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Determination of non-steroidal anti-inflammatory analgesics in solid dosage forms by high-performance liquid chromatography on underivatized silica with aqueous mobile phase

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SUMMARY

A high-performance liquid chromatography procedure for the determination of selected non-steroidal anti-inflammatory analgesics (acetylsalicylic acid, fenbufen, fenoprofen, ibuprofen, indomethacin, ketoprofen, naproxen, sulindac and tolmetin) from pharmaceutical dosage forms has been developed. The individual analytes are extracted from the dosage forms with 0 to 10% aqueous acetonitrile and chromatographed on a 22-cm underivatized silica column at ambient temperature (23 ± 1°C). The mobile phases consisted of 5 m*M* aqueous sodium phosphate–phosphoric acid buffer, pH 2.6 containing 0 to 10% acetonitrile. Accuracy and precision of the method were shown to be excellent. This study was performed to extend the applicability of underivatized silica stationary support with aqueous eluents to the analysis of acidic compounds.

INTRODUCTION

Reversed-phase high performance liquid chromatography (RP-HPLC) on bonded stationary phases has developed into a major analytical tool for separation and quantitation of analytes. Recently, reports have appeared in the scientific literature describing the separation of basic[1–8] and neutral[9] compounds on underivatized silica using typical reversed-phase mobile phases. These systems showed considerable improvement in peak shape, plate numbers and efficiency as compared to conventional bonded phase chromatography. The predominant retention mechanism for basic compounds was determined to be cation exchange with the silica surface[2–8]. Hydrogen bonding or other non-specific forces were cited for the retention of neutral compounds on silica in the reversed-phase mode[9]. When used with aqueous buffered eluents, the silica surface is deactivated by several layers of adsorbed water over a layer of strongly hydrogen bonded water[10–12]. This makes the silica surface capable of interacting with neutral and possibly acidic analytes.

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This study was designed to explore the applicability of underivatized silica to the analysis of acidic drugs from pharmaceutical dosage forms. Non-steroidal anti-inflammatory analgesics were chosen as test compounds due to their wide use and availability. Chromatographic procedures suitable for dosage form assays of these pharmaceutical preparations were developed using underivatized silica with aqueous buffered acetonitrile eluents.

EXPERIMENTAL

Reagents and chemicals

The structural formulae of the compounds studied are shown in Fig. 1. Fenoprofen calcium and naproxen sodium were purchased from United States Pharmacopeial Convention (Rockville, MD, U.S.A.). Acetylsalicylic acid and salicylic acid were obtained from Aldrich Chemical Company (Milwaukee, WI, U.S.A.). Fenbufen, fenoprofen, ibuprofen, indomethacin, ketoprofen, sulindac and tolmetin were purchased from Sigma (St. Louis, MO, U.S.A.). Commercial tablet and capsule dosage forms of the various non-steroidal anti-inflammatory analgesics were obained at a local pharmacy.

Acetonitrile, methanol and water were HPLC grade (J.T. Baker, Phillipsburg, NJ, U.S.A.). Monobasic and dibasic sodium phosphate and concentrated phosphoric acid were Baker analyzed reagents.

Instrumentation

Chromatography was performed on an HPLC system consisting of two Varian Model 2510 HPLC pumps (Walnut Creek, CA, U.S.A.) connected to a Varian Model 2584 static mixer, a Rheodyne Model 7125 injector equipped with a 10-μl loop (Cotati, CA, U.S.A.) and a Varian Model 2550 variable-wavelength UV detector. The analytical wavelength was set to the absorbance maximum of each particular analyte. Data acquisition and reduction were performed on a Spectra-Physics Model SP4290 recording integrator (San Jose, CA, U.S.A.).

