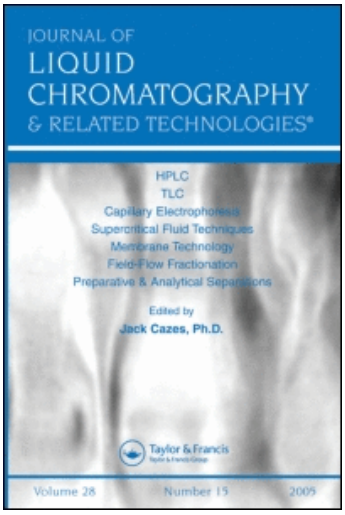

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High Performance Liquid Chromatographic Analysis of Tolmetin, Indomethacin and Sulindac in Plasma

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF TOLMETIN, INDOMETHACIN AND SULINDAC IN PLASMA

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ABSTRACT

Isocratic and gradient reversed phase high-performance liquid chromatographic (HPLC) methods for the quantitation of tolmetin, indomethacin, and sulindac and their respective metabolites in plasma were developed. Only the determination of the parent drugs was possible using the isocratic technique. Specific, simultaneous determination of each drug and its respective metabolites was achieved using the gradient technique. The effect of pH and ionic concentration of the mobile phase on retention time was studied. Statistical analysis demonstrated excellent precision and linearity over the following ranges: 1-40, 0.1-3, and 0.1-3 ug/ml plasma for tolmetin, indomethacin, and sulindac respectively. Both methods have been applied to the analysis of patient samples.

INTRODUCTION

The nonsteroidal, anti-inflammatory drugs are used in the treatment of arthritis and for the relief of mild to moderate pain (1). Many adverse reactions are common to all these drugs. Almost every patient experiences some symptoms of gastrointestinal toxicity, but even when such symptoms are absent, GI lesions are often present (2,3). Reduction of renal function has also been reported (4). Severe and even life threatening adverse reactions have been reported (5,6).

Direct spectrophotometric (7-10) and spectrofluorometric (8,11,12) determination of these drugs have been reported. These methods lack specificity due to interference from metabolites and salicylic acid. A TLC separation with quantitation by a TLC scanning spectrophotometer has been described (13). Several GLC procedures have been reported which require derivatization (7,14-22) and/or special detectors such as electron capture (15-20) or mass spectroscopy (21-23). Also described in the literature are radioimmunoassay methods (24,25). Quantitation has been performed by high-performance liquid chromatography (HPLC). The reported methods involve precipitation of plasma proteins followed by injection of the supernatent (26-28) or have not been applied to the analysis of patient samples (29,30). An HPLC method for the screening of solid dosage forms using dual UV detectors has been reported recently (31).

This paper describes a rapid, specific method for the determination of indomethacin, sulindac and tolmetin by isocratic elution. Also described are alternate methods using gradient elution making it possible to determine these drugs and their metabolites. Both methods are by HPLC using a reversed-phase octadecylsilane column and a 254 nm detector. These methods are applicable to the direct determination of plasma levels even in the presence of other drugs. The applicability

was demonstrated by the analysis of plasma from patients taking oral indomethacin, sulindac or tolmetin.

MATERIAL AND METHODS

Instrumentation - Isocratic - A Waters Associates Model 202 liquid chromatograph was equipped as follows: an M6000 pump, a U6K Universal injector, a Zorbax ODS column (25.0 cm x 4.6 mm), a Schoeffel Model SF770 variable wavelength UV detector and a Perkin-Elmer Model 56 recorder. The detector was set at a wavelength of 254 nm. The degassed mobile phase was pumped through the column at 1.5 ml/min (22.0-23.4 MPa) at ambient temperature until a stable baseline was obtained.

Gradient - A Hewlett-Packard Model 1084B liquid chromatograph with a variable wavelength UV detector was equipped as follows: A Model 79850B LC terminal, a Zorbax ODS column, and an automatic injector. The wavelength was set at 254 nm. The degassed mobile phase set at the proportions used initially was pumped through the column at 1.5 ml/min (20.7-25.5 MPa) at 40°C until a stable baseline was obtained.

Chemicals and Reagents - Reagent grade sodium hydroxide, sodium acetate, sodium phosphate, acetic acid, phosphoric acid, sulfuric acid, citric acid, methylene chloride, chloroform, 2-propanol, hexane, ethyl acetate, diethylether, diphenylacetic acid and p-phenylphenol were

used. HPLC grade methanol (Fisher) was used. Indomethacin, sulindac, and tolmetin and their respective metabolites were obtained courtesy of the manufacturer.

Mobile Phases - Sodium acetate buffers of pH 4 and 5 (0.01,0.02,0.03,0.04,0.06,0.08,0.10,0.12 and 0.15M) and acetic acid solutions (0.01,0.02 and 0.03M) were prepared and an appropriate amount was added to methanol. All mobile phases were degassed under vacuum. The effect of ionic concentration and pH on retention time was studied.

Drug Solutions - Methanolic solutions of each drug and metabolite were prepared at the appropriate concentrations to be used for the preparation of plasma standards.

Internal Standard Stock Solution - A solution of 3 mg p-phenylphenol in 100 ml methanol was prepared and kept refrigerated.

