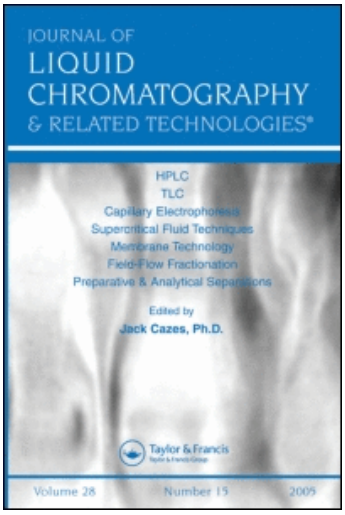

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HPLC Post-Column Ion-Pair Extraction of Acidic Drugs Using a Substituted α-Phenylcinnamonitrile Quaternary Ammonium Salt as a New Fluorescent Ion-Pair Reagent

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HPLC POST-COLUMN ION-PAIR EXTRACTION OF ACIDIC DRUGS USING A SUBSTITUTED α-PHENYLCINNAMONITRILE QUATERNARY AMMONIUM SALT AS A NEW FLOURESCENT ION-PAIR REAGENT

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ABSTRACT

A reversed-phase HPLC post-column ion-pair extraction system was developed for the analysis of carboxylic acid drugs and their salts using α-(3,4-dimethoxyphenyl)-4'-trimethylammonium-methylcinnamonitrile methosulfate (DTM) as a new fluorescent ion-pair reagent. The on-line post-column extraction system was optimized with respect to the reagent concentration, extraction coil length and internal diameter, ionic strength of mobile phase, extraction solvent, and the membrane phase separator. Sodium salicylate, ketoprofen, ibuprofen, probenecid, and valproic acid were used as model compounds to evaluate the ion-pair extraction system. The method was applied to pharmaceutical dosage forms containing ketoprofen and valproic acid. Other acidic compounds evaluated using the ion-pair reagent showed that lipophilic acids produced more extractable ion-pairs and higher sensitivities than hydrophilic acids.

INTRODUCTION

Post-column ion-pair extraction was introduced into high performance liquid chromatography (HPLC) to enhance detectability and selectivity (1-2) and has been widely used for the determination of basic compounds and quaternary ammonium surfactants (3-7). The technique is based on ion-pair formation between a separated analyte and a counter ion present in the mobile phase, and the extraction of the resulting ion-pair from the mobile phase into an organic phase. The organic phase containing the extracted ion-pair is then directed to the chromatographic detection system.

Detection is usually based on some optical property of the counter ion. When analytes have no UV absorbance and are weakly or non-fluorescent, the ion-pairing technique would offer excellent possibilities for high detectability and selectivity when using an appropriate counter ion. The counter ion is also selected based on its structural properties such that the lipophilicity of the resulting ion-pair is greatly enhanced over the extractability of the analyte alone into the organic phase. Therefore, the proper choice of a counter ion is an extremely important factor in any post-column ion-pair extraction system. (8) Fluorescent ion-pair reagents reported in the literature include dimethoxyanthracene sulfonate (DAS) (4,9),α-(3,4-dimethoxyphenyl)cinnamonitrile-2'-sulfonate (DPS) (10), acridinium chloride (3), protriptyline hydrochloride (11), and acridine orange (12). DAS and DPS are good fluorescent ion-pair reagents for cationic compounds, acridinium

chloride has been used for anionic surfactants and both protriptylium ion and acridine orange have been employed for carboxylic acid drugs.

In this laboratory, substituted α-phenylcinnamonitrile quaternary ammonium triflate salts were synthesized (13) and their fluorescence evaluated for use as ion-pair reagents. It was found that α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile triflate (trifluoromethanesulfonate) exhibited the highest fluorescence intensity of the compounds synthesized. Since the anionic portion of an ion-pair reagent was shown in this laboratory to affect not only its ion-pairing ability but also its extractability, the iodide, hydroxide, and methosulfate salts of the cinnamonitrile were also synthesized and evaluated versus the triflate salt. Sodium salicylate, ketoprofen, ibuprofen, probenecid, and valproic acid were selected as model carboxylic acid drugs to aid in the development of the general assay method.

The method was then applied to the analysis of pharmaceutical dosage forms containing ketoprofen and valproic acid. Ketoprofen [2-(3-benzoyl-phenyl) propionic acid], is a widely used non-steroidal anti-inflammatory drug (14,15) and valproic acid (2-propylpentanoic acid), is an antiepileptic drug used to control several type of seizures (16). Ketoprofen is weakly fluorescent and valproic acid is a poor UV/VIS absorber that also has no fluorescent properties. Gas chromatography (GC) (17) and HPLC (18-20) methods reported for ketoprofen give sensitivities of 2 ug/ml and 2 - 20 ng/ml, respectively. The most sensitive assays

for valproic acid have been obtained using GC-mass spectrometry (21) and fluorescence immunoassay (22,23). Because these methods are expensive and laborious, GC (24,25) and HPLC (25) have been utilized as alternative methods. However, the existing GC and HPLC procedures only have a sensitivity around 1 - 2 ug/ml.

