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DETERMINATION OF INDOMETHACIN IN SERUM BY AN EXTRACTIVE ALKYLATION TECHNIQUE AND GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A method for determining indomethacin in serum has been developed, involving extractive alkylation, in which indomethacin is converted into its ethyl ester, and subsequent gas chromatographic determination of the ester. The method is specific and permits the determination of amounts down to 50 ng/ml in serum.

The indomethacin concentration in serum was followed for 24 h after oral and rectal application and the results are compared with those reported in the literature.

INTRODUCTION

A photofluorimetric method has been described[1] for the determination of indomethacin in biological fluids, but it is not specific and metabolites and salicylates, if present, are included in the result.

Previous methods used for separating indomethacin from metabolites and salicylates are column chromatography[2,3] and thin-layer chromatography[4]. The indomethacin content is measured, after separation, by photometry[2] or photofluorimetry[3,4]. However, these methods are time consuming and not sufficiently sensitive for the determination of indomethacin in serum following therapeutic doses.

The determination of indomethacin in plasma or serum has also been carried out by means of gas chromatography[5–7] and high-performance liquid chromatography (HPLC)[8]. These methods are both specific and sensitive, but they involve the use of explosive reagents (diazomethane or diazoethane) or a special detector (mass fragmentometer).

Radioactive methods have also been described for the determination of indomethacin[9–11], but of these methods only Duggan *et al.*'s isotope dilution technique[11] is able to separate and determine indomethacin and its metabolites. This method, however, is not applicable in clinical pharmacological studies using commercial preparations.

In the method described here, the determination involves extractive alkylation, followed by gas chromatography. Indomethacin is converted into an anionic form and transferred as an ion pair with a quaternary ammonium ion into an organic solvent

(dichloromethane). In dichloromethane the indomethacin is converted by means of ethyl iodide into the ethyl ester, which is then determined by gas chromatography.

EXPERIMENTAL

Materials and reagents

The column material was 3% E 350 (SE-52) on diatomite CQ (100–120 mesh) obtained from J.J.'s (Chromatography), Kings Lynn, Great Britain. The carrier gas was a mixture of argon (99.996% pure) and methane (99.95% pure) (9:1), obtained from Dansk Ilt og Brintfabrik, Copenhagen, Denmark. Trimethylchlorosilane was of a specially purified grade from Pierce, Rockford, Ill., U.S.A., and *n*-hexane and dichloromethane were specially purified (nanograde) from Mallinckrodt, St. Louis, Mo., U.S.A. Tetrahexylammonium hydrogen sulphate was obtained from Labkemi Ltd., Stockholm, Sweden. Ethyl iodide "for synthesis" from E. Merck, Darmstadt, G.F.R., was used without further purification.

Apparatus

The gas chromatograph was a Pye Series 104 instrument, equipped with an electron-capture detector (^{63}Ni) and a 5-ft. coiled glass column with an I.D. of $^1/_4$ in. The column was packed with E 350 on diatomite CQ. Before use, the column was conditioned at 300° for 72 h and finally silanized with trimethylchlorosilane (10 × 10 μl) at 70°. The temperature was 350° in the detector, 275° in the column and 300° at the injection port. The flow-rate of the carrier gas was 60 ml/min.

Printing of the chromatogram and calculation of the peak area were performed with an electronic integrator from Hewlett-Packard, Avondale, Pa., U.S.A. (No. 3080A). Calculation of the area under the serum curves was carried out with Hewlett-Packard No. 9815A calculator.