Extraction Solutions - A. (0.12 ug/5ml) - A 0.2 ml aliquot of the p-phenylphenol stock solution was diluted to 250 ml with methylene chloride.

B. (0.3ug/5ml) - A 0.5 ml aliquot of the p-phenylphenol stock solution was transferred to a 250 ml volumetric flask and brought to volume with methylene chloride.

C. (8.5ug/5ml) - A solution containing 1.7 mg diphenylacetic acid in 1 liter methylene chloride was prepared.

D. (50ug/5ml) - A solution was prepared containing 5 mg diphenylacetic acid in 500 ml methylene chloride.

Analytical Procedure - To a known volume of heparinized plasma in 15 ml screw capped centrifuge tube, an aliquot of the methanolic solution of the drug (and its metabolite(s) if gradient elution is used) was added. The plasma was acidified with 1.0M sulfuric acid and 5 ml extraction solution containing the appropriate internal standard was added. The tubes were vortexed for 10 sec and centrifuged for 5 min at 500xg. A 4 ml volume of the organic phase was transferred to a Concentratube^{R} (32) and evaporated to dryness at ambient temperature under a gentle stream of nitrogen.

The residue was dissolved in 100 ul methanol and an aliquot was injected (Table 1).

HPLC Separation - Isocratic Analysis - Ambient temperature was maintained during all assays. The mobile phases used were as follows: for indomethacin, 60:40 methanol: 0.10M acetate buffer, pH5; for sulindac, 60:40 methanol:0.04M acetate buffer, pH4; and for tolmetin, 60:40 methanol: 0.10M acetate buffer, pH5.

Gradient Analysis - All Analyses were performed at an oven temperature of 40^{o}C. Indomethacin and its metabolites - The initial mobile phase consisted of 45% methanol and 55% 0.10M acetate buffer, pH5. This proportion was maintained for 3 min followed by a linear gradient

TABLE 1

Sample Preparation Parameters

Parameters	Drug		
	Indomethacin	Sulindac	Tolmetin
Plasma volume in ml	0.5	0.5	0.25
Concentration of Standards, μg/ml plasma	0.1-3.0	0.1-3.0	1-40
Volume of 1.0M H_2SO_4 in ml	0.25	0.25	0.125
Extraction Solution			
For Isocratic	B	B	D
For Gradient	A	C	D
Injection Volume in μl			
For Isocratic	20-30	20-30	5-8
For Gradient	20	20	10

to 62% methanol in 2 min. This percentage was continued for 10 additional min.

Sulindac and its metabolites - For 10 min a mobile phase consisting of 51% methanol and 49% 0.10M acetate buffer, Ph5, was used. Using a linear gradient, the methanol was increased to 80% in 3 min. This ratio was maintained for an additional 6 min.

Tolmetin and its metabolite - The initial composition of the mobile phase was 22% methanol and 78% 0.10M acetate buffer, pH5. This proportion was used for 5 min, then the methanol percentage was increased to 53% in 2 min and was continued as such for 8 additional min.

Quantitation - A standard curve was constructed by injecting plasma extracts simulating concentrations of the drug (and metabolite(s) if gradient elution). The chromatograms were recorded at a chart speed of 0.5 cm/min. The ratios of peak heights (drug or metabolite to internal standard) were calculated and plotted against the concentration in micrograms per milliliter plasma.

Interferences - The possible interference of normal plasma constituents was tested by the analysis of blank plasma samples. The interference of other drugs was tested by direct injection of methanolic drug solutions or by the analysis of extracts of plasma samples containing therapeutic concentrations.

Recovery - For the recovery study, plasma standards were prepared as described under Analytical Procedure. After evaporation, the residue was dissolved in methanol containing a known concentration of the drug or the metabolite being studied. An aliquot was injected onto the column.

Patient Sample Preparation and Assay - Heparinized plasma samples from patients receiving oral indomethacin, sulindac or tolmetin were processed in duplicate as described under Analytical Procedure. The amount of drug was calculated by comparison with standards prepared daily.

RESULTS AND DISCUSSION

Both the isocratic and gradient procedures require an extraction step. In both cases methylene chloride extracts of plasma acidified with 1.0M sulfuric acid were selected based on optimal recovery of drugs and metabolites (Table 2) with minimum interference from plasma constituents (Figures 1 & 2).

To determine the optimum chromatographic conditions for each drug and its metabolite(s), the effects of pH and molarity of mobile phase buffers or acids on the capacity factor was studied. It can be concluded that:

1. As acidity increases the capacity factor increases and the peaks become broader. Generally a

TABLE 2

Recovery of Drugs and Metabolites

Drug or Metabolite	Recovery,% ± Standard Error
Indomethacin	66.41 ± 0.71
des(chlorobenzoyl) metabolite	100.43 ± 4.99
desmethyl metabolite	97.63 ± 5.66
Sulindac	69.04 + 1.13
Sulfide metabolite	77.61 ± 1.70
Sulfone metabolite	100.03 ± 4.14
Tolmetin	73.06 ± 2.65
Dicarboxylic acid metabolite	93.83 ± 2.88

mobile phase of pH 5 afforded adequate resolution and a reasonable assay time (Figure 3); however, a pH 4 mobile phase was required to resolve sulindac from its sulfone metabolite (Figure 4).

