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The high performance liquid chromatography electrospray ionization mass spectrometry analysis of diverse basic pharmaceuticals on cyanopropyl and pentafluorophenylpropyl stationary phases

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Abstract

Cyanopropyl (CN) and pentafluorophenylpropyl (PFPP) modified silica columns give good retention and good peak shape for the high performance liquid chromatography/electrospray ionization/mass spectrometry (HPLC/ESI/MS) analysis of several classes of basic drugs. These phases enhance the ESI-MS signal by providing good retention of basic drugs with a mobile phase containing 90% acetonitrile. With C18 columns, in order to achieve good retention of basic drugs, only a mobile phase containing less than 40% acetonitrile can be used. Higher concentrations of acetonitrile produce a larger MS signal in the ESI process; the MS signal was a factor of 9 and 12 times greater on the CN and PFPP phases when compared with the C18 phase for the analysis of codeine. The C18 phase required only 4.0–6.0% acetonitrile to obtain the same retention time for codeine. The CN and PFPP stationary phases can be used for the analysis of a range of basic drugs, including many compounds which are poorly retained on the popular C18 and C8 stationary phases. Applications of CN and PFPP columns in the HPLC/ESI/MS of basic drugs include the analysis of antimalarials, such as quinine, bronchodialators, such as salbutamol and tulobuterol, cardioactive drugs, such as procainamide and β-blockers, tricyclic antidepressants (TCAs), such as protriptyline and trimipramine and alkaloids, such as morphine and codeine. The CN and PFPP phases are also useful for the analysis of bufuralol and its metabolite, hydroxy-bufuralol. All the above analyses were performed using the same mobile phase, 90% acetonitrile; thus the HPLC method development process was expedited. The CN and PFPP phases also gave reproducible retention times and peak shape after more than 8 h of analyses.

Keywords: HPLC/MS; Stationary phases; Basic pharmaceuticals

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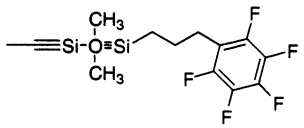
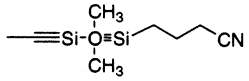
1. Introduction

Bonded C8 and C18 phases have been widely used for the HPLC analysis of basic drugs [1,2]. However, buffer salts and various additives, such as pairing- or counter-ions, which include alkylsulphonates, alkylamines or quaternary ammonium compounds, are often needed with these hydrophobic phases to achieve good peak shape and good retention [3,4]. In ESI-MS, volatile buffers and additives must be used; thus many conventional additives and ion-pairing agents must be avoided. Even when ion-pair agents, such as trifluoroacetic acid [5], or ion suppressing agents, such as triethylamine [6], are used the ESI-MS signal is decreased. To analyze drugs such as codeine or morphine by HPLC without the use of silanol suppressing agents is problematic because these basic molecules (pK_a 8.2 and 9.9, respectively) interact with the silanols to produce tailing peaks [7]. In addition, drugs such as these are hydrophilic; thus, with reversed-phase columns (C8 or C18), low concentrations ($< 10\%$) of organic solvent or ion-pairing agents must be used to provide adequate retention of the solutes [8]. However, it has been reported that when the concentration of the aqueous solvent in the mobile phase is increased, the ESI-MS signal is decreased [9–11]. Therefore, because of the poor peak shape and inefficient desolvation in the ESI process due to high concentrations of aqueous solvent when C18 columns are used, the overall MS response is highly reduced.



(Pentafluorophenylpropyl)=PFPP



Cyanopropyl=CN

Both phases were endcapped with trimethylchlorosilane

Fig. 1. Illustration of the stationary phases.

