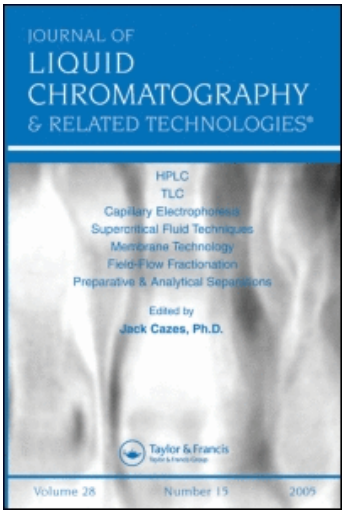

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Pharmaceuticals by Multi-Phase Chromatography: Combined Reversed Phase and Ion Exchange in One Column

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To cite this Article Slingsby, Rosanne W. and Rey, Maria(1990) 'Determination of Pharmaceuticals by Multi-Phase Chromatography: Combined Reversed Phase and Ion Exchange in One Column', Journal of Liquid Chromatography & Related Technologies, 13: 1, 107 — 134
To link to this Article: DOI: 10.1080/01483919008051791
URL: http://dx.doi.org/10.1080/01483919008051791

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(1), 107-134 (1990)

DETERMINATION OF PHARMACEUTICALS BY MULTI-PHASE CHROMATOGRAPHY: COMBINED REVERSED PHASE AND ION EXCHANGE IN ONE COLUMN

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ABSTRACT

Separations of various classes of pharmaceuticals using several HPLC retention modes simultaneously are described. The drugs including barbiturates, anti-inflammatories, cephalosporins and sulfa antibiotics are separated by a combination of reversed phase and ion exchange on a new multi-phase HPLC column. This column contains two types of particles, a macroporous substrate particle, which has adsorptive properties, and a monolayer of ion exchange latex particles which is attached to the substrate. The reversed phase and ion exchange retention modes operate independently and therefore can be controlled independently through selection of eluant components and gradient conditions. This approach to multi-mode chromatography is applied to accomplish assay reduction and on-line sample preparation. The determination of drugs in biomedical matrices such as urine, serum and saliva are discussed. Advantages of multi-mode chromatography over ion-pair reversed phase chromatography are discussed.

INTRODUCTION

Traditional methods used in the determination of many types of pharmaceuticals involve either ion suppression or ion pairing modes of HPLC. When the mobile phase is buffered to suppress the ionization of weakly acidic or weakly basic analytes, retention is based on the adsorptive i.e. solubility properties of the neutral analytes. The pH required for full suppression is often outside of the stable range of silica-based packings. This happens mostly when the analytes are weak bases such as many amine-type drugs. Another problem associated with ion suppression on silica-based columns is the uncontrolled interaction of some analytes e.g. carboxylic acids, with residual silanols. The result is often poor column-to-column reproducibility for these analytes, peak tailing and low efficiency.

Ion pairing is often chosen as the separation mode for weakly acidic and weakly basic drugs in an effort to control the effects of their ionization and obtain acceptable peak symmetry. Use of an ion pairing reagent essentially neutralizes any possibility of separations based on the ionic properties of the analytes. Although ion pairing is often a successful technique for controlling the separation of ionic analytes, it has inherently poor selectivity for higher valency analytes

of the same charge. For example ion pairing gives poor separations of divalent anions such as sulfate, oxalate, tartrate etc.

In considering the structural features of many pharmaceuticals, e.g. hydrophobic, polar and/or ionic and the scope of HPLC separation modes potentially available it seems that use of only one separation mode in an analysis is unnecessarily limiting. Packing materials with well-controlled multiple modes offer a larger "tool-box" for developing separations in complex matrices.

In the past there have been several approaches to combining different selectivities into one analysis. Some investigators have studied mixing ligands on silica to generate mixed-mode columns (1). Improvements in separation and peak shape were observed for several classes of basic drugs on C_8/cation exchange columns. This technique does not utilize the full potential of mixed-mode columns for weak acid/weak base analytes however because wide pH gradients cannot be used with the silica support.

Some investigators have used the column-switching approach to combine multiple column selectivities into one analysis. One type of application of column-switching in HPLC is the removal of matrix interferences i.e. serum proteins, on a precolumn before the

analytical separation (2). The precolumn is backflushed after each analysis with the use of automation. In some cases the selectivity of a precolumn is optimized for the proteins, while the analytical column is chosen for its selectivity for analytes of interest.

The combination of columns with different selectivities to either allow separation of chromatographically unrelated analytes (3) or to remove matrix interferences (4) has also been reported. Two RP-HPLC modes have also been combined in a column-switching scheme in order to remove matrix components (5).

In another application of column-switching, weak acids are separated from an unretained band of strong acids on an ion exclusion column (6-7). The band of strong acids are diverted to an ion exchange column for separation. The weak acids are separated on the ion exclusion column and detected. The scheme is particularly useful when the analysis requires the determination of low ppm levels of weak acids in the presence of high concentrations of chloride, sulfate etc.