2. Increasing the molarity affected the capacity factor in a complex manner (Figure 5).

3. Elution order can be altered by changing pH (Figure 6) or molarity (Figure 7).

Excellent separation with sharp peaks is possible with the use of reversed-phase chromatography on an octadecylsilane column. Isocratic elution with mobile phases of methanol and acetate buffers is used for the analysis of the parent drugs indomethacin, sulindac and tolmetin (Figure 8).

Isocratic elution also has the advantage of short assay time; however, it is not possible to deter-

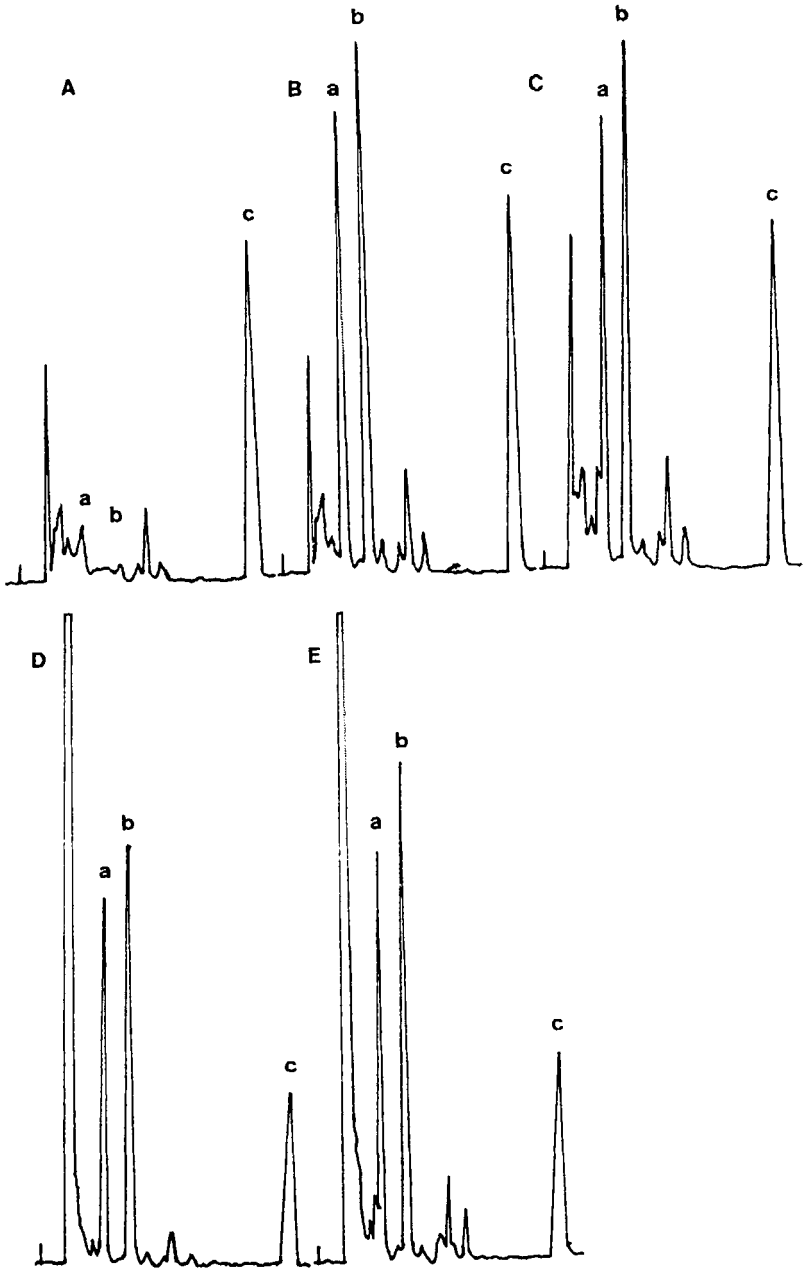


FIGURE 1 Chromatograms of plasma extracts containing tolmetin (3 µg/ml) (a), sulindac (3 µg/ml) (b) and indomethacin (3 µg/ml) (c) using the following solvents for extraction: A, Hexane; B, methylene chloride; C, chloroform containing 5% 2-propanol; D, diethylether, and E, ethyl acetate.