Ion suppression in the ESI interface has recently received much attention [12–15]. Ion suppression can occur when endogenous interferences are co-eluted with the analytes. These endogenous compounds can diminish the ionization of the analyte in the ESI interface. The final result is a reduced and imprecise MS signal. Improvements in the sample preparation [12] or the HPLC separation [14] or a combination of both can reduce ion suppression. It has been reported that capacity factors (k') greater than 4 were necessary to separate analytes from endogenous interferences and thus significantly decrease ion suppression [16]. Our goal is, therefore, to find HPLC stationary phases that provide good peak shape and good retention ($k' > 4$) for the HPLC/ESI/MS analysis of basic drugs using a mobile phase with high concentrations of organic solvent. We have investigated a number of stationary phases and found that cyanopropyl (CN) and pentafluorophenylpropyl (PFPP) stationary phases provided good peak shape and good retention for basic drugs with the use of 90% acetonitrile in the mobile phase [17,18]. By retaining the drugs with a high concentration of acetonitrile in the mobile phase, the CN and PFPP stationary phases provided signal enhancements greater than a factor of 10 when compared with a C18 stationary phase. We found other hydrophobic phases such as C8 and C4 had retention characteristics similar to C18 phases. However, only two classes of basic drugs were tested and it was not known whether the analyses obtained could be universally applied to all basic drugs. Thus, we report on the results of the use of a CN and PFPP stationary phase for the HPLC/ESI/MS analysis of a variety of basic drugs. Fig. 1 shows the structures of the CN and PFPP stationary phases.

2. Experimental

2.1. Reagents and standards

The drug, gepirone was obtained from Mr Bob

Behme of Scientific Resources, Inc. (Evansville, IN). Sumatriptan, bufuralol and hydroxybufuralol were obtained from Ms Jessica Dunn of Pfizer Inc. (Groton, CT). All other compounds were obtained from Sigma Chemical (St Louis, MO). Standard stock solutions (1.0 mg/ml) were prepared by dissolving a weighed amount of the compounds in H_2O/MeOH (90:10 v/v%). The solutions were sonicated in an Ultrasonicating Bath 3200 (Bransonic, Danbury, CT) for 10 min. Serial dilutions were made from the stock solutions to achieve the desired working concentrations of 0.05–10 μg/ml for the standards. All HPLC reagents (J.T. Baker, Phillipsburg, NJ) were of HPLC grade or better. Ammonium formate was obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA) and was of $>97\%$ purity. Formic acid was obtained from Acros (New Jersey) and was of 96% purity. All reagents used for synthesis of the stationary phases were obtained from Silar Laboratories (Scotia, NY). The PFPP reagent was obtained from Gelest, Inc. (Tullytown, PA). The reagents for the stationary phases were of $>97\%$ purity. All reagents were used without further purification.

2.2. HPLC columns

The HPLC columns were supplied by Restek Corporation (Bellefonte, PA) and were 3.0 or 1.0 cm length × 2.1 mm I.D. The stationary phases were monofunctional. Columns contained packings of 5 μm particles with 60 Å pores. The phases were endcapped by bonding with trimethychlorolsilane. All stationary phases are commercially available from Restek Corporation.

Capacity factors (k') were calculated using the following equation:

$$k' = (t_R - t_0)/t_0 \quad (1)$$

where t_R is the retention time of the analyte and t_0 is the retention time of the non-retained peak. The HPLC column hold-up time (t_0) was estimated by monitoring the first MS signal disturbance upon an injection. The hold-up time for all the HPLC columns was estimated to be 0.19 min.

The asymmetry factor (AF) was calculated at 10% peak height according to the following equation:

$$AF = a/b \quad (2)$$

where a is the tail of the chromatographic peak and b is the front of the peak.

2.3. HPLC conditions and apparatus

Two Jasco 980 series pumps (Tokyo, Japan) equipped with a vacuum membrane degasser delivered the mobile phase at a flow rate of 0.4 ml/min. The mobile phase consisted of mixtures of acetonitrile and 5 mM ammonium formate adjusted to pH 3.0 with formic acid. A CTC LEAP Technologies HTS PAL autoinjector (Carrboro, NC) injected 10 μl aliquots of the standards onto the HPLC columns.