In this paper, the utility of using α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (DTM) as a new fluorescent ion-pair reagent in an HPLC post-column ion-pair extraction system is described using carboxylic acid drugs as model compounds. The method is shown applicable to the assay of ketoprofen and valproic acid in pharmaceutical dosage forms.

EXPERIMENTAL

Apparatus

The HPLC system equipped with a post-column extractor is shown in Fig. 1. The system is composed of three pumps: an Alcott Chromatography Model 760 HPLC pump (Norcross, GA) for the mobile phase, a LDC/Milton Roy mini-Pump VS (Riviera Beach, FL) with pulse dampener (SSI Model LP-21, State College, PA) for the ion-pair reagent, and a Kratos Spectroflow 400 solvent delivery system (Ramsey, NJ) with pulse dampener for the extraction solvent, a Rheodyne Model 7125 injector (Cotati, CA) equipped with a 50 ul sample loop, and two mixing tees (Alltech, Deerfield, IL). The detector was a HP Model 1046A programmable Fluorescence Detector (Hewlett Packard, Waldbronn, Germany) set at an

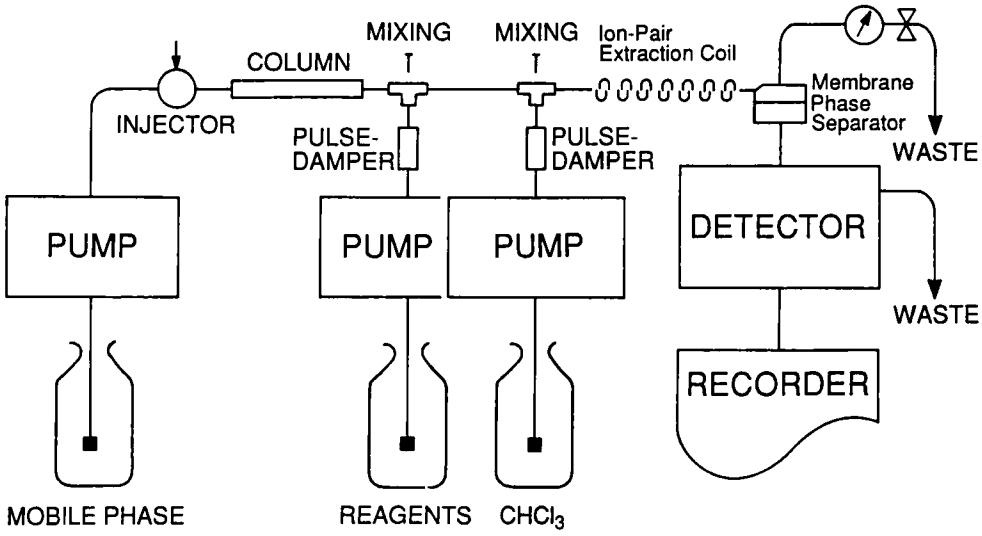


Figure 1 - Schematic of the HPLC Post-Column Extraction System

excitation of 355 nm and an emission of 460 nm (also equipped with a 408 nm emission cut-off filter). A HP 3390A integrator was used to record the resulting chromatograms.

The analytical column was a 5um RP-8 column (Spheri-5, 10 cm x 4.6 mm i.d., Brownlee Labs, Santa Clara, CA). The extraction coils were three dimensional knitted teflon coils (1.8 m x 0.3 mm i.d., LDC/Milton Roy, Riviera Beach, FL) knitted using a four pin "Strickliesel" (31). The phase separator shown in Fig. 2 is a membrane type phase separator constructed by the University of Georgia instrument shop (Athens, GA) out of a Kel-F rod (50 mm diameter) modified according to the designs of Backstrom et al. (32) and Quinn and Stewart (10). The separator accommodated a 13