In this paper we discuss separations and methods development using OmniPac multi-phase packing material (8). This material allows the controlled use of mixed separation modes within one analytical column. We apply this technology to classes of pharmaceuticals , to assay

reduction e.g. the combination of traditionally chromatographically unrelated analytes into one chromatographic analysis and to on-column sample preparation e.g. matrix elimination.

EXPERIMENTAL

Hardware

A model 4500i gradient ion chromatograph (Dionex Corp., Sunnyvale, CA, USA) was used for all separations. The instrument was equipped with 0.010 in. ID polyetherether ketone (PEEK) tubing and a polymeric rotary valve (Dionex Microinjection Valve). Two detectors in series were used when appropriate, consisting of a Dionex Rapid Scan multi-wavelength detector or a Dionex VDM-II UV/VIS detector and either a Dionex Pulsed Amperometric (PADII) or a Dionex Conductivity detector (CDMII). A Dionex AutoIon 450 data system was used for data collection and reduction.

Columns

The multi-phase columns used for this work were the Dionex OmniPac PAX-500 and a prototype OmniPac PCX-500 series column, both 250 x 4mm ID. These columns are composed of a pellicular layer of 60 A dia. ion exchange latex covalently bonded onto an 8 um macroporous ethylvinyl benzene/divinylbenzene substrate resin. The PAX-500 contains anion exchange latex and the prototype

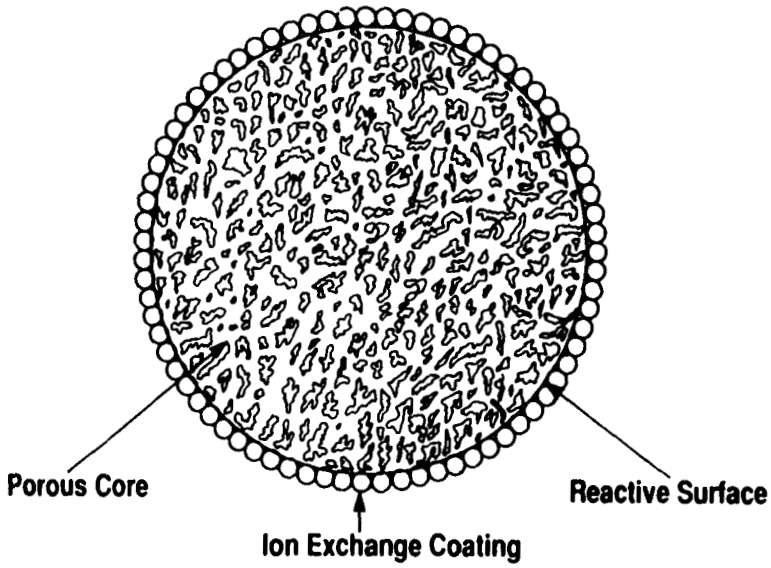


FIGURE 1. Schematic of Multi-phase pellicular ion exchange packing used in the OmniPac PAX-500 and the prototype PCX-500 columns.

PCX-500 column contains cation exchange latex covalently bonded to substrate resin (Fig. 1). The structure and fundamental properties of OmniPac PAX-500 have been discussed by Stillian etal. (8). The adsorptive properties of the macroporous substrate which allow reversed phase HPLC separations is derived from the surface area of the substrate, about 300 m^2/g. Ion pairing separations are also achieved using the substrate resin properties when ion pairing reagents are added to the mobile phase. Ion exchange occurs in the

pellicular layer of the attached latex. Ion suppression is achieved when the pH of the mobile phase is controlled to about two pH units above or below the pKa's of the analytes of interest. The prototype PCX-500 column is analogous to the PAX-500 column except that the latex has cation exchange properties.

The Dionex OmniPac PAX-100 (250 x 4mm ID) is a solvent-compatible anion exchange column with the same anion exchange selectivity as the PAX-500. Ion exchange selectivity in non-solvent-compatible substrate latex-based packing material has been discussed in detail (9). This column contains microporous substrate resin to which a pellicular layer of anion exchange latex is attached. It contains essentially no reversed phase character but has the same ion exchange selectivity as the PAX-500 column.

Standards and Chemicals

Drug Standards were obtained in methanol from Alltech/Applied Science (State College, PA 16801). Dosage forms were obtained from the pharmaceutical manufacturers. Urine was obtained from a healthy rat.

Acetonitrile was Optima (Fisher Scientific, Fair Lawn, N.J. 07410). Sodium carbonate and sodium acetate were from Mallinkrodt (Paris, KY 40631). Perchloric Acid was from J.T. Baker (Phillipsburg, PA).

Chromatography

The gradient conditions used in the figures are given below. All of the following gradients were re-equilibrated to initial conditions for 10 min. between injections. All injections were made at 0.2 min. except where noted.