2.4. Mass spectrometry

A Sciex API 3000 triple quadrupole mass spectrometer (PE-Sciex, Toronto, Ont.) equipped with a turbo ion spray interface (TISP) was used for the detection of analytes. Data was acquired in the positive ion mode with an ESI probe voltage of 5000 V. Nebulizer gas and curtain gas settings were 15 and 12 lb/in^2, respectively. The TISP interface was operated at a temperature of 150° C and a drying gas setting of 7000 ml/min. Data was collected by monitoring the ions in the selected ion monitoring (SIM) mode with window widths of 2–4 u. Multiple analyte detection was performed by scanning Q1 from 205–400 u. Scan times were 0.25–0.6 s/scan for the MS experiments. LC2Tune version 1.4, Sample Control version 1.4 and Multiview version 1.4 were used for data collection and analysis.

3. Results and discussion

Mass spectral, drug class and pK_a information for the solutes are shown in Table 1. All the solutes formed predominant protonated molecules $[M + H]^+$ in the ESI source.

Table 1
Major protonated molecular species observed from all solutes tested in positive ion ESI along with the drug class and pK_a information[a]

Drug	Drug class	pK_a	Protonated molecule $[M+H]^+$
Acebutolol	β-Blocker	9.2	337.2
Oxprenolol	β-Blocker	9.3	266.1
Protriptyline	Tricyclic antidepressant	10.7	264.1
Trimipramine	Tricyclic antidepressant	9.4	295.1
Quinine	Antimalarial	9.7	325.3
Salbutamol	Bronchodilaters	10.3	240.2
Tulobuterol	Bronchodilaters	10.4	228.6
Procainamide	Antiarrhythmic	9.2	236.3
Morphine	Analgesic (alkaloid)	9.9	286.2
Codeine	Analgesic (alkaloid)	8.2	300.3
Lidocaine	Anesthetic (local)	7.9	235.2
Procaine	Anesthetic (local)	8.9	237.3
Sumatriptan	Antimigraine	9.6	296.3
Buspirone	Anxiolytic	7.2	386.4
Gepirone	Anxiolytic	*	360.2
Bufuralol	Vasodialater	9.0	262.2
Hydroxy-bufuralol	Bufuralol metabolite	*	278.4

[a] Mobile phase consisted of mixtures of 90% acetonitrile and 5 mM ammonium formate, pH 3.0 at a flow rate of 0.4 ml/min. For those compounds with more than 1 pK_a just the highest pK_a value is reported.
* Information not found

To demonstrate the broad applicability of the CN and PFPP stationary phases for the HPLC/ESI/MS analysis of basic compounds, we chose basic drugs with a wide-range of characteristics. The basic drugs ranged in polarities, had a pK_a range from 7.2 to 10.7 and ranged in molecular weight from 227.7 to 385.5 Daltons. In addition, we chose bufuralol and the more polar metabolite, hydroxy-bufuralol to prove these phases useful for the HPLC/ESI/MS of drugs and their metabolites. The structures of the drugs are shown in Fig. 2.

Ninety percent by volume of acetonitrile was chosen as the mobile phase as a compromise to give adequate retention data and good dissolution of the solutes. The retention data are listed in Table 2. In this system a k' of 4 is equal to a retention time of 0.95 min. The CN and PFPP phase gave a $k' > 4$ for all the solutes tested with 90% acetonitrile mobile phase. Although, all solutes were eluted in the void volume when 90% acetonitrile was used with the C18 column, they had longer retention times on the CN stationary phase and even longer on the PFPP. With the C18 phase only 2–40% acetonitrile could be used to give a $k' > 4$ for the solutes. Polar solutes such as morphine are difficult to retain on C18 columns. For example, with many of the assays developed for morphine and other alkaloid compounds, the reversed-phase procedures require ion-pairing agents [19] or $>95\%$ aqueous mobile phase to provide adequate retention [8]. Neither ion-pairing reagents nor aqueous mobile phases produce optimum ESI-MS signal. In this study, morphine required $<2\%$ acetonitrile to obtain a retention time greater than 0.95 min on the C18 phase, whereas retention times of 1.51 and 2.83 min were obtained on the CN and PFPP stationary phases, respectively with the use of 90% acetonitrile. Typical chromatograms for the HPLC/ESI/MS analysis of morphine and quinine on a CN and PFPP stationary phase are shown in Fig. 3.