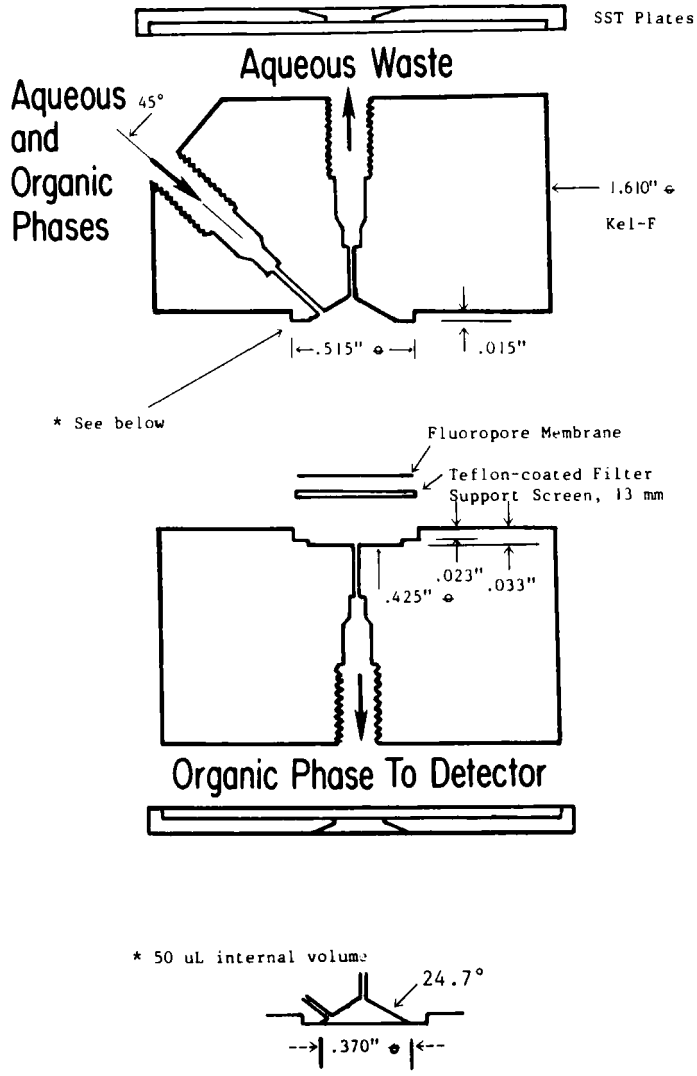


Figure 2 - Schematic Diagram of Membrane Phase Separator

mm teflon-coated support screen and a polyethylene backed 0.5 um Fluoropore membrane filter (Millipore Corp. Bedford, MA).

Reagents and Chemicals

Commercially available chemicals were used without further purification. Methanol, chloroform, and pentanol were HPLC grade (J. T. Baker, Phillipsburg, NJ) and the water used was double distilled in-house. Ketoprofen and valproic acid in capsule dosage form were purchased from a local pharmacy. Other drugs and chemicals used were of the highest purity grade available and obtained from various suppliers including: Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), and J. T. Baker (Phillipsburg, NJ).

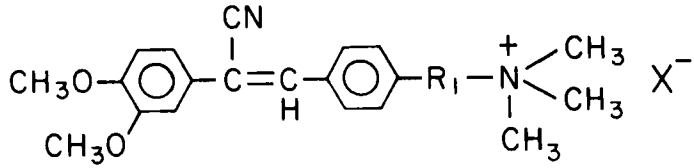
Synthesis of quaternary ammonium salts of substituted α-phenylcinnamonitriles

The quaternary ammonium salts studied and some of their important physical properties are shown in Table 1. The triflate salt (compound 1) and the iodide salt (compound 6) were synthesized previously by Stewart and Kim (13) and Sinsheimer et al. (33), respectively. Additional quaternary ammonium salts (compounds 2 - 5) were synthesized starting from the relevant tertiary amine compound. The compounds were identified by NMR, IR, and elemental analysis. (Atlantic Microlab, Inc., Atlanta, GA).

Synthesis of hydroxide salts 2 and 4: The respective iodide salt 5 or 6 (0.001 mol) was dissolved in 100 ml of absolute methanol contained in a 250 ml round bottom flask wrapped with aluminum foil to protect from light. An excess amount of finely

TABLE 1

Structures of quaternary ammonium salts of substituted α-phenylcinnamonitriles.



COMPOUND	R_1	X^-	Fluorescence[a] ex	em	QRU[b]	Relative Quantum[c] Efficiency
1	CH_2	CF_3SO_3	357	476	2.15	0.33
2	CH_2	OH	362	479	2.17	0.49
3	CH_2	CH_3SO_4	366	476	2.13	0.36
4	-	OH	367	493	1.96	0.60
5	CH_2	I	365	470	1.94	0.24
6	-	I	365	490	1.75	0.47

[a]Measured in a spectrophotofluorometer using 95% ethanol as solvent

[b]Quinine reference unit, see Ref. 33

[c]Measured by the method of Parker and Rees, Analyst, 85 (1960) 587.

ground silver oxide (0.002 mol) was added followed by stirring for 2 hours at ambient temperature. Each resulting solution, containing approximately 1 x 10^{-2} M of hydroxide salt, was used as the stock solution.