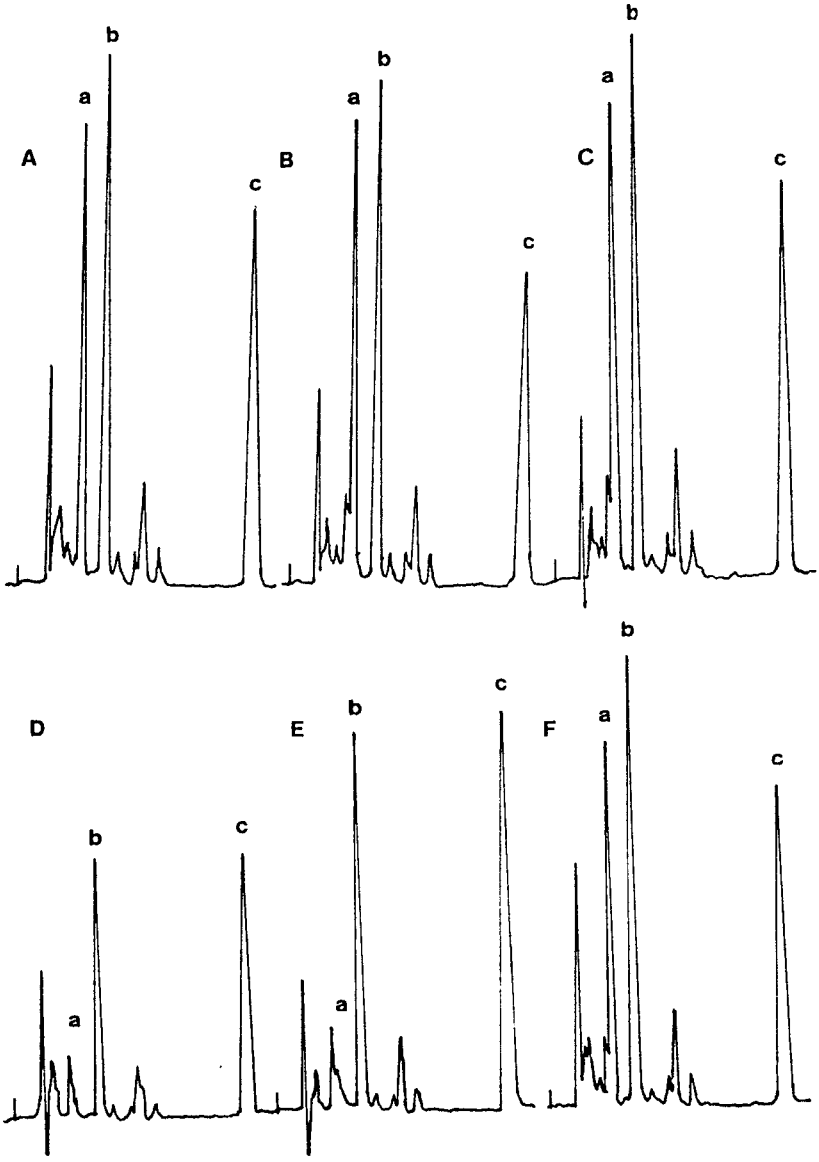


FIGURE 2 Chromatograms of plasma extracts containing tolmetin (3 µg/ml) (a), sulindac (3 µg/ml) (b) and indomethacin (3 µg/ml) (c) using methylene chloride for extraction. Plasma acidified with the following: A, 1.0M sulfuric acid: B, 1.0M phosphoric acid; C, 0.1M acetate buffer (pH4); D, 0.1M phosphate buffer (pH3); E, 0.1M phosphate buffer (pH4); and F, 0.1M citrate buffer (pH 2.5).