To demonstrate that improved peak shape is obtained on the CN and PFPP phase compared with a C18 phase, Fig. 4 shows the HPLC/ESI/MS analysis of procaine on all three phases. The asymmetry factor (AF) on the CN, PFPP and C18 phases are 0.97, 1.04 and 1.53, respectively for this analysis.

To illustrate that the CN and PFPP stationary phases are advantageous for solutes that are difficult to analyze by reversed-phase HPLC, we chose codeine as an example to show the increase in ESI-MS signal when the CN and PFPP phases are compared with a C18 phase. For the analysis of codeine the C18 phase required 4.0 and 6.0% acetonitrile in the mobile phase to obtain the same retention times on the CN ($t_R = 1.51$ min) and PFPP ($t_R = 2.83$ min) phases which used a mobile phase of 90% acetonitrile. The increased concentration of acetonitrile provides better desolvation in the ESI source and thus the MS signal was a factor of 9.4 and 12 times greater on the CN and PFPP phase, respectively, than the signal that had been achieved on the C18 phase in the

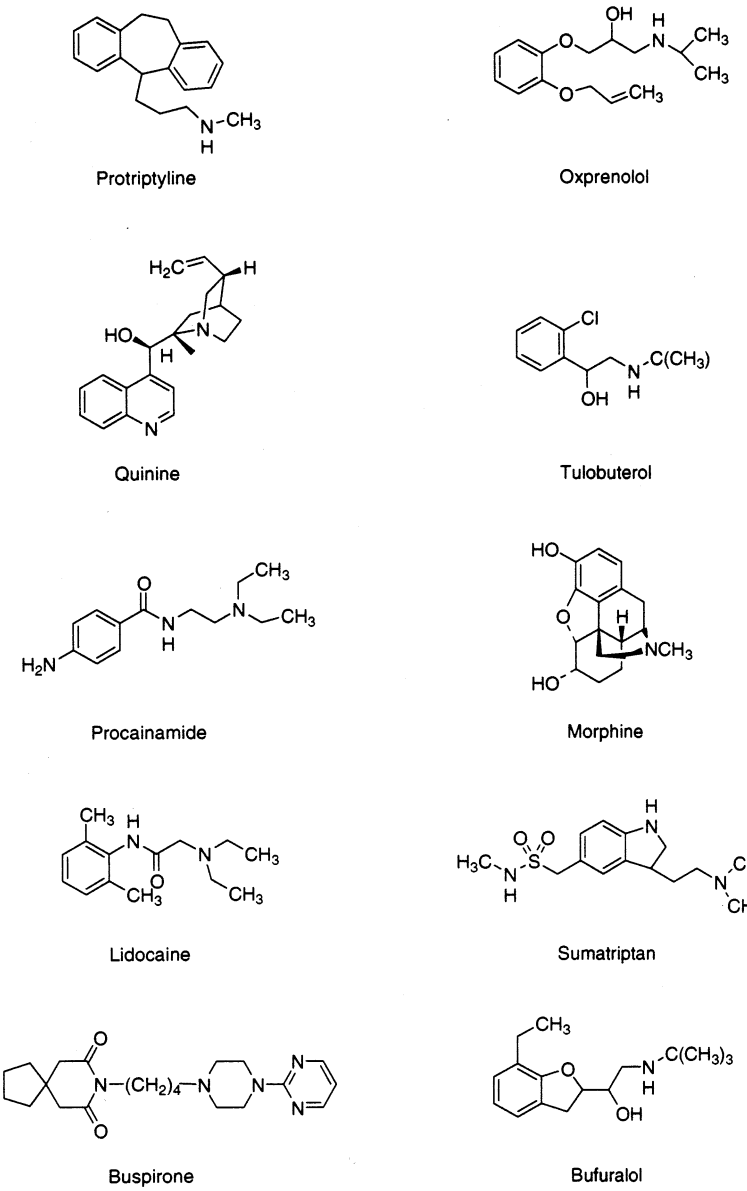


Fig. 2. Chemical structures of the solutes (one structure from each class of drugs).

