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Separation of non-steroidal anti-inflammatory agents using supercritical fluid chromatography

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ABSTRACT

Supercritical fluid chromatography (SFC) was investigated for the separation of non-steroidal anti-inflammatory agents (NSAIs). Three different stationary phases (SB-methyl-100, SB-biphenyl-30, and SB-cyanopropyl-50) were compared for the separation of the compounds. Baseline separation of a flufenamic acid, mefenamic acid, fenbufen and indomethacin mixture was achieved on the SB-biphenyl-30 column using a pressure gradient. A mixture containing flufenamic acid, mefenamic acid, acetylsalicylic acid, ketoprofen and fenbufen and another mixture containing ibuoprofen, fenoprofen, naproxen, ketoprofen and tolmetin were well separated on the SB-cyanopropyl-50 column using pressure gradients. Typical analysis time for a mixture of NSAIs on the biphenyl or cyanopropyl column was approximately 20–25 min. Application of the method using the biphenyl column to the determination of NSAIs present in selected commercial dosage forms was demonstrated.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a complementary technique to high-performance liquid chromatography (HPLC) and gas chromatography (GC). The advantages of SFC include the possibility of analysis of thermally labile compounds and the use of both HPLC and GC type detectors including the UV–Vis and flame ionization detectors. Other advantages of SFC are that a supercritical fluid possesses solvating properties similar to those of a liquid, and the solute diffusion coefficients are more than two orders of magnitude greater than those found in liquids [1]. Commercial SFC instruments are available that can utilize both open-tubular capillary and packed columns. Capillary SFC offers the advantage of GC-type efficiency yielding a high number of theoretical plates.

There have been reports of the use of SFC in pharmaceuticals [2–6]. Wong and Dellafera [2] demonstrated the use of capillary SFC in therapeutic drug monitoring of phenobarbital in serum using a polymethylsiloxane stationary phase and a carbon dioxide mobile phase. Later *et al.* [3] have reported the analysis of steroids, antibiotics and cannabinoids on polymethylsiloxane capillary columns using carbon dioxide mobile phase. Crowther and Henion [4] demonstrated the SFC–mass spectrometric analysis of codeine, caffeine, cocaine, phenylbutazone and methocarbamol by using packed amino and silica columns and a modified direct liquid-introduction interface. The mobile phase was carbon dioxide modified with methanol. Smith and Sangi [5] have reported the SFC analysis of barbiturates using polystyrene–divinylbenzene or octadecylsilane stationary phases with methanol-modified carbon dioxide as mobile phase. Perkins *et al.* [6] have reported the analysis of veterinary antibiotics (levamisol, furazolidone, chloramphenicol and lincomycin) on an amino-bonded stationary phase also utilizing carbon dioxide with methanol modifier.

In this paper, the separation of non-steroidal anti-inflammatory agents (NSAIs) using capillary SFC was explored. NSAI analgesics were chosen as

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model compounds due to their wide use and applicability. These studies were concerned with separation of NSAI mixtures using three different stationary phases (SB-methyl-100, SB-biphenyl-30 and SB-cyanopropyl-50) with carbon dioxide as mobile phase. The SFC method using the biphenyl column was then applied to the determination of NSAIs in selected dosage forms.

EXPERIMENTAL

Reagents and chemicals

HPLC-grade absolute methanol was purchased from J. T. Baker (Phillipsburg, NJ, USA). Puradisc 25TF, 0.45-μm filters were obtained from Whatman (Maidstone, UK). Supercritical fluid chromatography grade carbon dioxide was obtained from Scott Specialty Gases (Plumsteadville, PA, USA).