Synthesis of methosulfate salt 3: A mixture of 0.01 mol of α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile, 0.02 mol of dimethyl sulfate, and 5 g of potassium carbonate in 50 ml of acetone was refluxed for 1 hour and cooled to room temperature. The precipitate was filtered and dried in a vacuum oven at room temperature overnight. The residue was recrystallized from

chloroform containing 2-3 drops of 95 % ethanol, 2.8 g, 62.5 % yield, mp 212 - 215° C. Calcd for C_{22} H_{28} N_2 O_6 S: C, 58.91; H, 6.29; N, 6.25 Found: C, 58.93; H, 6.31; N, 6.17.

Synthesis of iodide salt 5: The methosulfate salt 3 (0.005 mol) was dissolved in 50 ml of water. Aproximately 3 g of solid potassium iodide was added to the aqueous solution of the methosulfate salt followed by stirring with a glass rod. The iodide salt immediately precipitated and was filtered. The residue was dried and recrystallized from 95 % ethanol, (2.2 g, 94.8 % yield), mp 203 - 204°C. Calcd for C_{21} H_{25} N_2 IO_2: C, 54.32; H, 5.43; N, 6.03 Found: C, 54.37; H, 5.44; N, 6.02.

Preparation of mobile phase and ion-pair reagent solutions

The mobile phase was prepared as an absolute methanol - pH 7 aqueous phosphate buffer mixture (30:70 v/v) and the flow rate was set at 1 ml/min. The pH 7 phosphate buffer was prepared by mixing 0.004M dibasic sodium phosphate and 0.007M monobasic potassium phosphate. The ionic strength of the buffer was 0.033 u.

A stock solution of α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (DTM) (4.46×10^{-4} M) was prepared by dissolving 20 mg in 100 ml of mobile phase and stored protected from light.

The mobile phase, reagent, and extraction solutions were all filtered through a 0.45 um nylon 66 membrane filter (Magna, Honeoye Fall, NY) and sonicated for 5 minutes under vacuum prior to use.

Preparation of standard solutions

Stock solutions (100 ug/ml) of sodium salicylate, ketoprofen, ibuprofen, probenecid, and valproic acid were prepared by dissolving weighed quantities of each substance in mobile phase. Working solutions were prepared immediately prior to use by serial dilution of the stock solutions with mobile phase.

An ibuprofen (internal standard) stock solution was also prepared by dissolving 50 mg of the drug in 100 ml of absolute methanol. A working solution (50 ug/ml) was prepared by diluting 10 ml of the stock solution to 100 ml with mobile phase.

Preparation of dosage form samples

A ketoprofen sample was prepared by weighing five ketoprofen capsules and transferring the combined capsule contents to a mortar. The empty capsules were then weighed and the average net weight per capsule was calculated. An accurately weighed portion of the powder equivalent to 50 mg of ketoprofen was transferred to a 100 ml volumetric flask followed by the addition of 80 ml of absolute methanol. The flask was placed in an ultrasonic bath for 10 minutes. The sample was diluted to volume with absolute methanol and filtered. A 4 ml aliquot of filtered solution was further diluted to 100 ml with mobile phase. A 10 ml portion of this diluted solution and 10 ml of internal standard (ibuprofen) working solution were then transferred to a 100 ml volumetric flask and diluted to volume with mobile phase. 50 ul of sample solution was injected into the HPLC system.

A valproic acid sample was prepared by weighing five valproic acid capsules. The capsules were then opened with a sharp blade

and the combined capsule contents were transferred to a 50 ml beaker. The empty capsules were washed with n-hexane to remove any adhering substances and dried in an air stream until the odor of n-hexane was no longer perceptible. The empty capsules were then weighed and the average net weight per capsule was calculated. An accurately weighed portion of the combined capsule contents equivalent to 250 mg of valproic acid was transferred to a 100 ml volumetric flask followed by the addition of 80 ml of absolute methanol. The flask was placed in an ultrasonic bath for 10 minutes. The sample was diluted to volume with absolute methanol and filtered. The contents were allowed to settle for 30 minutes. A 4 ml aliquot of the solution was diluted to 100 ml with mobile phase. Then 20 ml of the diluted solution and 10 ml of the internal standard (ibuprofen) working solution were transferred to a 100 ml volumetric flask and diluted to volume with mobile phase. Fifty microliters of the sample solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Ion-pair reagent

The ideal fluorescent ion-pair reagent should be soluble in the mobile phase, possess good fluorescence properties in the extraction solvent, and have suitable ion-pairing ability with any proposed analytes. The resulting ion-pair should be well extracted into the extraction solvent. Various quaternary ammonium salts of substituted α-phenylcinnamonitriles were synthesized in this laboratory and evaluated for their use as a fluorescent ion-pair

reagent for the carboxylic acid functional group. It was found that the anion form of the compound influenced both background noise and ion-pairing ability as shown in Table 2. When the anion is hydrophilic, the reagent is more soluble in the mobile phase than it is in the extraction solvent. This greatly influenced background noise which is an important factor in the limit of detection. The data in Table 2 also indicate that these cinnamonitriles give comparable results to acridine orange, a highly fluorescent reagent for anionic compounds that we have studied in this laboratory (12).

