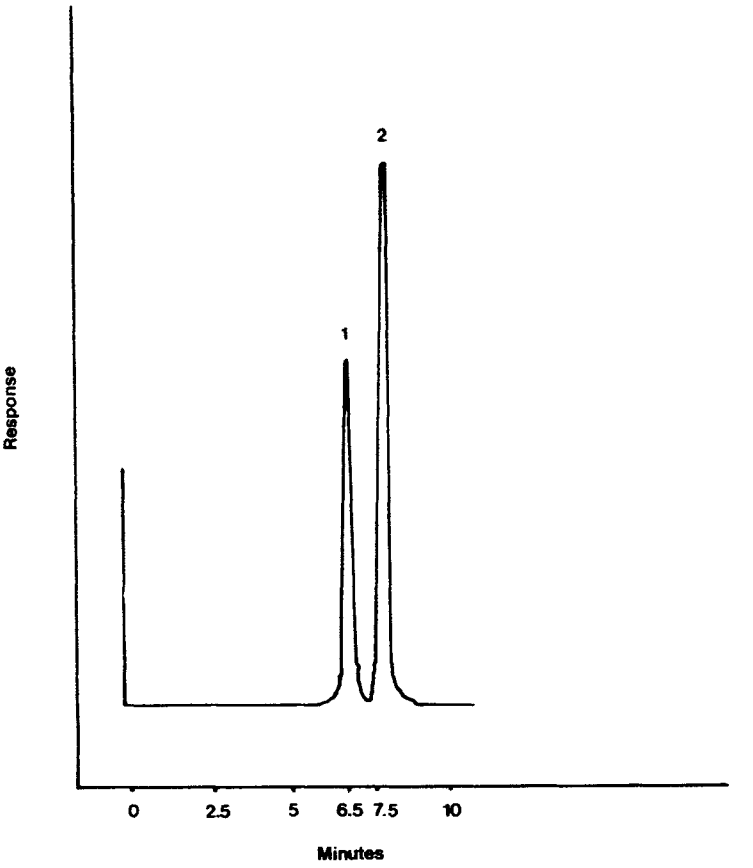

Figure 2 – Chromatogram of indomethacin capsules obtained under the assay conditions.
Key: 1, internal standard; 2, indomethacin

internal standard 6.5 min.

The described HPLC method has general applicability to most pharmaceutical dosage forms containing indomethacin, and provides a rapid, sensitive and precise assay.

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