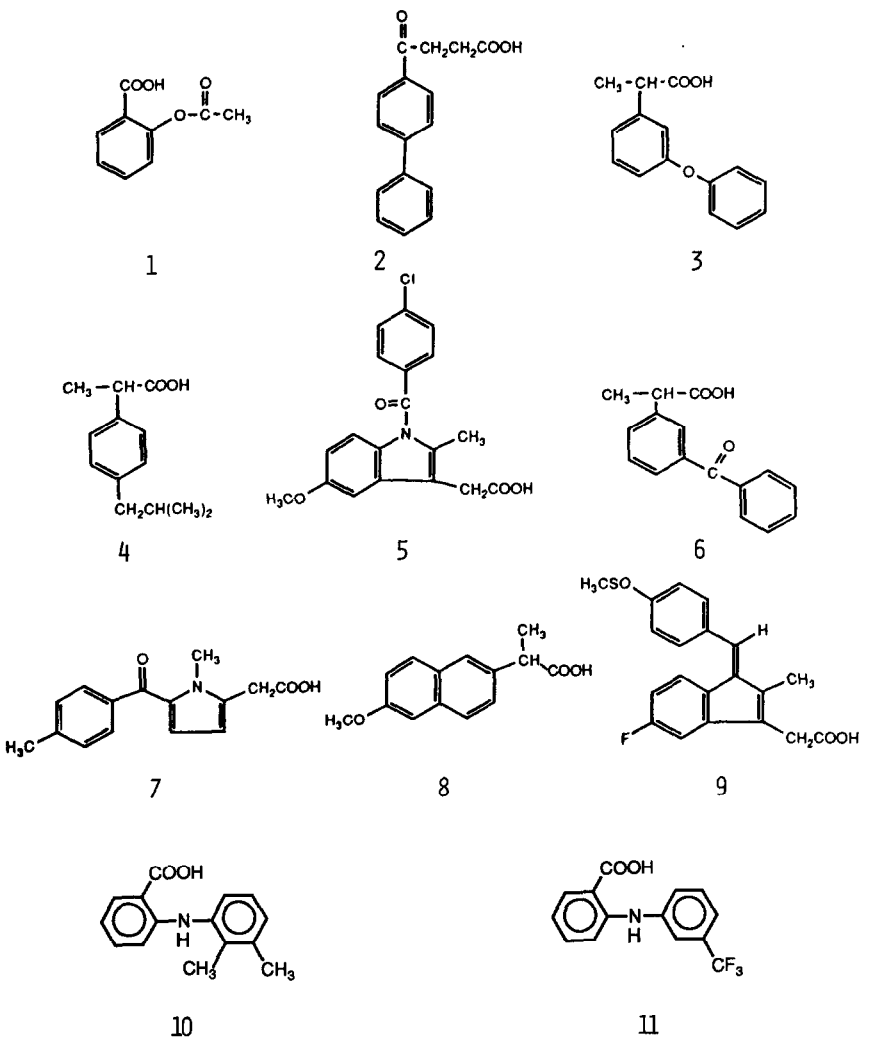

Fig. 1. Structural formulae of NSAI compounds studied. 1 = Acetylsalicylic acid; 2 = fenbufen; 3 = fenoprofen; 4 = ibuprofen; 5 = indomethacin; 6 = ketoprofen; 7 = tolmetin; 8 = naproxen; 9 = sulindac; 10 = mefenamic acid; 11 = flufenamic acid.

The structural formulae of the compounds studied are shown in Fig. 1. Fenoprofen calcium and naproxen sodium were purchased from the United States Pharmacopeial Convention (Rockville, MD, USA). Acetylsalicylic acid was obtained from Aldrich (Milwaukee, WI, USA). Fenbufen, ibuprofen, indomethacin, ketoprofen, sulindac, flufenamic acid and mefenamic acid were purchased from Sigma (St. Louis, MO, USA). Commercial tablet and capsule dosage forms of the various NSAI analgesics were obtained from a local pharmacy.

Instrumentation

Chromatography was performed on a Lee Scientific Model 600D supercritical fluid chromatograph (Salt Lake City, UT, USA) equipped with a pump, oven and flame-ionization detector and controlled by a Dell computer (ACI 600D, software version 2.2). SFC was performed on three different stationary phases: a 5 m × 100 μm I.D. SB-methyl-100 (200 μm O.D. and 0.25 μm film thickness), 10 m x 50 μm I.D. SB-biphenyl-30 and a SB-cyanopropyl-50 (both 195 μm O.D. and 0.25 μm film thickness). All three columns were purchased from Lee Scientific.

Preparation of drug solutions

Solutions of each NSAI drug were prepared by accurately weighing 5 mg of each drug and dissolving in 5 ml of absolute methanol to give a final concentration of approximately 1 mg/ml.

Chromatographic parameters

Pump program. Multilinear pressure program: 7 min hold at an initial pressure of 100 atm, then 25 atm/min ramp to 250 atm, followed by a 4.0 atm/min ramp to 290 atm.