Separation was accomplished on a 5-μm silica column (220 mm × 4.6 mm I.D., Brownlee Labs., Santa Clara, CA, U.S.A.) fitted with a 7-μm silica precolumn (15 mm × 4.6 mm I.D., Brownlee). A second precolumn was placed between the static mixer and the injector to saturate the mobile phase with silica. The column was maintained at ambient temperature (23 ± 1°C).

UV spectra were obtained using a Beckman Model DU-7 scanning spectrophotometer (Fullerton, Ca, U.S.A.).

Mobile phases

Mobile phase buffers of various pH values and molarities were prepared using the Henderson-Hasselbach equation. The actual pH of each mobile phase was measured carefully for subsequent calculation of ionic strength. All mobile phases were filtered through a 0.45-μm nylon-66 filter (MSI, Westborough, MA, U.S.A.) and degassed by sonication. The flow-rate was set at 1–1.1 ml/min.

Preparation of standard solutions

Standard solutions of each drug were prepared by dissolving 0, 2, 4 and 6 mg of

the drug in 100 ml of aq. acetonitrile containing the same concentration of acetonitrile as the appropriate mobile phase. A four-point standard curve was constructed for each analyte.

Analysis of dosage forms

Capsule contents and tablets were weighed and finely ground. An accurately weight portion, equivalent to 4 mg drug substance was transferred to a 100-ml volumetric flask. Approximately 80 ml of aqueous acetonitrile containing the same concentration of acetonitrile as the mobile phase was added and the flask was placed in an ultrasonic bath for 5 min. The mixture was allowed to cool, diluted to volume and mixed in a mechanical shaker for 2–3 min. Any remaining solids in the mixture were allowed to settle. An aliquot of the solution was drawn up and filtered through a 0.2-μm nylon-66 syringe filter (Lida Manufacturing, Bensenville, IL, U.S.A.) prior to injection into the HPLC system. Quantitation was based on linear regression of peak heights.

RESULTS AND DISCUSSION

The goal of this study was to demonstrate the applicability of an underivatized silica stationary support and aqueous buffered eluents to the analysis of drug substances containing the carboxylic acid moiety. Non-steroidal anti-inflammatory analgesics were chosen as test compounds (Fig. 1). Not only are these drugs widely available, but they also exhibit enough variation in structure and functional group chemistry to provide a representative sample of acidic compounds of pharmaceutical interest.

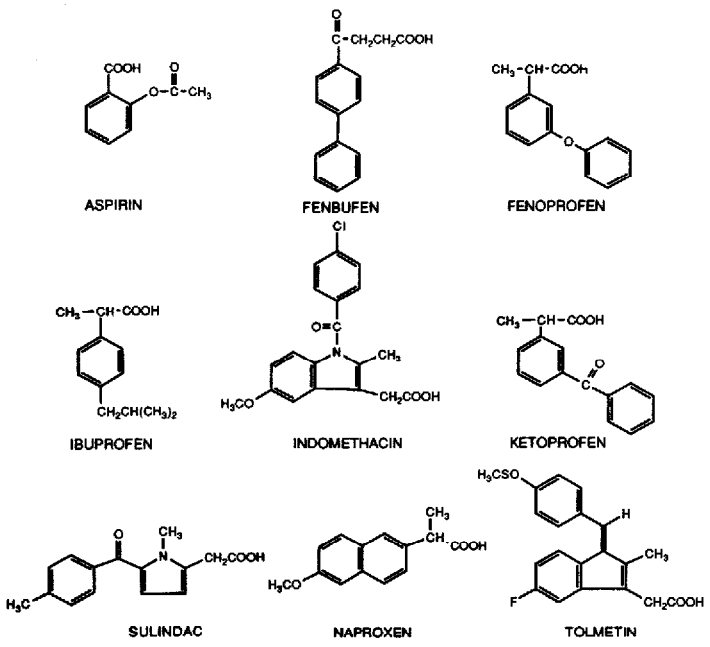


Fig. 1. Structures of non-steroidal anti-inflammatory analgesics.