Fig. 2a Barbiturates

Time 0.0 0.005 M Na_2CO_3/9% Acetonitrile
10.0 0.02 M Na_2CO_3/20% Acetonitrile
10.1 Re-equilibrate

Fig. 2b Barbiturates

Time 0.0 0.002 M Na_2CO_3/5% Acetonitrile
10.0 0.05 M Na_2CO_3/40% Acetonitrile
10.1 Re-equilibrate

Fig. 3a Anti-inflammatory Drugs

Time 0.0 0.01 M Na_2CO_3/18% Acetonitrile
10.0 0.05 M Na_2CO_3/33% Acetonitrile
10.1 Re-equilibrate

Fig. 3b Anti-inflammatory Drugs

Time 0.0 0.03 M $HClO_4$/0.02 M NaAcetate/36% Acetonitrile
6.0 0.06 M $HClO_4$/0.06 M NaAcetate/50% Acetonitrile
12.0 0.06 M $HClO_4$/0.08 M NaAcetate/54% Acetonitrile
15.0 0.06 M $HClO_4$/0.1 M NaAcetate/59% Acetonitrile
18.0 Same as Time 15.0
18.1 Re-equilibrate

Fig. 4 Cephalosporins

Time 0.0 0.09 M $HClO_4$/13.5% Acetonitrile
7.0 0.3 M $HClO_4$/45% Acetonitrile
11.0 Same as Time 7.0
11.1 Re-equilibrate

Fig. 5a Sulfonamide Antibiotics

Time 0.0 0.11 M $HClO_4$/0.02 M NaAcetate/27% Acetonitrile
5.0 0.11 M $HClO_4$/0.10 M Na Acetate/50% Acetonitrile
5.1 Re-equilibrate

Fig. 5b Sulfonamide Antibiotics

Time 0.0 0.005 M Na_2CO_3/20% Acetonitrile

10.0 0.01 M Na_2CO_3/50% Acetonitrile
10.1 Re-equilibrate

Fig. 6a and b Combined Analysis of Cefazolin, Two Diols and p-Toluenesulfonics

Time 0.0 0.03 M $HClO_4$/18% Acetonitrile
2.0 0.03 M $HClO_4$/67.5% Acetonitrile
2.1 Re-equilibrate

Fig. 7a and 7b Acebutolol in Rat Urine

Time 0.0 0.1 M Na_2CO_3/4.5% Acetonitrile
0.1 4.5% Acetonitrile and Inject
5.0 4.5% Acetonitrile
20.0 67.5% Acetonitrile
20.1 Re-equilibrate

RESULTS AND DISCUSSION

This paper discusses separations and methods development using OmniPac "multi-phase" HPLC columns, that is columns which contain two types of particles with different and independently controllable selectivities. These two types of particles, e.g. macroporous substrate particles with ion exchange latex particles covalently attached in a monolayer to the exterior (Fig. 1), can retain analytes by multiple retention modes i.e. multi-mode depending on the structural features of the analytes. Because the ion exchange particles form a pellicular layer on the substrate particles, the short diffusion paths for analytes into and out of the layer account for the good

efficiencies observed in this type of packing. The chromatographic properties of latex-based packings are similar to ODS phases where the C_{18} essentially forms a pellicular layer on the silica surface.

All modes of reversed phase chromatography including ion pairing and ion suppression, as well as ion exchange chromatography can be utilized in separations with OmniPac series -500 columns depending on the eluant conditions chosen for the analysis. In this paper we focus on the combined use of reversed phase including ion suppression and ion pairing modes and ion exchange to effect the separations of pharmaceuticals.

Pharmaceuticals are well suited to the use of multi-mode columns for separations because structurally many of them are weakly acidic or weakly basic and also contain hydrophobic moeities. For this type of compound use of ion exchange in addition to reversed phase retention allows complete and independent control of chromatography based on several different structural features of the analyte molecules simultaneously. Use of gradients which elute based on, for example two major retention modes for a molecule, allows higher efficiencies as well as better selectivity control than systems which try to minimize the effects of one retention mode while eluting based on the other mode.

Chromatography

There are a variety of combinations of ionic and solvent eluant components that have been used on OmniPac PAX-500 and PAX-100 columns (8). The eluant systems used for anion-exchange OmniPac columns, PAX-500 and PAX-100, in this paper are based on carbonate, hydroxide and acetonitrile. Carbonate is a convenient eluant anion because due to its divalent character it is able to more easily elute higher valency sample components, at lower eluant concentrations than monovalent eluting anions.

Carbonate is also very soluble in acetonitrile. When dual gradients are used the chromatographer must be careful to choose eluant components which are soluble within the range of the gradient. For example, sodium hydroxide is not soluble at acetonitrile concentrations over about 65%. Acetonitrile is a good reversed phase eluant but is not stable in sodium hydroxide for extended periods of time. Carbonate is also a "suppressible" eluant component in that it can be removed using an Anion Micromembrane Suppressor . This is useful when it is necessary to recover analytes in a salt-free matrix.