TABLE 2

Comparison of ion-pair reagents with selected drugs.

	Compound[a]				
	1	2	3	4	Acridine Orange
Excitation(nm)	355	355	355	360	480
Emission(nm)	460	460	460	460	520
PMT GAIN[b]	10	10	10	10	8
Valproic acid (10 ug/ml)	4.8[c]	10.4	15.3	5.2	13.6
Ketoprofen (2.5 ug/ml)	28.6	31.8	40.0	15.9	31.4
Ibuprofen (5.0 ug/ml)	12.1	25.2	32.2	13.4	31.6

[a]The structures of compounds 1 - 4 are listed in Table 1.

[b]PMT GAIN : Sensitivity of HP 1040A Fluorescence detector.

[c]Peak height (x 10^4) obtained from HP 3390A integrator.

It was observed that compound 3, α-(3,4-dimethoxyphenyl)-4' -trimethylammoniummethylcinnamonitrile methosulfate (DTM), gave the highest fluorescent intensity results and, therefore, it was selected as the fluorescent ion-pair reagent to investigate for the determination of carboxylic acid compounds. Maximum fluorescence intensity of DTM in the on-line system was obtained using an excitation of 355 nm and an emission of 460 nm.

Although the fluorescence intensity of an analyte increased as the concentration of DTM increased (Fig. 3), background noise increased at a much faster rate than the analyte signal. Therefore it was decided that an ion-pair reagent concentration of 1.12×10^{-4} M (5 mg/ 100 ml of mobile phase) was the most appropriate in order to minimize the contribution of background noise in the system.

Chromatographic system

The post-column ion-pair extraction system utilized three pumps as shown in Fig. 1: one each for the mobile phase, ion-pair reagent solution, and extraction solvent. The ion-pair reagent was mixed with the column eluent at the first mixing tee followed by addition of the organic extraction solvent at the second mixing tee. The mixture of two immiscible phases was then passed through a three dimensional knitted extraction coil where the reagent-analyte ion-pair was extracted from the mobile phase into the extraction solvent. The organic phase containing the extracted ion-pair was then separated from the largely aqueous mobile phase

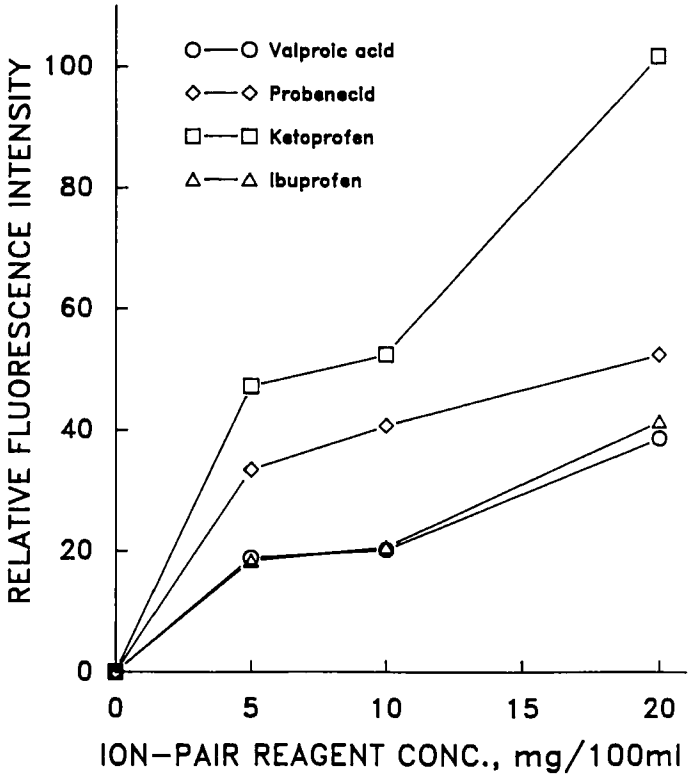


Figure 3 - Effect of DTM Concentration on the On-Line Post-Column Extraction of Valproic Acid (10 ug/mL), Probenecid (1 ug/mL), Ketoprofen (2.5 ug/mL), and Ibuprofen (2.5 ug/mL).

using a phase separator and directed to the fluorescence detector. The separated aqueous phase containing any remaining ion-pair reagent, drug or extraction solvent was sent to waste. For the most efficient operation, the extraction solvent was always pumped into the system for 30 min. prior to pumping the mobile phase and ion-pair reagent. The extraction solvent pump was also the last to

be turned off. This was necessary in order to properly wet the teflon membrane in the phase separator with organic phase.