Oven program. Isothermal at 130°C.

Columns. 5 m × 100 μm SB-methyl-100, 10 m × 50 μm SB-biphenyl-30 and 10 × 50 μm SB-cyanopropyl-50.

Injection type. Time split set at 200 ms. Injection ratio approximately 20:1, giving an injection volume of approximately 25 nl.

Detector. Flame ionization at 375°C.

Mobile phase. Supercritical fluid chromatography grade carbon dioxide. Analysis time: 20–25 min.

RESULTS AND DISCUSSION

The goal of this study was to investigate the separation of NSAIs using capillary SFC. These widely used drugs exhibit variation in structure and functional group chemistry to provide a representative sample of acidic compounds of pharmaceutical interest. There have been no reports in the scientific literature describing the use of SFC in the analysis of these compounds.

Three different capillary columns, SB-methyl-100, SB-biphenyl-30 and SB-cyanopropyl-50, were compared for the separation of these drugs. The SB-methyl-100 column is coated with 100% methylpolysiloxane and is cross-linked for SFC use. Several different temperature and pressure gradients were investigated. The NSAIs could not be chromatographed on this column due to poor peak shape and overlapping of peaks.

The SB-biphenyl-30 column is coated with 30% biphenyl and 70% methylpolysiloxane and is also cross-linked for SFC use. After investigating several pressure gradients (25 atm/min to 3 atm/min), the gradient described above in the Experimental section was found to give the best separation of NSAIs. The SFC oven temperature was investigated in the range 80–140°C in the isothermal mode.

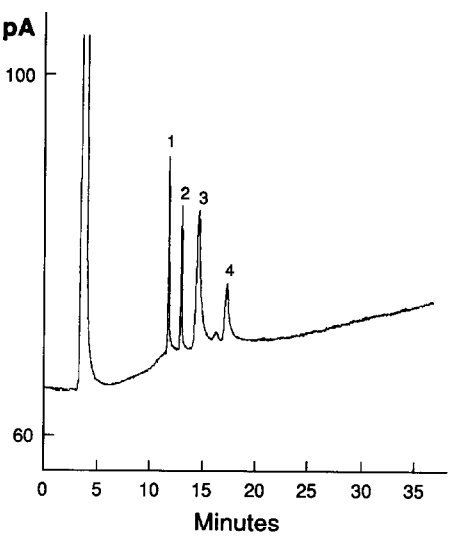

Fig. 2. Typical SFC separation of flufenamic acid (1), mefenamic acid (2), fenbufen (3) and indomethacin (4) on a SB-biphenyl-30 column.

TABLE I

ANALYTICAL FIGURES OF MERIT WITH SB-BIPHENYL-30 COLUMN

Compound	Retention time (min)	Tailing factor[a]	LOD (μg/ml)[b]	Amount injected on column (ng)
Ibuprofen	11.8	1.05	100	2.5
Flufenamic acid	11.9	1.00	75	1.8
Fenoprofen	12.4	1.25	100	2.5
Mefenamic acid	13.1	1.06	75	1.8
Naproxen	13.5	1.43	100	2.5
Tolmetin	14.6	1.40	150	3.8
Fenbufen	14.7	1.50	100	2.5
Ketoprofen	15.0	1.13	100	2.5
Indomethacin	17.5	1.50	100	2.5
Sulindac	21.5	0.63	250	6.3

[a] Calculated according to the USP XXII method (ref. 7).
[b] Signal-to-noise ratio 3.

The separation of the compounds on the biphenyl column was adequate, but the compounds were not all separated in a single injection. However, certain groups of NSAIs can be efficiently separated. Fig. 2 shows the SFC separation of a flufenamic acid, mefenamic acid, fenbufen and indomethacin mixture on the biphenyl column. The analytical figures of merit for each drug on the biphenyl column are shown in Table I. Tailing factors were generally in the 1.0–1.5 range except for sulindac, which exhibited some frontal tailing. The limits of detection (LOD) based on a signal-to-noise ratio of 3 were in the range of 75–250 μg/ml corresponding to approximately 1.8–6.3 ng of analyte injected on col-