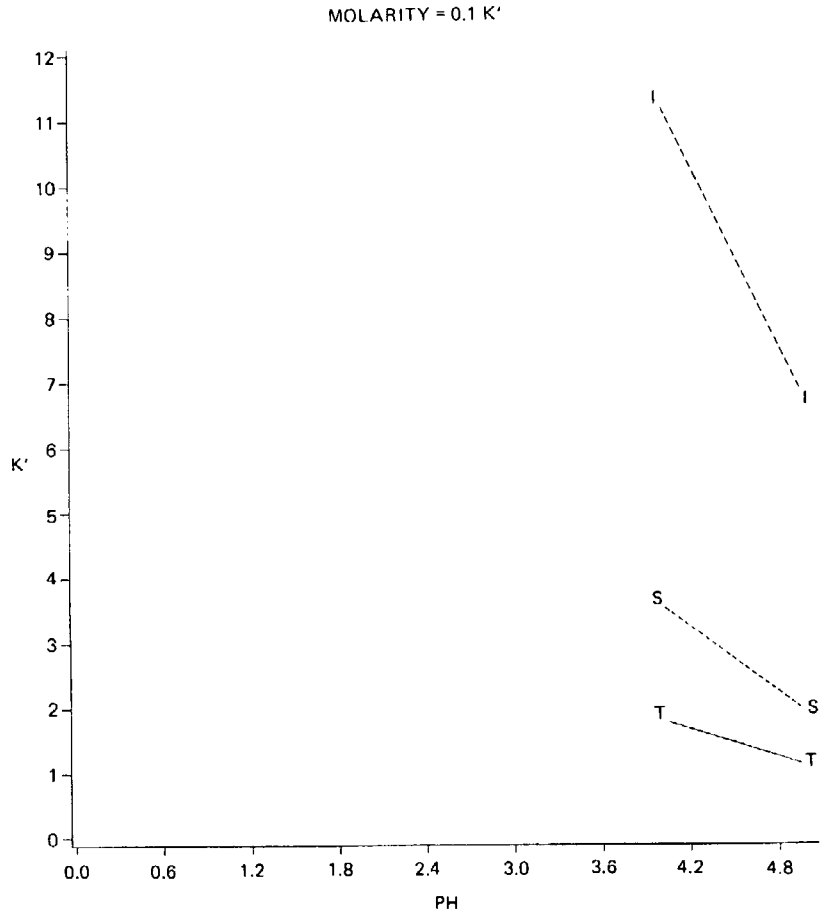
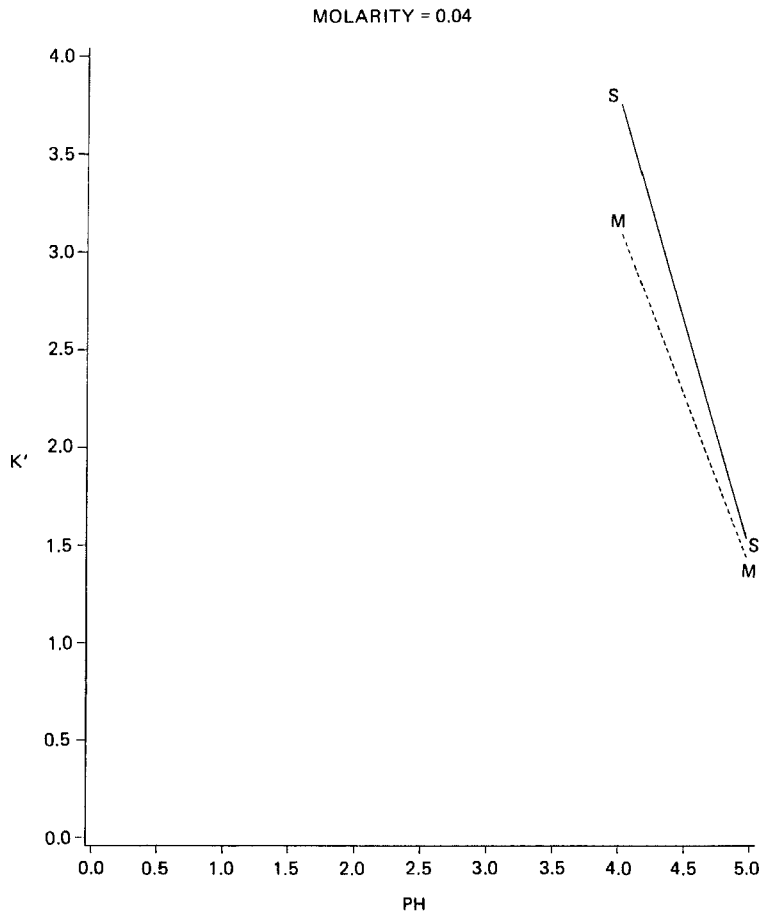
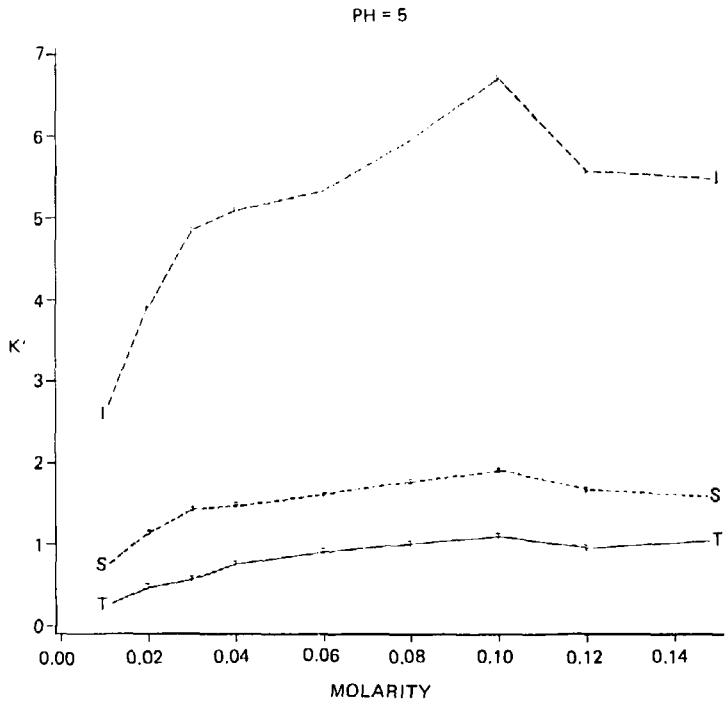


FIGURE 3 Effect of Ph on K'



KEY: S = K' FOR SULINDAC
M = K' FOR SULFONE METABOLITE

FIGURE 4 Effect of Ph on K'