Procedure

To 0.5 ml of serum or a dilution thereof, add 2 ml of a 5 m*M* aqueous solution of tetrahexylammonium hydrogen sulphate and 5.00 ml of a 0.5 *M* solution of ethyl iodide in dichloromethane. Shake the mixture in a water-wath at 37° for 40 min and, after centrifuging (*ca.* 1500 *g*) for 5 min at 10°, remove a 3.00-ml volume from the dichloromethane phase and evaporate it to dryness at 40° with a gentle stream of nitrogen. Re-dissolve the residue in 1000 μl of *n*-hexane and centrifuge for 5 min at 10° (*ca.* 1500 *g*), in order to precipitate the excess of tetrahexylammonium hydrogen sulphate. Remove 800 μl of the supernatant and evaporate it to dryness at 40° with nitrogen. Immediately before the measurement, dissolve the residue by shaking it in at least 500 μl of *n*-hexane, then inject 2 μl of the resulting solution into the gas chromatograph.

Read off the amount of indomethacin in the serum samples from a calibration graph constructed from control sera containing known amounts of indomethacin.

Sampling

Six healthy subjects (five women and one man in the age range 18–45 years) each received one capsule containing 50 mg of indomethacin (Confortid). Another seven healthy subjects (five women and two men in the age range 20–48 years) re-

ceived a suppository containing 50 mg of indomethacin (Confortid). None of the subjects were receiving other drugs and all fasted from the preceding evening and until 2 h after the administration.

Blood samples were drawn immediately before and 1, 2, 3, 4, 8 and 24 h after administration. The serum centrifuged off was stored at −18° until required for analysis.

RESULTS

The response of the gas chromatograph to ethylindomethacin is illustrated in Fig. 1. A 2-μl volume of *n*-hexane solutions containing various concentrations of ethylindomethacin was injected and the peak areas in arbitrary units were plotted against amount of ethylindomethacin injected.

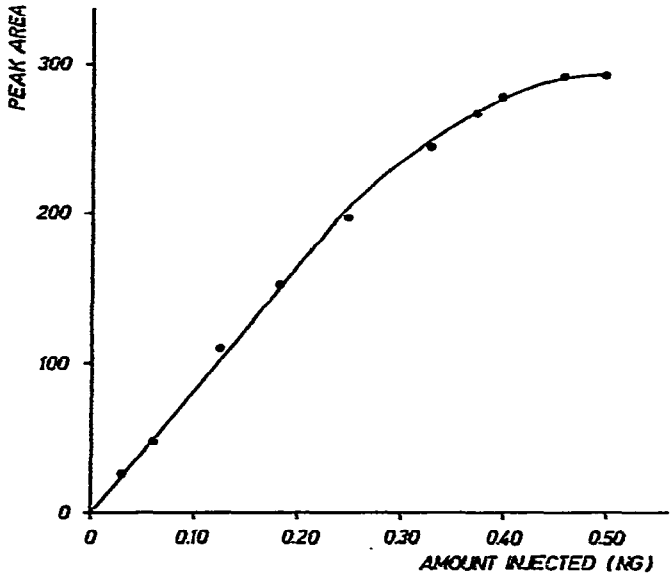

Fig. 1. Response of the gas chromatograph to ethylindomethacin.

Fig. 2 is a calibration graph for indomethacin in control serum. The samples were diluted to different extents prior to injection into the gas chromatograph, so that the amount of ethylindomethacin injected never exceeded about 0.3 ng.

Chromatograms of control serum (A) and of control serum with indomethacin (150 ng/ml) added (B) are shown in Fig. 3. The retention time of indomethacin was 9.4 min.

O-Desmethylindomethacin, a metabolite of indomethacin[11], was added to serum which was then analysed by the method described. The retention time for O-desmethylindomethacin was 10.4 min (Fig. 4).

Interference by salicylic acid was studied. Fig. 5 shows a chromatogram obtained with serum to which salicylic acid had been added.

