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Note

# Determination of indomethacin in plasma and urine by direct quantitative thin-layer chromatography

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Several methods for quantitative analysis of the antirheumatic drug indomethacin in biological fluids have been utilized in the past. These include radioactive [1—3], spectrophotometric [1] and spectrofluorometric [4] methods which, except for the isotope dilution technique of Duggan et al. [3] lack specificity due to interference from metabolites or salicylates. Recently, a specific gas chromatographic method has been published [5]. However, since indomethacin does not have an adequate vapour pressure to permit its direct gas—liquid chromatography (GLC) at temperatures below 300° the method involves a derivation step with diazomethane.

Indomethacin has been shown to undergo extensive biotransformation to 0-desmethylindomethacin, N-deschlorobenzoylindomethacin, 0-desmethyl-N-deschlorobenzoylindomethacin and their glucuronides in man and all laboratory animals so far examined [1, 3, 6]. All of these metabolites are present in significant amounts in plasma but are devoid of anti-inflammatory activity [7].

A method for separation of indomethacin, 0-desmethylindomethacin and N-deschlorobenzoylindomethacin using anion-exchange chromatography and spectrophotometry for detection of the individual compounds has been described [8] but the method lacks the sensitivity needed for clinical pharmacological studies.

The direct quantitative thin-layer chromatography (TLC) method described in this paper permits specific determination, without any derivation step, of

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indomethacin in plasma and urine at concentration levels far below the levels obtained from therapeutic doses of indomethacin.

## MATERIALS AND METHODS

### *Apparatus*

A Zeiss spectralphotometer with TLC scanning equipment KM 3 (Carl Zeiss, Oberkochen/Württemberg, G.F.R.) linked to a Servogor SB RE 646 recorder (Goerz Electro, Vienna, Austria) was used. The apparatus was equipped with two photomultipliers for simultaneous measurement of remission and transmission. The monochromator was set at 330 nm and the remission—transmission ratio used was 100:30. The scanning speed was 120 mm/min.

### *Chemicals*

The following chemicals were used: 1,2-dichloroethane (Reinst), methanol (analytical grade), chloroform (analytical grade), citric acid (analytical grade) from E. Merck (Darmstadt, G.F.R.); sodium hydroxide (analytical grade) from Elektrokemiska (Bohus, Sweden); indomethacin from Dumex (Copenhagen, Denmark); metabolites of indomethacin from Alfred Benzon (Copenhagen, Denmark); filter paper Whatman No. 3 from A.G. Frisenette and Sons (Ebeltoft, Denmark).

### *Thin-layer chromatography*

TLC was carried out using 20 × 20 cm pre-coated silica gel 60 thin-layer plates with a layer thickness of 0.25 mm and fluorescent indicator obtained from E. Merck. Twelve samples were spotted on each plate leaving 2 cm at the edges. Duplicate samples were always run on separate TLC plates and 4 of the 12 spots on each plate were extracts from spiked plasma samples used for preparing a standard curve. To ensure a reproducible spot size, the organic phase containing indomethacin was spotted, 10 μl at a time and the spot was completely dried under a stream of nitrogen before the next 10-μl portion was applied. The chromatography tank was lined with Whatman No. 3 chromatography paper. The mobile phase was chloroform—methanol (30:6). Indomethacin has an $R_F$ value of 0.37 in this solvent. After chromatography the plates were dried in a ventilated oven at 50° for 15 min. The chromatography was conducted with the exclusion of light.

### *Procedures*

One ml of plasma or urine was pipetted into each 10-ml PTFE-stoppered centrifuge tube and mixed with 1 ml citrate buffer (pH 5.0). A 7-ml aliquot of 1,2-dichloroethane was added and the tubes were shaken horizontally for 15 min at a rate of approximately 90 strokes per min. After centrifugation for 5 min at 1500 *g* the aqueous phase was removed by aspiration. A 3-ml volume of citrate buffer (pH 5.0) was added and the tubes were shaken for 5 min. The tubes were centrifuged and the aqueous phase was removed by aspiration. Five ml of the organic phase were then transferred to a conical centrifuge tube and evaporated to dryness under nitrogen, in a water bath at 40°. The residue was dissolved in 50 μl 1,2-dichloroethane using a Whirlimixer and the extracted material was spotted under nitrogen on the TLC plates using a 10-μl

constriction pipette. An additional 20-μl portion of 1,2-dichloroethane was added to each tube, shaken and spotted. After chromatography the TLC plates were scanned.