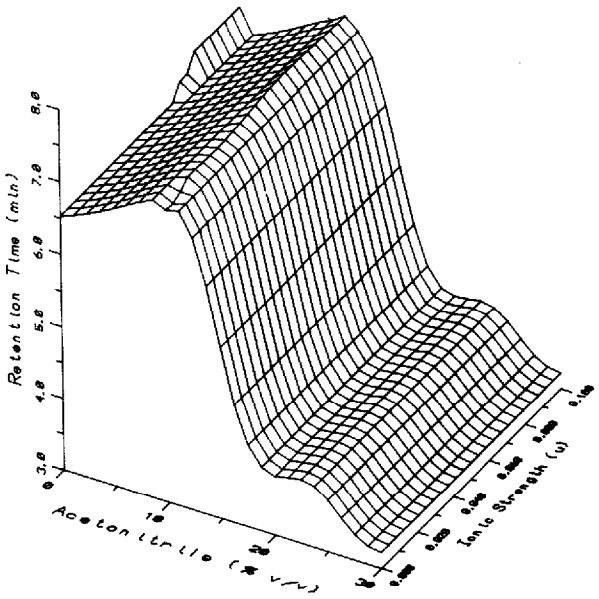


Fig. 2. Response surface of fenbufen. Response surface was generated from retention data, ionic strength and organic modifier concentration of the mobile phase. Mobile phase: sodium phosphate–phosphoric acid buffer, pH 2.5; flow-rate: 1 ml/min.

There are no reports in the scientific literature describing the reversed-phase separation of acidic compounds on silica. Therefore, several mobile phases differing in ionic strength, concentration and type of organic modifier and pH were prepared and tested for the chromatography of these non-steroidal anti-inflammatory analgesics.

It has been reported that not only the ionic strength but also the type of buffer and competing cation influences the retention of basic analytes on underivatized silica[13]. Therefore sodium phosphate buffers were chosen to prepare the mobile phases since phosphate buffer covers a wide pH range. The buffer pH of each mobile phase was not adjusted since the actual amounts of sodium phosphate and phosphoric acid were calculated using the Henderson–Hasselbach equation. The final pH of each mobile phase was then accurately measured and the ionic strength of the buffer was calculated. The pH varied from 2.5 to 7.5 with calculated ionic strengths of 0 to 0.1 U and 0 to 40% organic modifier.

Ionic strength of the mobile phase had a minor effect on retention as shown for fenbufen in Fig. 2. Increases in buffer concentration of the mobile phase only increased retention slightly. Increasing the ionic strength of the mobile phase shifted the equilibrium of the carboxylic acid to the unionized species. In this form, the analyte is able to interact more effectively with the stationary phase. To avoid excessive pump seal wear, the buffer strength was held to 5 m*M*.

As observed previously in this laboratory on the chromatography of neutral compounds[9], the concentration of organic modifier in the mobile phase was the

TABLE I

EFFECT OF ACETONITRILE CONCENTRATION AND MOBILE PHASE pH ON RETENTION OF SELECTED ANALGESICS ON UNDERIVATIZED SILICA

Compound	*Acetonitrile (%)*[a]			*pH*[b]		
	10	*20*	*30*	*2.5*	*5.1*	*7.5*
Tolmetin	6.3[c]	3.7	3.2	–[d]	–	–
Sulindac	–[d]	–	–	15.2[c]	12.1	4.5

[a] Mobile phase: 5 m*M* sodium phosphate/phosphoric acid pH 2.5–acetonitrile; flow-rate was 1 ml/min with detector set at 254 nm.
[b] Mobile phase: 20 m*M* sodium phosphate/phosphoric acid–acetonitrile, (90:10, v/v) flow-rate was 1 ml/min with detector set at 254 nm.
[c] Retention time in minutes.
[d] Not applicable.