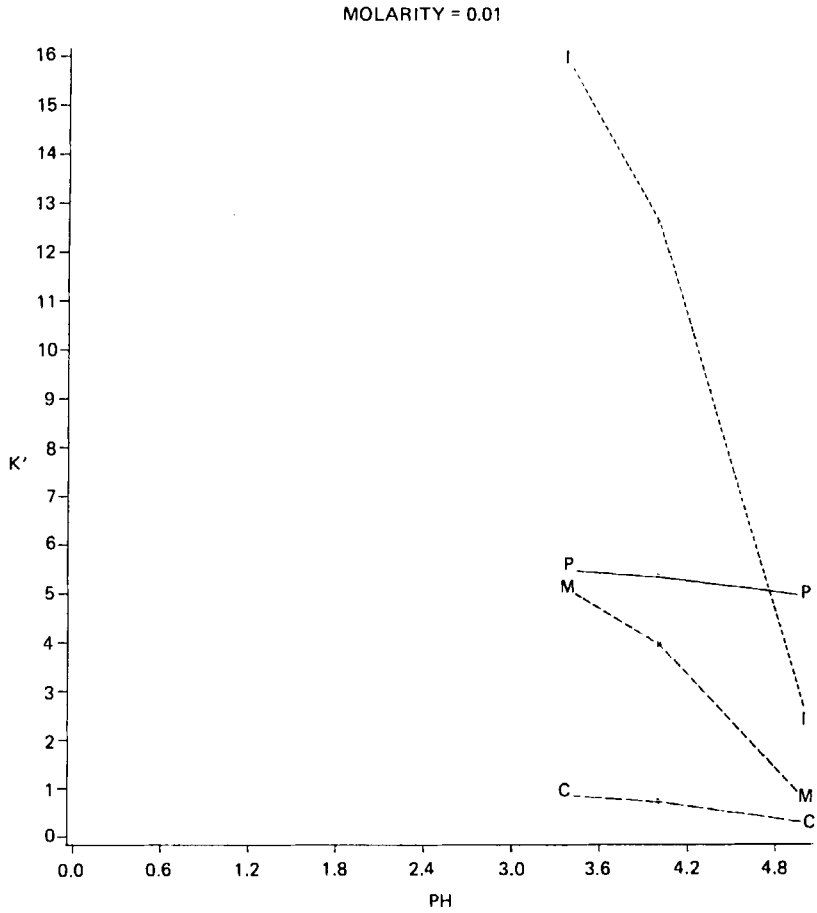


KEY: I = K' FOR INDOMETHACIN S = K' FOR SULINDAC
T = K' FOR TOLMETIN

FIGURE 5 Effect of Molarity on K'

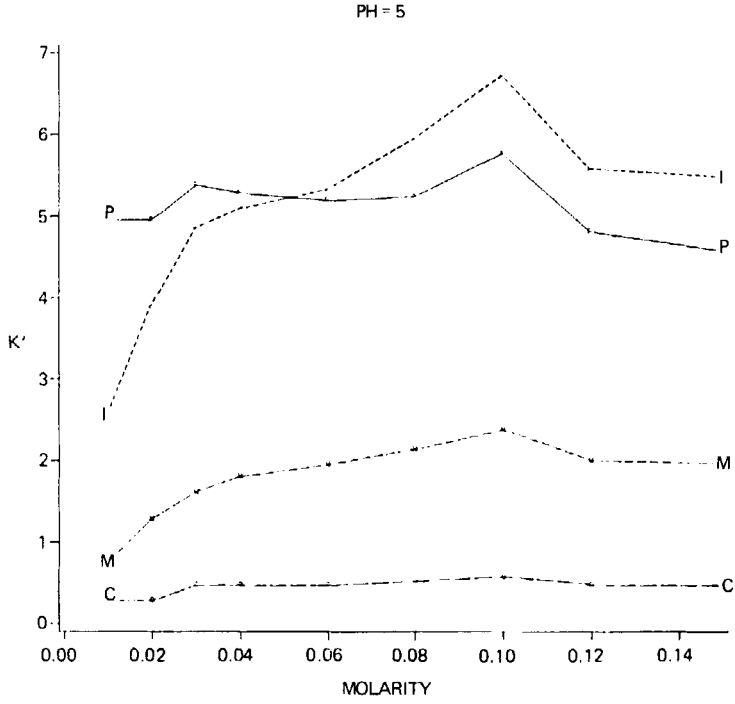
mine all the metabolites for the drugs considered here unless gradient elution is used (Figure 9).

The ratio of peak height of drug or metabolite to the peak height of the internal standard was calculated and plotted versus concentration. Statistical analysis by linear regression indicated excellent linearity (Table 3) and reproducibility (Tables 4,5).



KEY: I = K' FOR INDOMETHACIN
C = K' FOR (DESCHLOROBENZOYL) INDOMETHACIN
M = K' FOR DESMETHYLINDOMETHACIN
P = K' FOR P - PHENYLPHENOL

FIGURE 6 Effect of Ph on K'



KEY: I = K' FOR INDOMETHACIN
C = K' FOR (DESCHLOROBENZOYL) INDOMETHACIN
M = K' FOR DESMETHYLINDOMETHACIN
P = K' FOR P-PHENYLPHENOL

FIGURE 7 Effect of Molarity on K'

The retention times of the drugs, metabolites and internal standards used in this study under all the assay conditions are reported (Table 6). The possibility of interference due to other commonly used drugs was also studied (Table 7).

Patient samples were analyzed using the methods described here. A patient receiving oral tolmetin 400