Acetonitrile is not electro-chemically active. Electrochemical activity becomes important if there is a need to detect species such alcohols, carbohydrates,

alkyl amines, amino acids etc. in the sample. These species are easily detectable electrochemically (10-12). Other mobile phase solvents including tetrahydrofuran and methanol have also been used successfully on the PAX columns.

Eluants used in this paper for the multi-phase prototype OmniPac PCX-500 series column, include perchloric acid, hydrochloric acid, sodium perchlorate, sodium acetate and acetonitrile. The considerations in these systems are the following. Acid eluant components are used to generate pH gradients into order to use pK_a values of the analytes as a basis of separation. The effect of pH can be to suppress the ionization of for example, a weak acid to allow reversed phase retention on a cation-exchange type OmniPac, PCX-500, or to use the anionic character of carboxylic acids at high pH as the basis of separation by anion exchange. Weak acids are only retained by anion exchange when they are deprotonated. The hydronium ion is not a strong cationic eluting ion, therefore a second cation is usually used, e.g. ammonium, sodium, diaminopropionic acid, to elute strongly retained analytes.

The counterion chosen for the acid and the salt also have several characteristics of note. Perchlorate forms stable ion pairs with many cations such that its use adds ion pairing interaction to the separations

scheme with the prototype PCX-500 column. Acetate, a highly hydrated ion can be added to the mobile phase to aide salting-out effects.

Gradients for use with multi-phase columns are designed at two levels, as screening conditions and fine-tuning for difficult separations. As a screening tool a useful gradient system for the OmniPac PAX-500 column is 0.002 M - 0.100 M Na_2CO_3 and 5% - 80% acetonitrile in 10 min. After 10 min. the mobile phase can remain at the upper levels until no more peaks elute. This system is capable of eluting higher valency anions as well as fairly adsorptive species. As the ion exchange and reversed phase retention mechanisms operate independently in OmniPac -500 series columns, the ionic and solvating eluant components can be adjusted separately to resolve different types of coeluting analyte pairs. Retention behavior with different eluant and gradient systems can also be used to assign features to unknown analytes. For example, in the prototype PCX-500 column, an anionic analyte will elute earlier as an ionic gradient is made steeper although an amine-type analyte will be only effected by the solvent gradient.

A useful screening gradient for the prototype PCX-500 separations is 0.050 M HCl with a gradient of 0.10-0.40 M NaCl and 10-60% acetonitrile in 10 min. The rationale is similar to that described for PAX-500. The

Na^+ is a fairly strong eluting cation and can elute many divalent cations within 20 min. on this gradient. The HCl maintains weak base analytes such as amines in the protonated state.

Most gradients are designed so that the analytes elute during the ramp of the gradient. This technique yields the best peak efficiencies due to the band sharpening effects operating during gradient elution. In order to accomplish this, analytes that elute too early can be brought onto the gradient ramp by lowering the initial mobile phase concentrations. If some analytes elute too late, then the upper concentrations are increased if possible.

Fine-tuning gradients on multi-phase columns involves identifying the coeluting analytes if possible and then decreasing the concentration of the eluant component at the time of the coelution that is most likely to cause a difference between the analytes. For example, if two analytes coelute at 5.0 min. on a dual gradient of carbonate and acetonitrile, two approaches are possible. The chromatographer can decrease or increase the carbonate concentration or the acetonitrile concentration at 5.0 min. by 20% relative and observe the resulting elution behavior. This can be a pH adjustment to take advantage of pKa differences among analytes, a solvent adjustment to separate based on

adsorption characteristics or an eluting ion concentration change to separate based on ion exchange selectivity. These gradients will have a step at the time of a coelution.

APPLICATIONS

Barbiturates

Figs. 2a and 2b show the separations of barbiturate standards on OmniPac PAX-100 and PAX-500 respectively. Table 1 shows the structures of several barbiturates which undergo elution order reversal between the PAX-100 and the PAX-500 columns. Use of dual gradients, e.g. simultaneous carbonate and acetonitrile ramps, allows elution of analytes that are retained both by ion exchange and by adsorption.

The 5,5′-dialkyl substituted barbiturates have two pKa values, the first usually between 7 and 8 and the second between 12 and 13 (13). Definite pK_a values were found for several of the barbiturates listed in Table 1 (14). It is evident from these values that the elution order reversals observed in comparing Figs. 2a and 2b are not due to ion exchange effects resulting from the slightly different carbonate gradients.