predominant parameter affecting retention (*i.e.* fenbufen in Fig. 2, tolmetin in Table I). Furthermore, increases in the organic modifier beyond 30% were shown to move the analyte peak into the solvent front. This identical retention behavior was also observed with all the other analgesic compunds. The type of organic modifier in the mobile phase also affected retention. Substituting an equal concentration of methanol for acetonitrile caused an approximate doubling in retention and deterioration in peak shape. Peak width increased and tailing was evident on late eluting peaks (capacity factor, $k' > 5$). Similar chromatographic behavior was observed in our earlier study of neutral compounds on underivatized silica with reversed-phase eluents. As explained by Scott *et al.*[10–12], the deactivated silica stationary phase has one layer of water strongly hydrogen bonded to the surface silanol groups, 2 to 3 layers of water are more loosely held over the strongly held primary water layer. Being able to act both as a proton donor and acceptor, methanol can interfere with the formation of the secondary and tertiary water layer, replacing water molecules and, hence, altering the stationary phase. These changes result in a more lipophilic stationary phase that will alter the partitioning of the unionized acidic analytes into the stationary phase and behave analogous to non-polar bonded phase chromatography.

The pK_a values of these analgesic drugs range between 3.5 and 4.6. At a mobile phase pH greater than 5, the silanols are ionized and the analytes would be expected to pass unretained through the column due to charge–charge repulsion. As was shown for sulindac in Table I, it was surprising to observe retention of the drug on the silica column at a mobile phase pH of 7.5 where the analyte is totally ionized. Even though retention of all of the analgesics was shown to decrease considerably with increasing pH, there was enough interaction between each analyte and silica to allow retention.

It has been our experience that the silica column has shorter equilibration times, less prominent solvent fronts and is much more stable when operated at a low pH[8,9]. Since low pH was more applicable to these separations, the mobile phase pH was held at 2.5 for the analysis of the dosage forms.

Traditionally, silica has been used in conjunction with less polar mobile phases in normal phase chromatography. The current use of normal phase chromatography

TABLE II

COMPARISON OF RELATIVE RETENTION BEHAVIOR OF SELECTED ANALGESICS ON OCTADECYLSILANE *versus* UNDERIVATIZED SILICA

Octadecylsilane[a]		*Underivatized silica*[b]	
Compound	*k'*	*Compound*	*k'*
Tomletin	3.77	Sulindac	0.7
Sulindac	5.02	Fenoprofen	1.1
Ketoprofen	5.98	Ibuprofen	1.1
Naproxen	6.59	Naproxen	1.3
Fenbufen	7.46	Tolmetin	1.4
Fenoprofen	8.54	Ketoprofen	1.5
Indomethacin	8.86	Fenbufen	2.6
Ibuprofen	10.01	Indomethacin	3.6

[a] Values derived from solvent programming using acetonitrile–0.05 *M* acetate buffer pH 4.5 at a column temperature of 35°C; flow-rate of 0.8 ml/min and detector set at 254 nm (see ref. 17).
[b] See Table IV for chromatographic conditions.

in drug analysis is rather limited when compared to reversed-phase chromatography on bonded phases. The latter technique is employed for the vast majority of all analytical separations of drugs[14,15]. Factors governing analyte retention in an underivatized silica system are different from those in bonded phase chromatography. For the separation of basic compounds such as amines and/or quaternary ammonium ions, cation exchange has been identified as the predominant retention mechanism[1–8]. Neutral compounds have also been separated on underivatized silica with an acetonitrile–sodium phosphate buffer eluent. In this instance, the retention mechanism is most likely hydrogen bonding or other non-specific analyte–silica interactions[9]. In both cases, peak shape of the various analytes was equal or improved compared to conventional reversed-phase chromatography. In addition, exceedingly simple mobile phases were used consisting of small amounts of organic modifier in buffer. Columns are very stable and exhibit high efficiencies (up to 70 000 plates/m)[5].