*Calculations*

The amount of indomethacin was calculated by comparison of the peak areas, calculated as peak height × width at half-height, for samples and standards. The standard curve fits a polynomium of the form $y = a_2x^2 + a_1x + a_0$, where $y$ is the peak area and $x$ is the concentration. The quadratic regression using the least-square method is made on a programmable calculator (Texas Instruments SR-52 or similar) and typically the correlation coefficients obtained are 0.99 or better.

## RESULTS AND DISCUSSION

*Precision, accuracy and sensitivity*

The within-day as well as the day-to-day variations are shown in Table I.

When linear regression on added versus measured amount of indomethacin in the range 30—500 ng/ml plasma (72 samples) is made the correlation coefficient is 0.997 and the slope of the regression line is 0.97.

The recovery of indomethacin by extraction from plasma and urine with 1,2-dichloroethane has a maximum at pH 5.0. At this pH the recovery is 90%. It is important that the citrate buffer is freshly prepared every week.

The absorption spectrum of indomethacin when measured on a TLC plate in the remission mode, shows two maxima, one at 265 nm and one at 320 nm. However, to use the automatic baseline correction, inherent in the simultaneous measurement of remission and transmission, the lowest possible wavelength at which simultaneous measurement can be performed was chosen, i.e. 330 nm. Below this wavelength the absorption of transmitted light in the glass plate becomes too significant to allow simultaneous measurement. If the apparatus used is not equipped for simultaneous measurement it is advisable to measure in the remission mode at 265 nm.

No interference was found between indomethacin and the metabolites, O-desmethylindomethacin, N-deschlorobenzoylindomethacin, O-desmethyl-N-deschlorobenzoylindomethacin or the salicylates, in the analysis, because the $R_F$ values of these compounds differed from that of indomethacin. Fig. 1 shows a typical scan of two samples extracted from plasma, one containing 250

TABLE I

WITHIN-DAY AND DAY-TO-DAY VARIATIONS AT DIFFERENT INDOMETHACIN CONCENTRATIONS

| Concentration (ng/ml) | Within-day range | *n* | Day-to-day S.D. (%) | *n* |
|---|---|---|---|---|
| 31 | 27— 31 | 4 | 12.2 | 22 |
| 63 | 60— 65 | 4 | 6.5 | 17 |
| 125 | 120—130 | 4 | 4.4 | 18 |
| 250 | 245—257 | 4 | 6.3 | 11 |
| 500 | 469—524 | 4 | 3.0 | 5 |

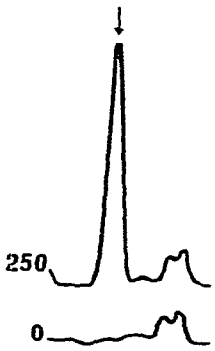


Fig. 1. Chromatogram scan showing 0 and 250 ng indomethacin, extracted from two 1-ml plasma samples.

ng and one no indomethacin. The lower limit for reliable quantitation of indomethacin in plasma and urine is 30 ng/ml using 1 ml samples (Table I). As no derivation step is involved the samples can be re-chromatographed in the same or in another solvent to yield a better separation, for example in case of interference from a new drug. From the following formula [9] $n = -1/\ln(1 - R_{F'})$, where $n$ is the number of runs and $R_{F'}$ is the average $R_F$ of the two solutes, the number of runs which yields maximum separation with a given solvent, can be calculated. In this case when the $R_F$ value is 0.37, $n = 2$.

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