The observed elution order reversals, e.g. between metharbital and allobarbital and among methohexital, secobarbital, mephobarbital and phenobarbital are due to

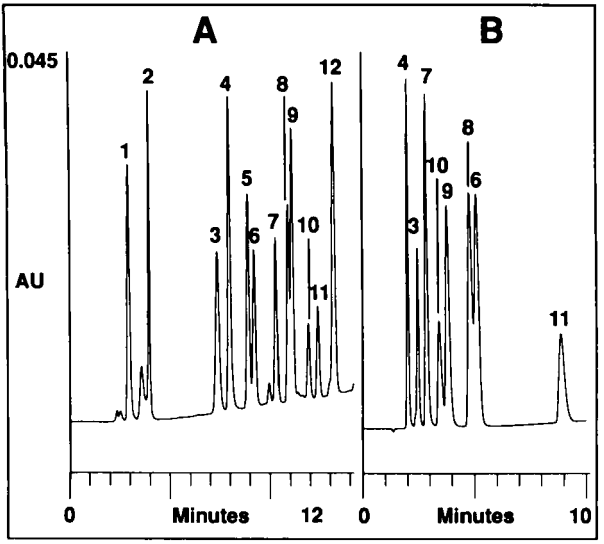


FIGURE 2a. Chromatogram of barbiturate standards on OmniPac PAX-500 column. Detection: UV 254nm; Flow rate: 1.0 mL/min; Peaks: 1, barbituric acid, 63ng; 2, barbital, 250ng; 3, allobarbital, 175ng; 4, metharbital, 188ng; 5, butabarbital, 125ng; 6, phenobarbital, 125ng; 7, amobarbital, 125ng; 8, mephobarbital, 125ng; 9, secobarbital, 125ng; 10, methohexital, 63ng; 11, diphenylhydantoin, 250ng; 12, thiamylal, 750ng. Eluant described in text.

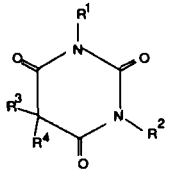
FIGURE 2b. Chromatogram of barbiturate standards on OmniPac PAX-100 column. Same detection and peak identification as in Fig. 2a. Eluant described in text.

the solvent elution of the more highly adsorbed barbiturates on the PAX-500 relative to the PAX-100, in which only ion exchange is operating.

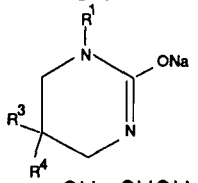
One feature of the analysis is that because the column packings are pH stable over the range of 0-14, use of high pH eluants is possible, eliminating the need for the addition of post column base (13). Post column

TABLE 1

Structures of Barbiturates Used in this Study

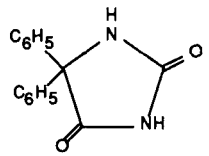


	R^1	R^2	R^3	R^4
Metharbital	CH_3	H	C_2H_5	C_2H_5
Allobarbital	H	H	$CH_2{=}CHCH_2$	$CH_2{=}CHCH_2$
Amobarbital	H	H	C_2H_5	$(CH_3)_2CHCH_2CH_2$
Mephobarbital	CH_3	H	C_6H_5	C_2H_5
Phenobarbital	H	H	C_2H_5	C_6H_5
Barbituric Acid	H	H	H	H
Barbital	H	H	C_2H_5	C_2H_5

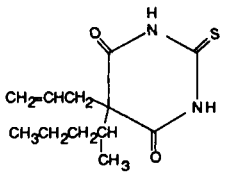


Secobarbital Na	H	–	$CH_2{=}CHCH_2$	$CH_3CH_2CH_2CH(CH_3)$
Methohexital Na	CH3	–	$CH_2{=}CHCH_2$	$CH_3CH_2C{\equiv}CCH(CH_3)$
Butabarbital Na	H	–	CH_3CH_2	$CH_3CH_2CH(CH_3)$

Diphenylhydantoin



Thiamylal



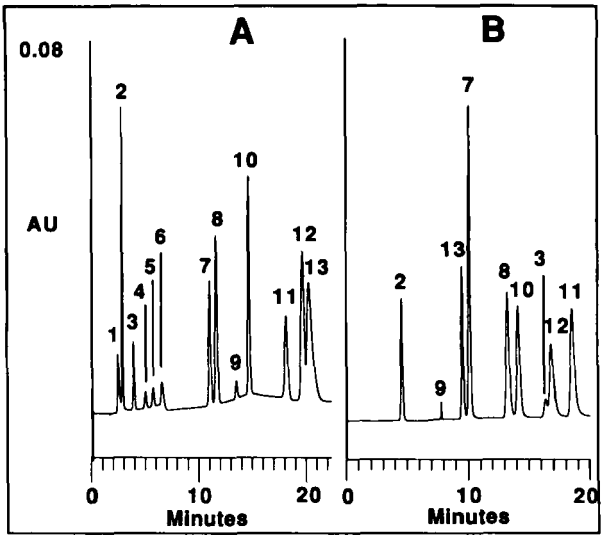


FIGURE 3a. Chromatogram of anti-inflammatory drug standards on OmniPac PAX-500 column. Detection: UV 254nm; Flow rate: 1.0 mL/min. Peaks: 1, impurity from aspirin; 2, aspirin; 3, ibuprofen; 4, impurity from ibuprofen; 5 and 6, impurities from indomethacin; 7, tolmetin; 8, naproxen; 9, unknown; 10, fenbufen; 11, indomethacin; 12, carprofen; 13, diflunisal. Eluant described in text.