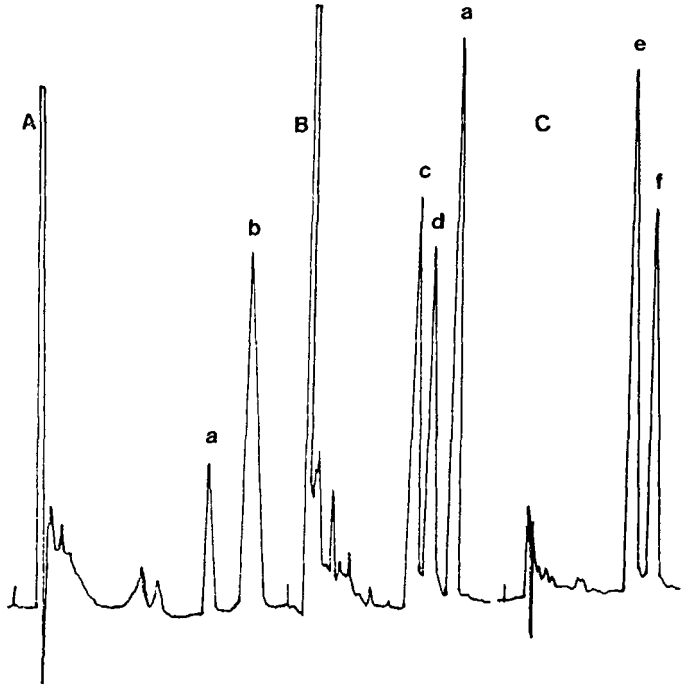


FIGURE 8 Isocratic analysis of antiarthritic drugs from plasma extracts. A, indomethacin (3 μg/ml); B, sulindac (2 μg/ml); and C, tolmetin (20 μg/ml). Key: a, p-phenylphenol (internal standard); b, indomethacin; c, sulfone metabolite; d, sulindac; e, tolmetin; and f, diphenylacetic acid (internal standard).

mg three times daily had a plasma level of 12.23 ± 1.40 μg/ml tolmetin and 7.62 ± 0.43 μg/ml dicarboxylic acid metabolite. On analyzing samples from 3 patients receiving oral indomethacin 25 mg three times daily no detectable levels of the des(chlorobenzoyl) metabolite were observed. Indomethacin was present at a level

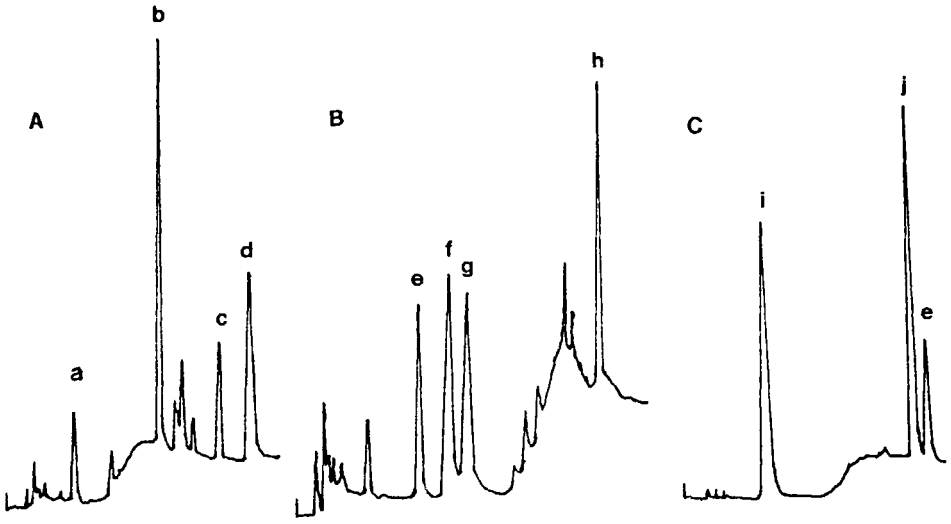


FIGURE 9 Gradient analysis of antiarthritic drugs from plasma extracts. A, indomethacin and metabolites (2 μg/ml); B, sulindac and metabolites (2 μg/ml); and C, tolmetin and metabolite (30 μg/ml). Key: a, des(chlorobenzoyl) metabolite; b, desmethyl metabolite; c, p-phenylphenol (internal standard); d, indomethacin; e, diphenylacetic acid (internal standard); f, sulfone metabolite; g, sulindac, h, sulfide metabolite; i. dicarboxylic acid metabolite; and j, tolmetin.

of 0.10-0.21 μg/ml and the desmethyl metabolite was determined to be 0.26-0.32 μg/ml. The range of plasma levels in 3 patients receiving oral sulindac 150 mg twice daily was 0.39-1.16 μg/ml sulindac, 0.35-1.57 μg/ml sulfide metabolite, an 0.41-1.28 μg/ml sulfone metabolite.

Major advantages of the proposed methods are their simplicity and rapidity. Both the parent drug

Table 3

Statistical Analysis by Linear Regression

Drug or Metabolite	Range of Standards (μg/ml plasma)	Correlation Coefficient, r	Slope	Intercept
Isocratic:				
Indomethacin	0.1-3.0	0.9957	0.7724 ± 0.0170	0.0425 ± 0.0270
Sulindac	0.1-3.0	0.9985	0.3419 ± 0.0043	-0.0170 ± 0.0067
Tolmetin	1-30	0.9985	0.0660 ± 0.0001	0.0027 ± 0.0134
Gradient:				
Indomethacin	0.5-2.0	0.9911	0.7931 ± 0.0376	-0.1278 ± 0.0522
des(chloro)	0.5-2.0	0.9956	0.3910 ± 0.0151	-0.0413 ± 0.0206
desmethyl	0.5-2.0	0.9932	1.7157 ± 0.0712	-0.2289 ± 0.0988
Sulindac	0.5-2.0	0.9945	0.5489 ± 0.0219	-0.0648 ± 0.0319
Sulfide	0.5-2.0	0.9913	0.6318 ± 0.0317	-0.1817 ± 0.0461
Sulfone	0.5-2.0	0.9793	1.8351 ± 0.1433	-0.7979 ± 0.2082
Tolmetin	5-30	0.9996	0.0911 ± 0.0010	0.0527 ± 0.0208
dicarboxylic acid	5-30	0.9985	0.0765 ± 0.0014	-0.0876 ± 0.0267