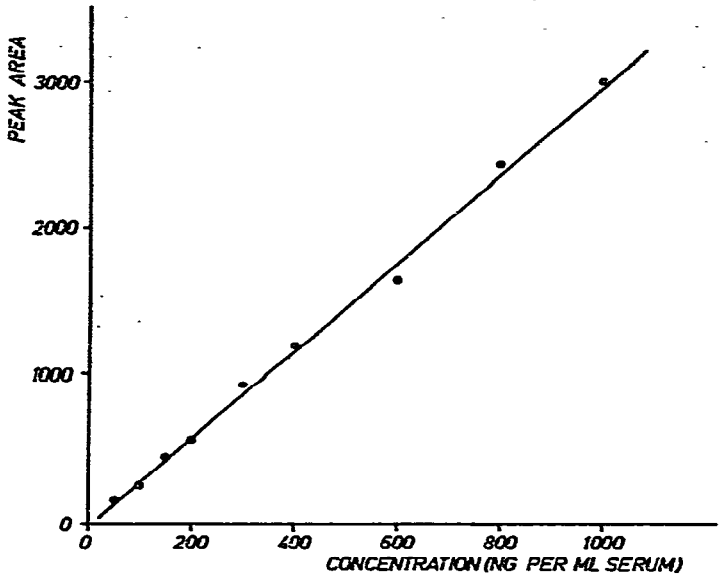

Fig. 2. Calibration graph for indomethacin in serum.

The minimal concentration of indomethacin that can be determined in serum is 50 ng/ml. The recovery of indomethacin in serum was investigated in the concentration range 50–1000 ng/ml (Table I). The mean recovery was 95%.

The reproducibility of the method was determined by analysing control serum to which had been added 500 ng/ml of indomethacin. The standard deviation was 6% ($n = 10$).

Application to clinical samples

Following oral administration of 50 mg of indomethacin to healthy subjects,

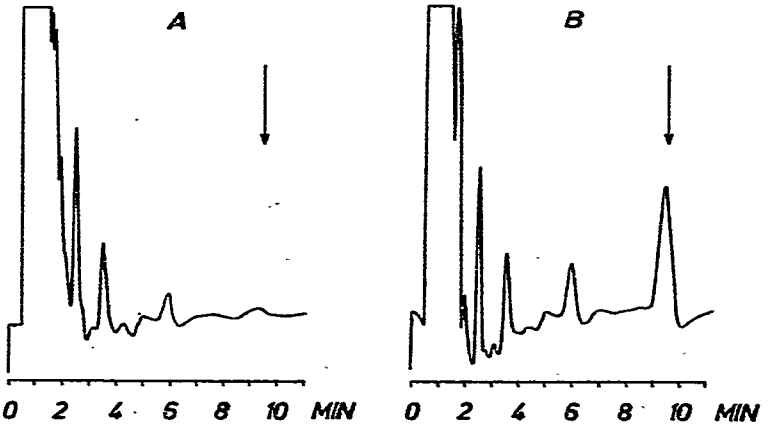

Fig. 3. Chromatograms of (A) control serum and (B) control serum to which 150 ng/ml of indomethacin had been added. The arrows indicate the retention time of indomethacin.

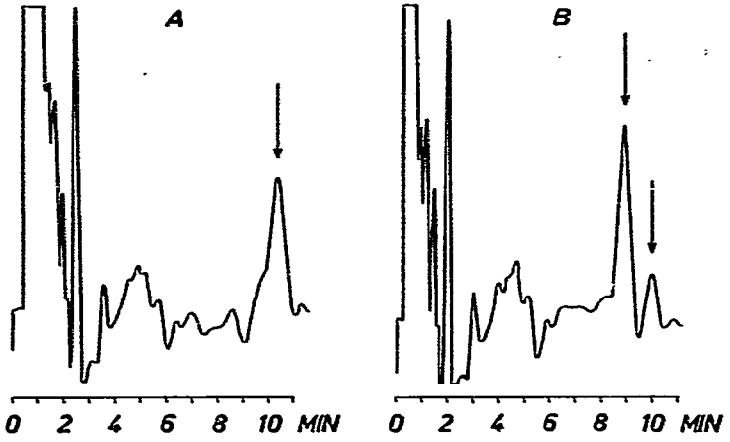

Fig. 4. (A) Chromatogram of control serum to which had been added 10 μg/ml of O-desmethylindomethacin (retention time 10.4 min); (B) chromatogram of control serum to which had been added 4 μg/ml of O-desmethylindomethacin (retention time 10.4 min) and 0.50 μg/ml of indomethacin (retention time 9.4 min).