FIGURE 3b. Chromatogram of anti-inflammatory drugs on the prototype OmniPac PCX-500 column. Same detection and peak identification as in Fig. 3a. Eluant described in text.

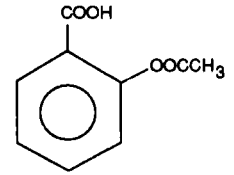
addition of base has been discussed in combination with reversed phase separations of barbiturates on ODS columns (15).

Anti-inflammatories

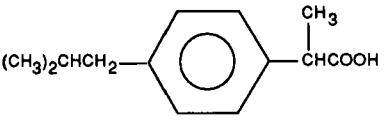
Many anti-inflammatory drugs are carboxylic acids and also possess enough hydrophobicity to be retained through reversed phase HPLC (Table 2). Fig. 3a

TABLE 2

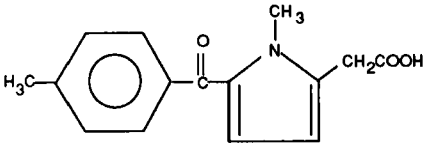
Structures of Anti-inflammatory Drugs Used in this Study



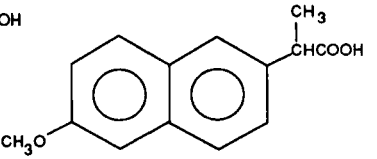
Aspirin



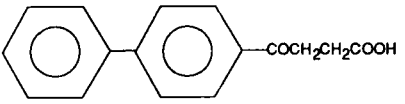
Ibuprofen



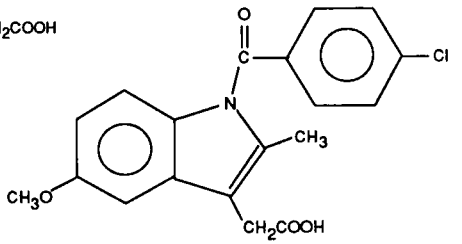
Tolmetin



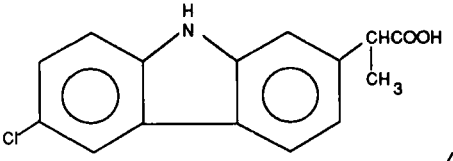
Naproxen



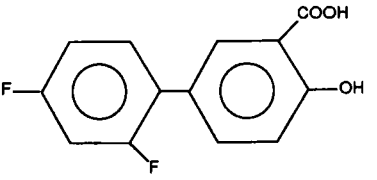
Fenbufen



Indomethacin



Carprofen



Diflunisal

shows the separation of 8 anti-inflammatory drugs through a combination of ion exchange and adsorption on OmniPac PAX-500. Of significance in this chromatogram is the wide range of structural properties among the analytes which can be eluted in about 20 minutes. In comparison, fewer of these drugs can be separated on the prototype OmniPac PCX-500 column (Fig. 3b) as the only retention mode operating is reversed phase adsorption, e.g. these drugs cannot be cationic. The elution order differences between these two chromatograms is due to the differences in retention modes operating in the two systems. The acid in the Fig. 3b eluant assures protonation of the carboxylic acids while the sodium acetate increases the ionic strength of the system enhancing adsorption-based retention.

Cephalosporin Antibiotics

Structures of cephalosporins indicate that they could be retained by cation exchange and/or adsorption. As these drugs are known to be less stable in base than in acid, separation on prototype PCX-500 was chosen as the column of choice, combining cation exchange and reversed phase in an acidic environment.

Fig. 4 shows the cation exchange and reversed phase separation of 10 cephalosporin antibiotics and precursors. The presence of the perchlorate in the eluant allows the ion pair separation of these drugs. Of