The ion-pair extraction portion of the system was composed of two mixing tees, a 1.8 m x 0.3 mm i.d. teflon extraction coil, and a phase separator. Three events occurred in this part of the total system: phase segmentation of organic and aqueous phase at the second mixing tee, extraction of the ion-pair in the extraction coil, and separation of the organic and aqueous phases at the phase separator. The flow rates of solvents and reagents and the design of the extraction coil and phase separator hardware components influenced the sensitivity and reproducibility of the system (27-32).

Band broadening caused by the extraction coil did not appear to be a problem in this extraction system because of the relatively short extraction coil lengths used and the use of solvent segmentation. It has been shown in this laboratory that the three dimensional knitted coil shape is advantageous over other geometrical shapes (34).

The length and diameter of the three dimensional knitted teflon coils were studied in relation to extraction efficiency. The coil diameters tested were 0.3, 0.5, and 0.8 mm i.d. Similiar fluorescent intensities were noted for all three diameters. The length of the coil as shown in Fig. 4 affects both analyte dispersion and extraction efficiency so that any increase in coil length beyond a certain point will result in increased analyte dispersion without a concurrent increase in extraction of analyte.

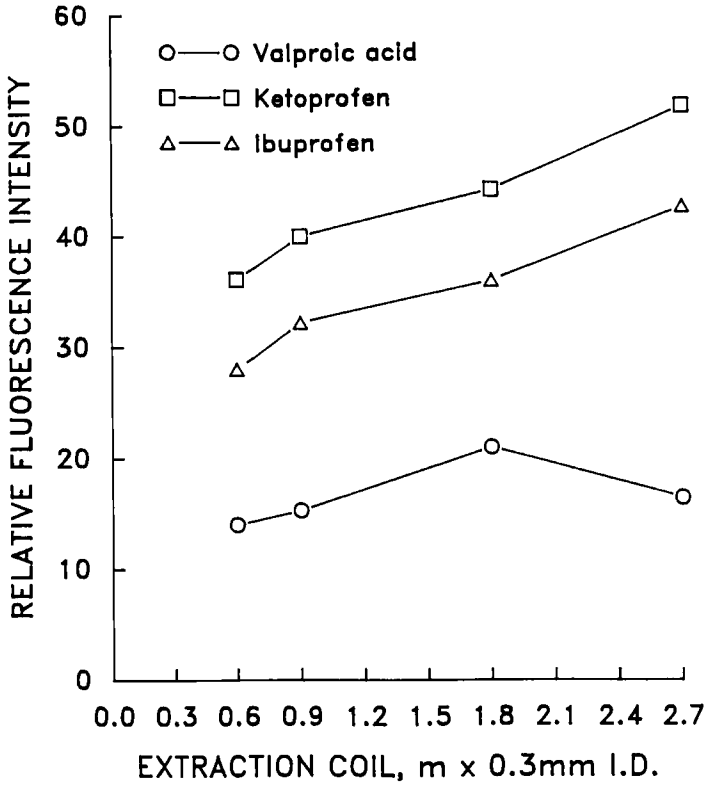


Figure 4 - Effect of Three Dimensional Knitted Coil Length on the On-Line Extraction of Valproic Acid (10 ug/mL), Ketoprofen (2.5 ug/mL), and Ibuprofen (5 ug/mL), and DPM (5 mg/100 mL).

A 1.8 m x 0.3 mm i.d. three dimensional knitted extraction coil was thus selected for use in this study to minimize band broadening.

The role of the phase separator is to separate the two immiscible phases on-line and to direct the organic phase to the detector. Since the phase separator can be a source of band

broadening in any extraction system, the phase separator should be designed such that its internal volume is as small as possible but large enough to produce an efficient phase separation. The membrane phase separator used in this study was modified on our campus by Quinn and Stewart (10) using a design originally reported by Backstrom et al. (32). The internal volume of the membrane phase separator was 50 microliters. In order to achieve a quantitative separation of aqueous and organic phases, backpressure on the membrane was controlled to around 65 - 75 psi by a backpressure gauge connected to the aqueous phase waste line of the separator. The outlet flow rate of the organic phase was also an important factor for sensitivity and reproducibility and was controlled to within 0.75 - 0.8 ml/min (75 - 80 % efficiency).