Table 2
Retention data of basic solutes with 90% acetonitrile in the mobile phase[a]

Drug	Retention time (min)		
	CN	PFPP	C18
Acebutolol	1.14	2.23	<0.25
Oxprenolol	1.35	3.41	<0.25
Protriptyline	1.78	5.84	<0.25
Trimipramine	1.86	7.29	<0.25
Quinine	1.32	4.24	<0.25
Salbutamol	1.18	1.98	<0.25
Tulobuterol	1.46	3.78	<0.25
Procainamide	1.36	2.59	<0.25
Morphine	1.17	2.57	<0.25
Codeine	1.51	2.83	<0.25
Lidocaine	1.02	3.05	<0.25
Procaine	1.45	3.26	<0.25
Sumatriptan	1.18	3.08	<0.25
Buspirone	1.14	3.47	<0.25
Gepirone	1.09	3.21	<0.25
Bufuralol	1.64	6.0	<0.25
Hydroxy-bufuralol	1.45	3.83	<0.25

[a] Mobile phase consisted of ACN/5 mM ammonium formate, pH 3.0 (90/10) at a flow rate of 0.4 ml/min.

HPLC/ESI/MS (Fig. 5). All ESI-MS signal enhancement measurements were performed in triplicate. For the analysis of 1.0 ng of codeine, the ESI-MS signal was 9.23×10^5 counts per second (cps) for the CN phase and 9.87×10^4 cps for the C18 phase. The ESI-MS signal was 6.71×10^5 cps and 5.77×10^4 cps on the PFPP and C18 phases, respectively, for the analysis of 1.0 ng of codeine. Further enhancement of the MS signal is possible with the use of 100% acetonitrile in the mobile phase. However, the solutes had irreproducible retention times with the use of 100% acetonitrile, possibly due to poor dissolution.

Combinatorial chemistry has caused an exponential increase in the numbers of new chemical entities that are synthesized each year in the pharmaceutical industry. These vast numbers of compounds need to be screened using analytical chemistry techniques. Due to the generality and selectivity of ESI-MS, this analytical technique is often the detection system of choice [13]. Presently the analysis times are so rapid (often < 6 min per injection) with HPLC/ESI/MS that the most time consuming step is usually the development of the HPLC method. Often with C18 phases, the mobile phase composition (% organic and pH) is adjusted accordingly to obtain the desired retention times of the solutes [20]. Others have reported that gradient HPLC/MS with a C18 phase can be used successfully on many classes of pharmaceutical compounds [21]. However, poor peak shapes are still obtained for compounds with basic moieties and the polar solutes are not always retained well on C18 stationary phases. The data we present in this report shows that with one