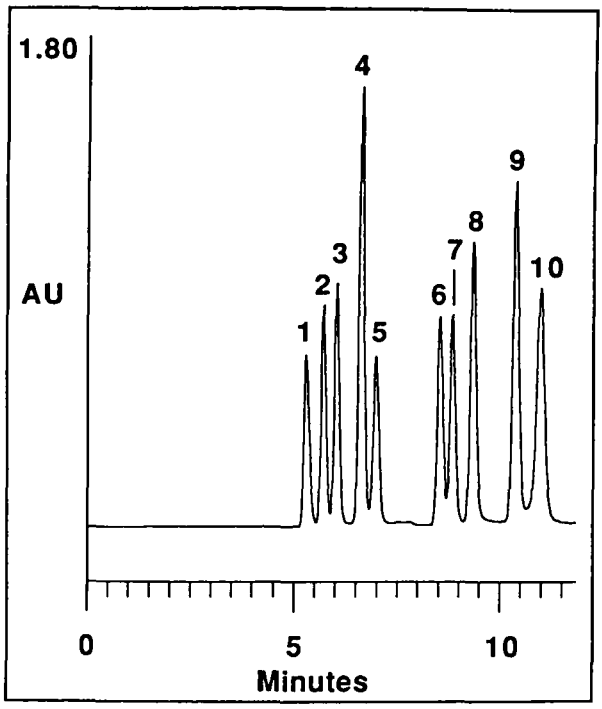


FIGURE 4. Chromatogram of cephalosporin antibiotics on the prototype OmniPac PCX-500 column. Detection: UV 254nm; Flow rate: 1.0 mL/min; Peaks: 1, D-hydroxyphenylglycine; 2, cephalosporin C; 3, 7-aminocephalosporanic acid; 4, cefadroxil; 5, cefazolin; 6, cephalexin; 7, cephapirin; 8, cefotaxime; 9, cephaloridine; 10, cephalothin. Eluant described in text.

significance in this chromatogram is the separation of cephalosporin C, 7-aminocephalosporanic acid and D-hydroxyphenylglycine, precursors of many of the antibiotics.

Sulfa Antibiotics

Sulfa drugs have sulfonamide structures. The pKa of the sulfonamide proton is in the vicinity of 2.7, making

these drugs easily retained by anion exchange. Most are also aromatic amines, making cation exchange another option and the ring structures essentially allow reversed phase adsorption.

Basically, sulfa drugs can be chromatographed on any of the columns under discussion in this paper. The choice of column is determined by sample matrix interferences etc. as the selectivity is different on each column. Figs. 5a, and 5b show the separation of sulfa drugs on prototype OmniPac PCX-500 and PAX-500 respectively. The elution reversal of sulfanilic acid and sulfanilamide between the PCX-500 and the PAX-500 columns is one example of the selectivity differences that can be used for specific applications. More sulfa drugs were separable on the PCX-500 than on the PAX-500 due to better selectivity by a combination of cation exchange and ion pairing for these structures. Interestingly, the elution order for this set of sulfa drugs in OmniPac PAX-100 is identical to that achieved on PAX-500. In other words, the ion exchange process is dominant for these analytes.

Assay Reduction

One possibility that multi-phase HPLC columns allow is the separation of analytes which can be retained by different retention modes. For example, timolol, a thiadiazole derivative is easily separated from its

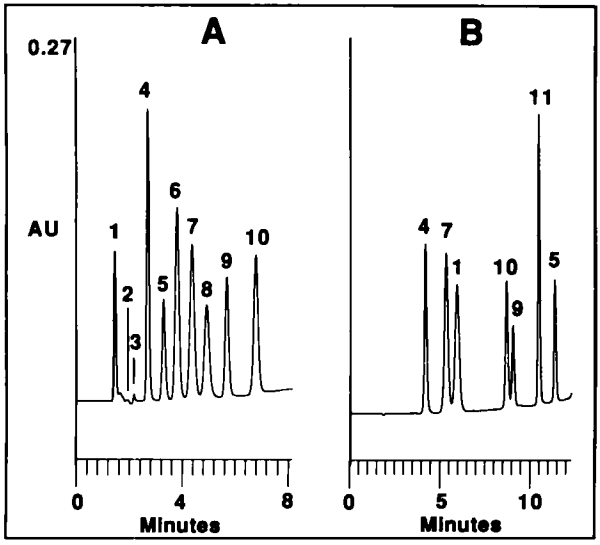


FIGURE 5a. Chromatogram of sulfa drugs on the prototype OmniPac PCX-500 column. Detection: UV 254nm; Flow rate: 1.0 mL/min; Peaks: 1, sulfanilic acid; 2 and 3, unknown; 4, sulfanilamide; 5, sulfathiazole; 6, sulfamethizole; 7, sulfamerazine ; 8, sulfamethazine, 9, sulfisoxazole; 10, sulfamethoxazole. Eluant as described in text.

FIGURE 5b. Chromatogram of sulfa drugs on OmniPac PAX-500. Peak identification as in Fig. 5a except peak 11, sulfamethizole. On-column injection for all analytes was 25 ng. Eluant described in text.

common divalent carboxylic acid counterion, maleate, using an isocratic eluant of 0.005 M Na_2CO_3 and 65% acetonitrile on PAX-500 in less than 4 min. The alkaline eluant maintains maleate in the anionic form for retention on the ion exchange latex while the timolol is retained by adsorption on the substrate particles and eluted by the acetonitrile.