Mobile Phase

Since carboxylic acids are weak acids, the pH of the mobile phase was adjusted to 7 so that the analytes of interest were at least 99% ionized. This pH is within the recommended operation range for bonded-phase HPLC columns. It was found that the ionic strength of the mobile phase should be at least 0.033 u since a lower ionic strength affected both the extraction of the ion-pairs and the retention time of the analytes. The methanol strength in the mobile phase influenced not only the retention time of the analytes but also their extraction efficiency. A 30:70 absolute methanol - 0.1 M pH 7 aqueous phosphate buffer mobile phase was used in this study since it showed a good separation of the model

carboxylic acids on a 5 um octylsilane column at a 1.0 ml/min flow rate.

Extraction Solvent

Chlorinated solvents such as chloroform and methylene chloride have been widely used in ion-pair extractions. Although methylene chloride is generally considered to be a very good extraction solvent, it has problems due to high background noise and volatility, which can cause air bubble formation in an HPLC system. Therefore, chloroform was chosen as the extraction solvent in this study. n-Pentanol can be added to the chloroform to enhance the extractability of certain hydrophilic compounds. The extracting ability of the organic phase can be varied by changing the proportion of n-pentanol to chloroform as shown in Fig. 5. For example, the extraction of sodium salicylate was enhanced about 30 fold by using 30 % n-pentanol in chloroform, whereas only a 3 fold effect was observed for ibuprofen and ketoprofen with the same solvent mixture. This latter increase is about the same as that noted for background noise with chloroform alone. Because of co-extraction of the ion-pair reagent into the extraction solvent, higher fluorescent backgrounds are observed when high percentages of n-pentanol were present in the chloroform.

Limit of Detection

The applicability of using DTM as a universal reagent was evaluated using a series of acidic compounds listed in Table 3.

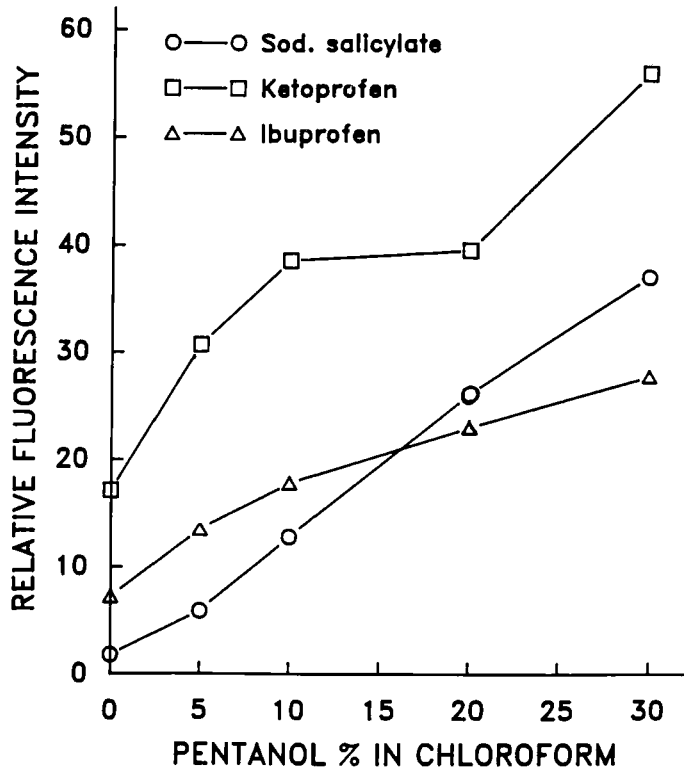


Figure 5 - Effect of n-Pentanol Added to Chloroform in the On-Line Extraction System. The Analyte Concentrations were: Sodium Salicylate (10 ug/mL), Ketoprofen (5 ug/mL), and Ibuprofen (5 ug/mL).

The limits of detection for the compounds using the post-column extraction system were determined at a signal-to-noise ratio of two under the system parameters established for valproic acid, probenecid, ketoprofen, and ibuprofen. The data indicate that DTM is a potent fluorescent ion-pair reagent for the determination of compounds containing carboxylic and sulfonic acid functional

TABLE 3

Detectability of miscellaneous acidic compounds with α-(3,4-dimethoxyphenyl)-4-trimethylammoniummethylcinnamonitrile methosulfate (DTM) as fluorescent ion-pair reagent.[a]

Compound	Capacity Factor(k')	Limit of Detection[b]	
		ng	ug/ml
Sodium Formate[c]	-	no response	no response
Sodium Acetate	0.2462	300,000	6,000
3-Bromopropionic acid	0.1846	1,000	20
6-Aminocaproic acid	1.4462	150,000	3,000
11-Bromoundecanoic acid	17.2791	100	2
1-Heptanesulfonic acid	0.6667	1	0.02
p-Nitrophenylacetic acid	0.2093	2,500	50
Sodium Benzoate	0.0923	500	10
Sodium Salicylate	0.2791	50	1
Valproic acid	0.3256	5	0.1
Probenecid	1.2791	0.5	0.01
Naproxen	0.9690	5	0.1
Ketoprofen	1.5504	1	0.02
Ibuprofen	4.1240	5	0.1
Mefenamic acid	7.5039	100	2
Flufenamic acid	12.1938	25	0.5
Cefuroxime Sodium	0.1008	250	5

[a]Data were determined using post-column extraction conditions established for the model carboxylic acids.