Whereas the predominant retention of basic compounds on underivatized silica is cited to be cation exchange, a different mechanism must be proposed to explain the retention of these acidic analytes. Based on our results, there is most likely a mixed retention mechanism of hydrogen bonding and quasi reversed-phase retention[9,16].

A marginal disadvantage of this underivatized silica system lies in the limited amount of analyte that can be injected at one time. Once the analyte size exceeds 0.5 to 1 μg on column, the tailing factor increases slightly (*ca.* 0.05 to 0.15) and a decrease in retention time is observed (*ca.* 0.2 min). A comparison of the relative retention behavior of selected analgesic compounds on octadecylsilane *versus* underivatized silica is shown in Table II.

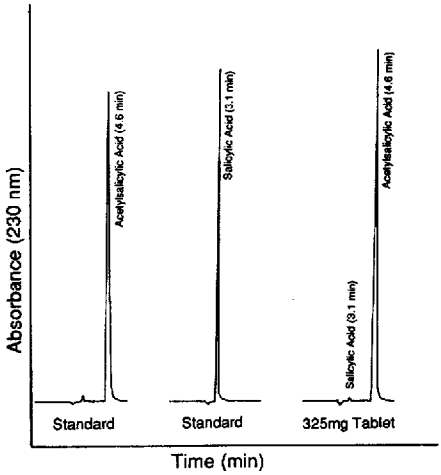
Accuracy and precision of the method were evaluated using spiked samples of selected anti-inflammatory drugs. The results are shown in Table III. The above described chromatographic system was then applied to the analysis of acetylsalicylic acid, fenoprofen, ibuprofen, ketoprofen, naproxen and tolmetin in commercialy available dosage forms. The mobile phase was optimized for each compound to

TABLE III

ACCURACY AND PRECISION FROM SPIKED DRUG SAMPLES

	Concentration		*Accuracy (%)*	*R.S.D. (%)*
	Added (μg/ml)	*Found[a] (μg/ml)*		
Aspirin	2.50	2.55 ± 0.05	2.00	1.91
	5.50	5.52 ± 0.04	0.36	0.73
Fenoprofen	2.50	2.45 ± 0.31	2.00	1.24
	5.50	5.53 ± 0.11	0.55	0.81
Ibuprofen	2.50	2.53 ± 0.09	1.20	1.01
	5.50	5.51 ± 0.07	0.18	0.68
Ketoprofen	2.50	2.49 ± 0.12	0.40	0.84
	5.50	5.50 ± 0.06	0.00	0.72
Naproxen	2.50	2.54 ± 0.12	1.60	1.00
	5.50	5.52 ± 0.03	0.36	0.87
Tolmetin	2.50	2.52 ± 0.09	0.80	1.02
	5.50	5.49 ± 0.08	1.64	0.56

[a]Based on $n = 3$.



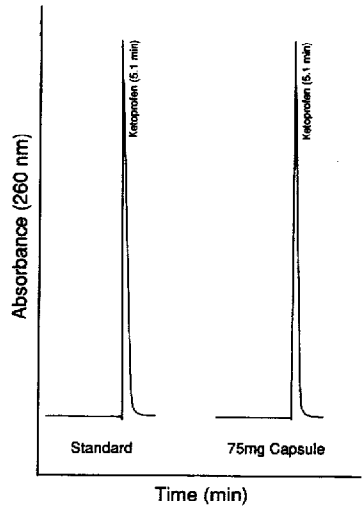


Fig. 3. Typical chromatograms of acetylsalicylic acid and salicylic acid in standard solutions and a solid dosage form (chromatographic conditions in text and Table IV).

Fig. 4. Typical chromatograms of ketoprofen in standard solution and dosage form (chromatographic conditions in text and Table IV).