TABLE 4

Assay Precision of Isocratic Technique

	Theoretical µg/ml	Experimental[a] µg/ml	Standard Error
Indomethacin	0.1	0.092	0.039
	0.3	0.328	0.016
	0.6	0.559	0.057
	1.0	0.966	0.034
	1.5	1.487	0.068
	2.0	2.125	0.008
	3.0	2.940	0.067
Sulindac	0.1	0.146	0.005
	0.3	0.318	0.004
	0.6	0.569	0.040
	1.0	0.986	0.017
	1.5	1.460	0.038
	2.0	1.983	0.038
	3.0	3.039	0.033
Tolmetin	1	1.16	0.023
	3	3.07	0.087
	5	5.15	0.231
	10	9.93	0.191
	15	14.46	0.647
	20	20.11	0.554
	30	30.07	0.237
	35	35.53	0.309
	40	40.02	0.622

[a]Mean of 4 Determinations

and its metabolites can be determined with no interference from many commonly used drugs in a single assay using a standard single wavelength UV detector. The methods described here can be recommended for routine patient monitoring or for pharmacokinetic studies.

TABLE 5

Assay Precision of Gradient Technique

	Theoretical µg/ml	Experimental[a] µg/ml	Standard Error
Indomethacin	0.5	0.47	0.01
	1.0	1.07	0.02
	1.5	1.48	0.02
	2.0	2.00	0.09
Des(chloro) Metabolite	0.5	0.50	0.05
	1.0	1.03	0.02
	1.5	1.44	0.02
	2.0	2.03	0.05
Desmethyl Metabolite	0.5	0.51	0.02
	1.0	1.02	0.03
	1.5	1.44	0.04
	2.0	2.02	0.07
Sulindac	0.5	0.55	0.02
	1.0	0.96	0.01
	1.5	1.42	0.02
	2.0	2.05	0.02
Sulfide Metabolite	0.5	0.60	0.02
	1.0	0.98	0.00
	1.5	1.38	0.05
	2.0	2.09	0.03
Sulfone Metabolite	0.5	0.53	0.01
	1.0	0.94	0.01
	1.5	1.48	0.06
	2.0	2.06	0.00
Tolmetin	5	4.75	0.00
	10	10.08	0.01
	20	20.46	0.09
	30	29.80	0.13
Metabolite	5	5.15	0.23
	10	9.85	0.26
	20	19.91	0.17
	30	30.07	0.64

[a]Mean of 3 Determinations

TABLE 6

Retention Time of the Nonsteroidal Anti-Inflammatory Drugs, Metabolites and the Internal Standards Used in this Study.

Drug, Metabolite or Internal Standard	Retention Time in Minutes					
	Isocratic Elution of			Gradient Elution of		
	Indomethacin	Sulindac	Tolmetin	Indomethacin	Sulindac	Tolmetin
Diphenyl acetic acid*	4.7	9.6	9.7	7.95	6.97	13.93
Indomethacin	14.0	>20	>20	13.83	15.44	>15
des(chlorobenzoyl)	3.3	3.7	3.7	3.98	3.15	10.37
desmethyl	7.1	9.9	16.9	8.89	10.92	>15
p-Phenylphenol*	11.6	9.9	>20	12.43	15.02	>15
Sulindac	6.1	8.5	15.8	8.70	9.52	>15
Sulfide	>20	>20	>20	>20	17.42	>15
Sulfone	5.8	7.6	14.8	8.38	8.47	>15
Tolmetin	4.4	6.0	7.4	7.46	5.90	13.11
Dicarboxylic Acid	S	S	S	S	S	4.65

*Internal Standard

S = Solvent Front

TABLE 7

Drugs Studied for Possible Interference Under the Assay Conditions

	Retention Time in Minutes					
	Isocratic Elution of			Gradient Elution of		
Drug	Indomethacin	Sulindac	Tolmetin	Indomethacin	Sulindac	Tolmetin
Acetaminophen	S	S	S	S	S	4.09
Caffeine	3.4	3.8	4.5	5.11	4.02	10.96
Carbamazepine	7.2	11.1	16.5	9.95	12.51	18.20
Ethosuximide	S	2.8	3.0	3.35	2.69	9.00
Fenoprofen	10.1	15	20	11.82	14.79	> 15
Ibuprofen	>20	>15	> 20	> 20	16.20	> 15
Naproxen	6.6	13.2	16.2	9.45	11.37	> 15
Metabolite	3.2	4.6	4.4	5.96	3.42	10.80
Phenobarbital	3.2	4.0	5.1	5.93	4.24	11.38
Phenytoin	4.6	6.8	9.8	8.08	7.31	14.40
Primidone	2.0	3.5	4.3	4.57	3.49	10.44
Quinidine	S	S	S	2.15	2.02	4.12
Salicylic Acid	S	S	S	S	S	4.06
Theophylline	S	2.6	2.9	2.91	2.54	8.21
Valproic Acid	NA	NA	NA	NA	NA	NA

S = Solvent Front

NA = No Absorbance

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