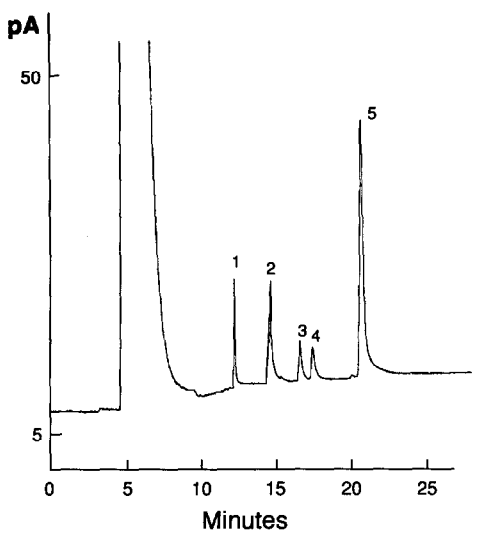

Fig. 3. Typical SFC separation of ibuprofen (1), fenoprofen (2), naproxen (3), ketoprofen (4) and tolmetin (5) on a SB-cyanopropyl-50 column.

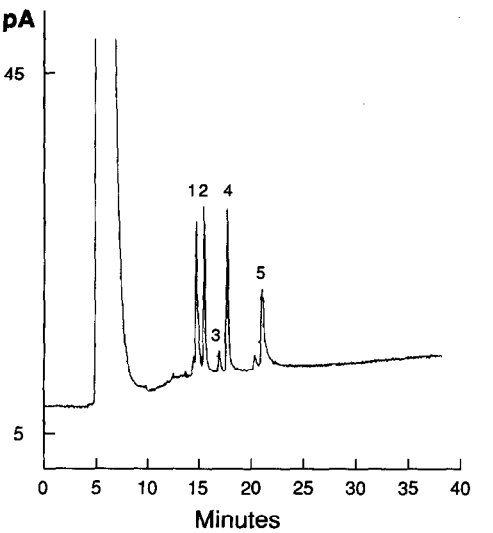

Fig. 4. Typical SFC separation of flufenamic acid (1), mefenamic acid (2), acetylsalicylic acid (3), ketoprofen (4) and fenbufen (5) on a SB-cyanopropyl-50 column.

TABLE II

ANALYTICAL FIGURES OF MERIT WITH SB-CYANOPROPYL-50 COLUMN

Compound	Retention time (min)	Tailing factor[a]	LOD (μm/ml)[b]	Amount injected on column (ng)
Ibuprofen	12.3	1.25	100	2.5
Sulindac	13.9	1.50	250	6.3
Flufenamic acid	14.5	1.00	80	2.0
Fenoprofen	14.7	1.38	100	2.5
Mefenamic acid	15.2	1.05	80	2.0
Naproxen	16.7	1.31	200	5.0
Acetylsalicylic acid	16.7	1.13	400	10.0
Ketoprofen	17.5	1.33	80	2.0
Fenbufen	20.8	1.50	150	3.8
Tolmetin	20.8	1.76	150	3.8

[a] Calculated according to the USP XXII method (ref. 7).
[b] Signal-to-noise ratio 3.

umn. One of the disadvantages of SFC instrumentation at the present time is its inability to allow for larger sample volumes to be injected on column and hence, improve the sensitivity of the method.

The SB-cyanopropyl-50 column is coated with 50% cyanopropyl and 50% methylpolysiloxane. It is considered the most polar column among the commercially available SFC columns. The same chromatographic parameters that were applied to the SB-biphenyl-30 column above were also used for comparison purposes. Retention increased for all of the analytes compared to that obtained on the

TABLE III

COMPARISON OF RELATIVE RETENTION BEHAVIOR OF SELECTED NSAIs ON OCTADECYLSILANE AND UNDERIVATIZED SILICA HPLC COLUMNS *VERSUS* SB-BIPHENYL AND SB-CYANOPROPYL SFC COLUMNS