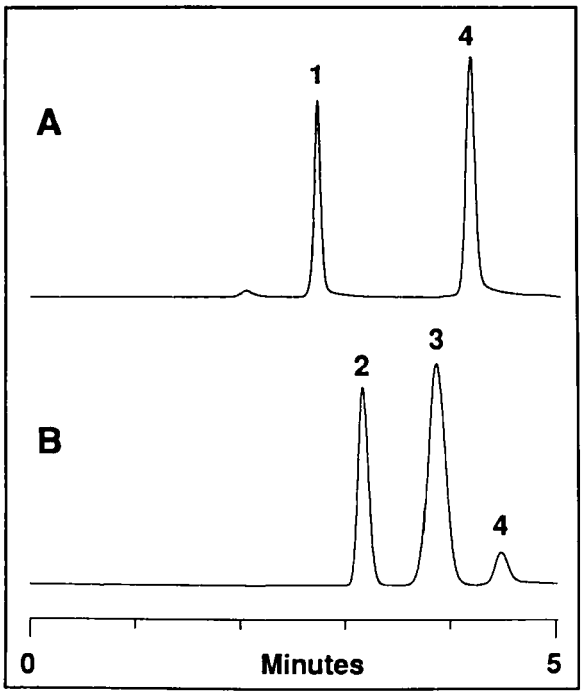


FIGURE 6a and 6b. Example of assay reduction. Column: prototype OmniPac PCX-500. Detection: 6a, UV 254nm; 6b Pulsed Amperometric;. Flow rate: 1.0 mL/min. Peaks: 1, p-toluenesulfonic acid; 2, 1,6-hexanediol; 3, 1,4-cyclohexanedimethanol; 4, cefazolin. Eluant described in text.

Another example utilizes two detectors in series. In this example (Figs. 6a and 6b) two diols and p-toluenesulfonic acid are retained by adsorption on the PCX-500 substrate while cefazolin, a cephalosporin antibiotic is retained by cation exchange and adsorption. All analytes are detected with the combination of UV and Pulsed Amperometric Detectors.

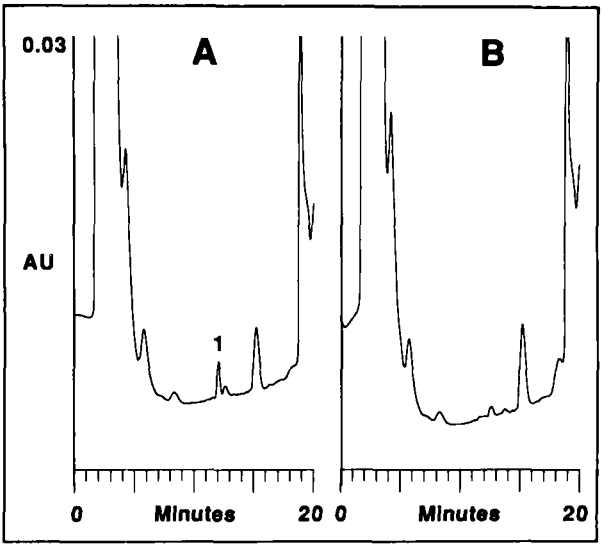


FIGURE 7a. Example of on-column sample preparation of rat urine. Column OmniPac PAX-500. Detection: UV 254 nm. Flow rate: 1.0 mL/min. Peak: acebutolol, 60ng. Eluant described in text.

FIGURE 7b. Blank chromatogram of rat urine used in Fig. 7a.

On-column sample preparation

The multi-mode nature of the PAX-500 and the prototype PCX-500 columns allows manipulation of sample matrix components as well as analytes. This allows on-column sample preparation which in some circumstances eliminates the need for solid phase extraction. Although this aspect of multi-mode chromatography remains under investigation, Fig. 7 is an example. Fig. 7 shows the analysis of acebutolol in rat urine using a PAX-500 column.

Gradients used for on-column sample preparation are designed to remove sample matrix interferences when the interferences differ from the analytes in hydrophobicity or ionizability. For example many UV-active components of urine are retained primarily by anion exchange while many drugs are nonionic at some pH. In the gradient used for Fig. 7 the injection is made when the column is equilibrated at a high carbonate level with no solvent in the eluant. The carbonate level is dropped and the solvent gradient is begun to elute the nonionic analytes such as adrenergic drugs.

SUMMARY

Multi-phase HPLC columns have been used for the analysis of a wide variety of polar and ionic organic species. The use of multi-phase OmniPac columns was studied using groups of pharmaceuticals and biomedical matrices because many analytes in these areas are polar and/or ionic in structure. The ability to use multiple retention mechanisms for separations of pharmaceuticals expands the selectivity-tuning "tool-box" available to the chromatographer. Multi-phase columns in combination with dual detectors also allow the combined analyses of structurally unrelated compounds such as anions, amine drugs and alcohols. Multi-mode capability also allows on-column sample preparation when the matrix interferences differ chromatographically from the analytes of interest.

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