[b]Based on S/N = 2

[c]Concentrations up to 10 mg/ml were investigated

groups, with lipophilic compounds giving more extractable ion-pairs than hydrophilic compounds.

Applications

Ketoprofen and valproic acid in capsule dosage form were analyzed utilizing the new post-column ion-pair extraction system.

TABLE 4

Chromatographic and Post-column Extraction parameters for ketoprofen and valproic acid.

Column	: 5 um RP-8 (10 cm x 4.6 mm i.d.)
Mobile phase	: pH 7 aqueous phosphate buffer:absolute methanol (70:30 v/v)
flow rate	: 1.0 ml/min.
Reagent	: 1.12×10^{-4} M (5 mg/100 ml) DTM in mobile phase
flow rate	: 0.5 ml/min.
Extraction solvent	: chloroform
flow rate	: 1.0 ml/min.
Extraction coil	: three dimensional knitted teflon tubing (1.8 m x 0.3 mm i.d.)
Detector	: fluorescence Ex. 355 nm Em. 460 nm Cutoff filter 408 nm PMT GAIN 10
Injection volume	: 50 ul

Table 4 summarizes the chromatographic and post-column extraction conditions used in both analyses. Fig. 6 shows typical chromatograms for ketoprofen and valproic acid. The results of the dosage form assays are shown in Table 5.

The linear dynamic range for valproic acid was 3.47×10^{-5} M (5 ug/ml) to 3.47×10^{-4} M (50 ug/ml) and 1.97×10^{-6} M (0.5 ug/ml) to 3.93×10^{-5} M (10 ug/ml) for ketoprofen under the established HPLC conditions using ibuprofen as internal standard. Linear regression analysis of drug/internal standard peak height ratios versus drug concentration data gave correlation coefficients (r) of 0.9994 for valproic acid and 0.9999 for ketoprofen (n=4). Relative standard deviations for valproic acid

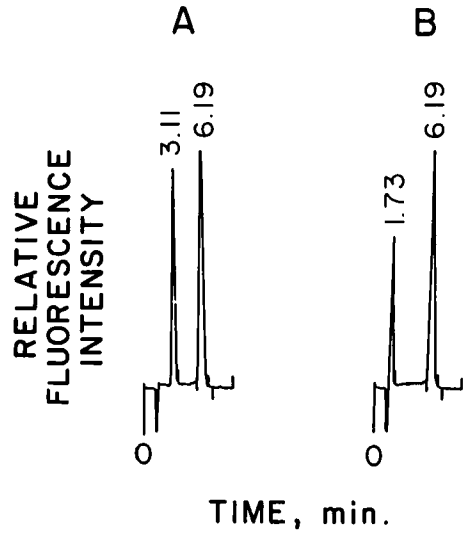


Figure 6 - Typical HPLC Chromatograms of Dosage Form Samples on a RP-8 Column (5 um, 10 cm X 4.6 mm i.d.) Using 70:30 v/v Aqueous pH 7 Phosphate Buffer-Absolute Methanol At A Flow Rate of 1 mL/min. The Extraction Solvent was Chloroform. The Ion-Pair Reagent was DTM (5 mg/100 mL).
A: Ketoprofen - 3.11 min
Internal Standard - 6.19 min
B: Valproic Acid - 1.73 min
Internal Standard - 6.19 min

TABLE 5

Analysis of valproic acid and ketoprofen in pharmaceutical dosage forms using the post-column Ion-pair extraction system.

Drugs	Labelled Amount (mg)	Amount[a] Found (mg)	% of Labelled Amount Found	RSD %
Valproic acid	250	258.5 ± 6.46	103.39	2.50
Ketoprofen	75	73.7 ± 1.33	98.23	1.80

[a]Based on n = 3

and ketoprofen were 2.50 and 1.80 %, respectively, based on triplicate injections.

In summary, α-(3,4-dimethoxyphenyl)-4'-trimethylammonium-methylcinnamonitrile methosulfate (DTM) has been demonstrated to be an excellent fluorescent ion-pair reagent for the assay of carboxylate and/or sulfonate containing compounds using a post-column ion-pair extraction system developed in this laboratory. The method was found to be useful in the assay of ketoprofen and valproic acid in pharmaceutical dosage forms and should be applicable to other acidic compounds in similar matrices.

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