HPLC Columns				SFC Columns			
Octadecylsilane[a]		Underivatized Silica[b]		SB-biphenyl[c]		SB-cyanopropyl[c]	
Compound	k'	Compound	k'	Compound	k'	Compound	k'
Tolmetin	3.77	Sulindac	0.7	Ibuprofen	1.4	Ibuprofen	0.8
Sulindac	5.02	Fenoprofen	1.1	Fenoprofen	1.5	Sulindac	1.0
Ketoprofen	5.98	Ibuprofen	1.1	Naproxen	1.7	Fenoprofen	1.1
Naproxen	6.59	Naproxen	1.3	Tolmetin	1.9	Naproxen	1.4
Fenbufen	7.46	Tolmetin	1.4	Fenbufen	2.0	Aspirin	1.4
Fenoprofen	8.54	Ketoprofen	1.5	Ketoprofen	2.0	Ketoprofen	1.5
Indomethacin	8.86	Fenbufen	2.6	Indomethacin	2.6	Fenbufen	2.0
Ibuprofen	10.01	Indomethacin	3.6	Sulindac	3.3	Tolmetin	2.0

[a] Solvent programming using acetonitrile–0.05 *M* acetate buffer, pH 4.5; column temperature 35°C; flow-rate 0.8 ml/min; detector set at 254 nm (ref. 8).
[b] 5 m*M* Sodium phosphate buffer, pH 2.6–acetonitrile (95.5, v/v); 1 ml/min; and detector set at 254 nm (ref. 9).
[c] See chromatographic parameters, Experimental section.

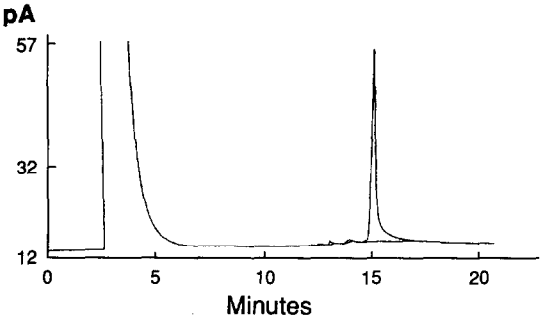

Fig. 5. Typical SFC chromatogram of ketoprofen in a dosage form on a SB-biphenyl-30 column.

SB-biphenyl-30 column. Figs. 3 and 4 show a comparison of chromatographic separations of two groups of NSAIs on the SB-cyanopropyl-50 column. The analytical figures of merit for each drug on the cyanopropyl column are shown in Table II. Tailing factors were generally in the 1.0–1.76 range. Limits of detection based on signal-to-noise ratio of 3 were in the range of 80–400 μg/ml corresponding to 2.0–10.0 ng of analyte injected on column.

A comparison of the relative retention behavior of selected NSAIs using two HPLC stationary phases *versus* our SFC stationary phases is shown in Table III. Capacity factors (k') are being used here only for comparison purposes since the SFC separations all involved pressure gradients and one of the HPLC separations was obtained under solvent programming conditions. However, the SFC capacity factors can be utilized as system suitability parameters for day-to-day SFC operations.

Since our studies indicated that both the biphenyl and cyanopropyl columns were suitable for the separation of NSAIs, the biphenyl column was arbitrarily chosen to demonstrate the applicability of the SFC method to selected pharmaceutical dosage forms. Linear calibration curves in the range 0.5–4 mg/ml were obtained for each of the three NSAIs chosen for study. The correlation coefficients calculated were better than 0.99 ($n=4$). A typical chromatogram obtained for a ketoprofen dosage form is shown in Fig. 5. The results obtained in Table IV suggest that the proposed SFC method can be a useful procedure for the routine determination of

TABLE IV

AMOUNTS OF NSAIs IN PHARMACEUTICALS

The contents of a NSAI tablet or capsule were dissolved in absolute methanol with the aid of sonication, filtered if necessary, and injected into the SFC chromatograph. R.S.D. = Relative standard deviation.

Compound	Labeled strength (mg)	Amount found ± S.D. (mg) ($n=3$)
Ibuprofen[a]	800	760.5 ± 38.70 (R.S.D. 5.1%)
Ketoprofen[b]	75	77.1 ± 3.45 (R.S.D. 4.5%)
Mefenamic acid[c]	250	252.4 ± 8.37 (R.S.D. 3.3%)

[a] IBU, Boots, Lot No. B6132.
[b] Orudis, Wyeth Lab., Lot No. 9880476.
[c] Ponstel, Parke-Davis, Lot No. 06449FA.

NSAIs in pharmaceuticals. No claim is made that the assay is stability-indicating since the separation of by- and degradation products of each drug was not investigated.

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