TABLE IV

ANALYTICAL FIGURES OF MERIT

	r^{2a}	*System suitability*[b]	*LOD*[c]	k'^{d}	*Theoretical plates*[d]	*Tailing factor*[e]
Aspirin	0.9995	0.95	1 ng at 230 nm	1.4	4957	1.03
Fenbufen	—[f]	1.08	—[f]	2.6	5028	1.07
Fenobrufen	0.9999	1.05	1 ng at 272 nm	1.1	2750	1.04
Ibuprofen	0.9994	1.12	0.5 ng at 225 nm	1.1	2052	1.11
Indomethacin	—[f]	1.10	—[f]	3.6	6352	1.10
Ketoprofen	0.9997	0.99	0.3 ng at 260 nm	1.5	4758	1.02
Naproxen	0.9995	0.99	0.5 ng at 260 nm	1.3	4571	1.01
Salicylic acid	—[f]	—[f]	—[f]	0.6	3462	1.02
Sulindac	—[f]	1.16	0.5 ng at 230 nm	0.7	5963	1.03
Tolmetin	0.9999	1.01	0.4 ng at 260 nm	1.4	3973	1.04

[a] Range examined from 0–60 μg/ml, $n = 4$. Mobile phase consisted of 5 m*M* sodium phosphate buffer, pH 2.6–acetonitrile (95:5, v/v) at 1 ml/min, except for aspirin (100% sodium phosphate buffer).
[b] R.S.D. (%) of 6 replicate injections at analyte concentration of 40 μg/ml.
[c] Limit of detection on column, signal-to-noise ratio of 3.
[d] Determined with mobile phase of 5 m*M* sodium phosphate buffer, pH 2.6–acetonitrile, (95:5 v/v), 1 μg/ml analyte solution in 5% acetonitrile, 220 mm × 4.6 mm I.D., 5 μm Brownlee silica column.
[e] Calculated at 10% peak height.
[f] Not applicable.

obtain a short retention time ($k' < 2.5$) for high sample throughput. For aspirin, the mobile phase consisted of 5 m*M* sodium phosphate–phosphoric acid buffer, pH 2.5 (Fig. 3); for all the other analgesics studied, 50% (v/v) acetonitrile was added to the mobile phase as shown for ketoprofen in Fig. 4. Quantitative recoveries were ob-

TABLE V

RESULTS OF TABLET AND CAPSULE DOSAGE FORM ASSAYS

Compound	*Label strength (mg)*	*Amount found (mg)*[a]	*Percentage of label claim*[a]
Aspirin[b]	325	328.7 ± 2.9	101.1 ± 0.9
Fenoprofen[c]	300	304.8 ± 0.8	101.6 ± 0.3
Ibuprofen[d]	200	201.1 ± 0.5	100.5 ± 0.3
Ketoprofen[e]	75	78.8 ± 0.2	105.0 ± 0.2
Naproxen[f]	250	252.2 ± 0.6	100.9 ± 0.2
Tolmetin[g]	400	398.0 ± 0.7	99.5 ± 0.2

[a] Mean ± S.D. based on $n = 4$.
[b] Enteric coated aspirin, Rugby, Lot No. 010-0488T.
[c] Nalfon, Dista, Lot No. 1FA78A.
[d] Advil, Whitehall Lab., Lot No. 9E09.
[e] Orudis, Wyeth Lab., Lot No. 9880476.
[f] Naprosyn, Syntex, Lot No. 11150.
[g] Tolectin, McNeil Pharmaceuticals, Lot No. DP8613P.

tained for all analytes from the dosage forms. The analytical figures of merit are summarized in Table IV. The results of the dosage form assays are listed in Table V.

CONCLUSION

Underivatized silica with aqueous eluents was shown to be amenable for the separation and quantitation of non-steroidal anti-inflammatory drugs in pharmaceutical dosage forms. This HPLC system has advantages of using simple and inexpensive mobile phases and a comparatively inexpensive and very stable silica column. This study suggests that the use of underivatized silica can be expanded to solve other separation problems where selectivities other than those found in bonded phases are needed.

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