the peak serum concentrations after 1–2 h were 1.3–2.0 μg/ml (mean 1.4 μg/ml). Following rectal administration of 50 mg of indomethacin, the peak serum concentrations after 1–2 h were 0.6–1.9 μg/ml (mean 1.1 μg/ml). Mean concentrations with standard errors of the means following administration of capsules and suppositories are illustrated in Fig. 6. After 24 h the serum concentration was very low (<0.05 μg/ml) both following oral and rectal administration.

The areas under the serum curves were calculared for each subject by the trapeze method, and the results are given in Table II.

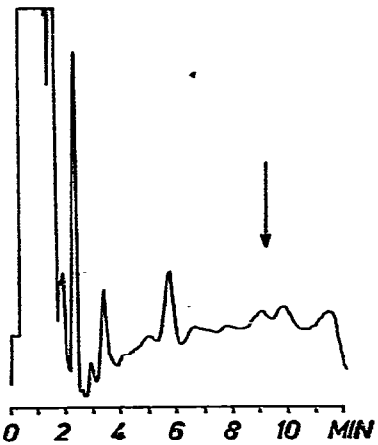

Fig. 5. Chromatogram of control serum to which had been added 2 μg/ml of salicylic acid. The arrow indicates the retention time of indomethacin.

TABLE I

RECOVERY OF INDOMETHACIN IN SERUM

Amount of indomethacin (ng)		*Recovery (%)*
*Added**	*Found*	
25	25	100
40	38	94
75	68	91
100	88	88
150	125	83
200	179	90
250	230	92
300	319	106
350	326	93
400	398	100
500	536	107
	Mean:	95

* To 0.5 ml of serum.

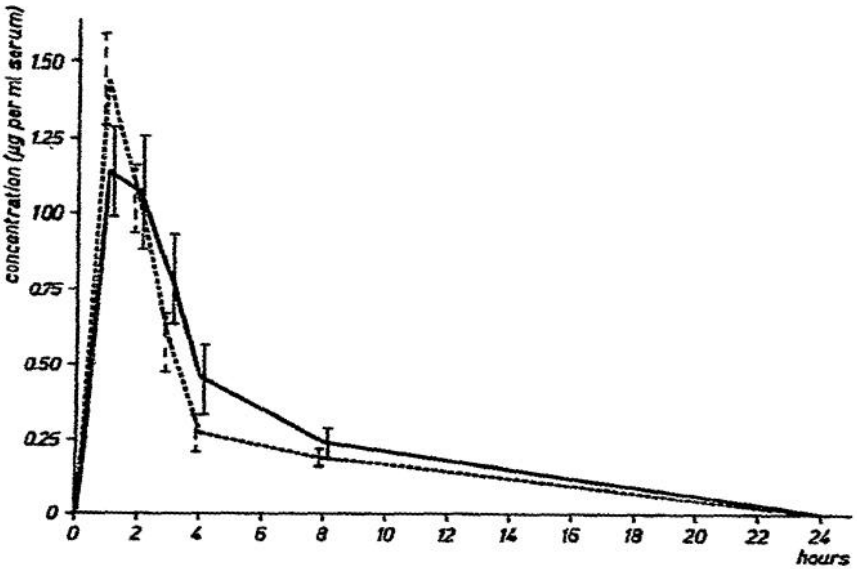

Fig. 6. Mean concentrations of indomethacin in serum following oral (six subjects) (broken line) and rectal administration (seven subjects) (solid line) of 50 mg of indomethacin as a single dose. Vertical bars are standard errors of the means.