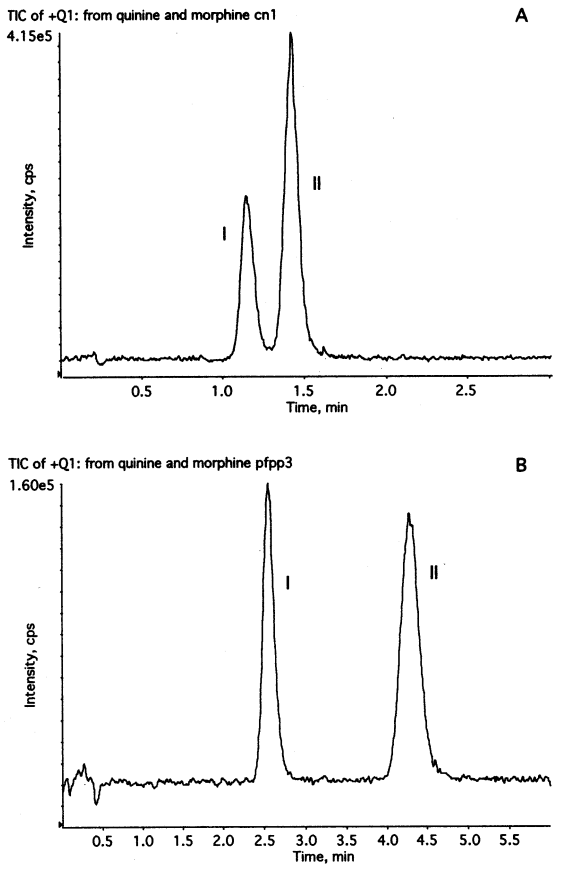


Fig. 3. Typical chromatograms for the HPLC/ESI/MS analysis of quinine and morphine on a CN and PFPP phase with 90% acetonitrile in the mobile phase (10% 5 mM ammonium formate, pH 3.0, 0.4 ml/min). (A) Chromatogram for the HPLC/ESI/MS analysis of morphine (I) and quinine (II) on a CN stationary phase. (B) Chromatogram for the HPLC/ESI/MS analysis of morphine (I) and quinine (II) on a PFPP stationary phase.

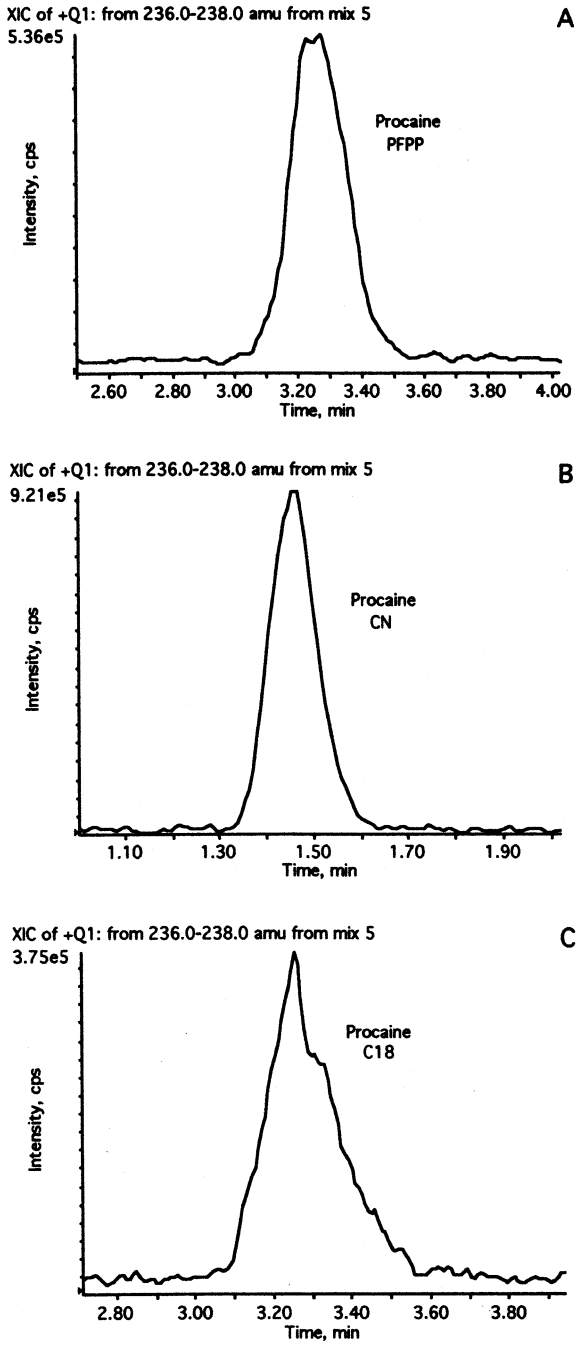


Fig. 4. Chromatograms to compare the peak shape for the HPLC/ESI/MS analysis of procaine by the use of mixtures of acetonitrile/5 mM ammonium formate, pH 3.0 on a CN, PFPP and C18 phase at 0.4 ml/min.

isocratic mobile phase composition, solutes were eluted with good peak shape with retention times between 1.09 and 7.3 min on a CN or PFPP stationary phase with the use of 30×2.1 mm columns. By significantly reducing the need to adjust the mobile phase composition to achieve the desired retention and peak shape of the solutes, the CN and PFPP stationary phases expedite the development of HPLC methods on an ESI-MS system when compared with a C18 stationary phase.