DISCUSSION

The gas chromatographic determination of indomethacin requires derivation of the substance prior to injection into the gas chromatograph. Helleberg[6] and Ferry *et al.*[5] converted indomethacin into the ethyl ester before performing the gas chromatographic analysis. Both used diazoethane, which is explosive and toxic[12].

TABLE II

AREA UNDER SERUM CURVES IN SUBJECTS FOLLOWING ORAL AND RECTAL ADMINISTRATION OF 50 mg OF INDOMETHACIN

Preparation	*Subject*	*Area under curve (h·μg/ml)*
Suppositories	A	9.7
	B	3.2
	C	9.7
	D	10.3
	E	4.4
	F	6.5
	G	2.6
	Mean	6.6
Capsules	I	7.0
	J	4.4
	K	6.5
	L	6.2
	M	3.8
	N	5.7
	Mean	5.6

Palmér *et al.*[7] employed gas chromatography–mass fragmentography for the determination of indomethacin. Before the measurement, the substance was converted into its methyl ester using diazomethane, which has the same disadvantages as diazoethane[12].

In this study, indomethacin was converted into its ethyl ester by extractive alkylation[13–15], which does not involve the same risks as the above methods.

The method is specific for indomethacin. O-Desmethyl indomethacin, which is a metabolite of indomethacin[11], shows, after 10.4 min, a peak separated from that of indomethacin (retention time 9.4 min). Another two metabolites of indomethacin, desbenzoylindomethacin and desmethyldesbenzoylindomethacin[11], cannot be determined by this method because of their lack of electronegativity.

Salicylic acid, which is often used in combination with indomethacin in clinical studies, does not give interfering peaks on the chromatogram.

The reproducibility of 6% ($n = 10$) with 500 ng/ml of indomethacin compares with Helleberg's value of 3% ($n = 10$) for serum samples containing 500 ng/ml of indomethacin[6] and Palmér *et al.*'s value of 7% ($n = 2$).

The minimal concentration of indomethacin that can be determined 50 ng/ml compares with Helleberg's value of 50 ng/ml and Palmér *et al.*'s value of 25 ng/ml. Skellern and Salole[8], using HPLC, reported a limit of detection of 100 ng/ml of indomethacin in serum.

The recovery of 95% within the concentration range 50–1000 ng /ml of indomethacin in serum compares with recoveries of 95% by Helleberg[6], 96% by Ferry *et al.*[5] and at least 85% by Skellern and Salole[8].

Application to clinical samples

After administration of capsules containing 50 mg of indomethacin, a peak serum concentration of 1.4 μg/ml (mean) was obtained after 1 h. Arnold and Brynger[16]

found a peak serum level of 3.0 μg/ml (mean) 2 h after oral administration of 100 mg of indomethacin to ten patients.

One hour after the application of suppositories containing 50 mg indomethacin, the maximal serum concentration was 1.1 μg/ml (mean). Arnold and Brynger[16], 1 h after rectal application of 100 mg of indomethacin to ten patients, found a maximal serum concentration of 2.8 μg/ml (mean). Lindquist *et al.*[4], 1 h after rectal administration of 100 mg of indomethacin to six subjects, found a peak serum level of 2.1 μg/ml (mean). Kaldestad *et al.*[17] obtained a maximal serum concentration of 2.0 μg/ml (mean) 1 h after rectal administration of 100 mg of indomethacin to 14 patients.

The areas under the serum curve for each subject were calculated to be 3.8–7.0 (mean 5.6) h·μg/ml following oral administration of 50 mg of indomethacin and 2.6–10.3 (mean 6.6) y·μg/ml following rectal administration of 50 mg of indomethacin. Thus, the absorption of indomethacin after administration of capsules and of suppositories is approximately the same.

CONCLUSION

A sensitive and specific method was used for the determination of serum concentration of indomethacin following oral and rectal administration of 50 mg to healthy subjects. The serum concentrations found have confirmed the results of previous absorption studies.

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