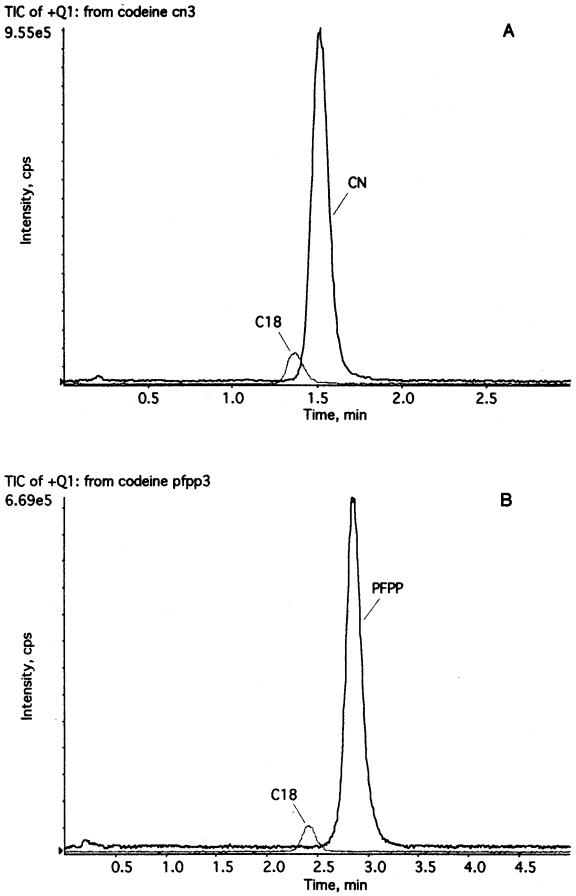


Fig. 5. The increase in the ESI-MS signal when phases are used that give retention of solutes at higher acetonitrile concentrations than hydrophobic chain phases. (A) Similar retention yet increased signal on a CN phase (90% acetonitrile) compared with a C18 phase (6% acetonitrile) for the HPLC/ESI/MS analysis of codeine ($\sim$ 1 ng) at 0.4 ml/min. (B) Similar retention yet increased signal on a PFPP phase (90% acetonitrile) compared with a C18 phase (4% acetonitrile) for the HPLC/ESI/MS analysis of codeine ($\sim$ 1 ng) at 0.4 ml/min.

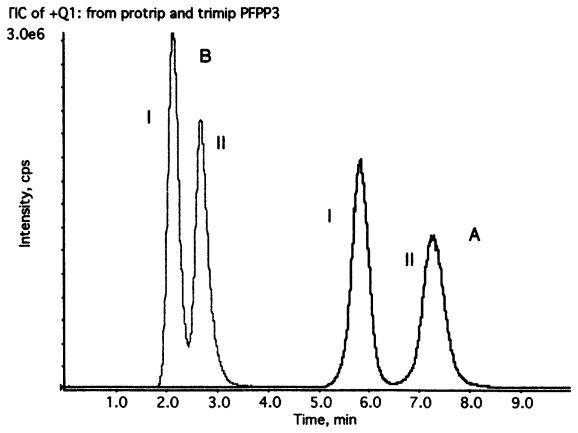


Fig. 6. The reduction of analysis times by the use of shorter columns at 0.4 ml/min on a PFPP phase. (A) 3 cm × 2.1 mm I.D. column I = trimipramine, II = protriptyline. (B) 1 cm × 2.1 mm I.D. column I = trimipramine, II = protriptyline.

With the use of one mobile phase composition, the range of retention times of the solutes was a compromise between high-speed analysis with adequate retention to separate the solutes from endogenous interferences that can cause ion suppression. However, if retention times are too long when 90% acetonitrile is used, than higher flow rates or shorter columns (1 cm length) can be used to shorten the analysis time for each set of particular compounds. Since the TCAs had the longest retention times on the PFPP phase, chromatograms for the HPLC/ESI/MS analysis of protriptyline and trimipramine at 0.4 ml/min on a PFPP phase with 3 and 1 cm × 2.1 mm I.D. columns were compared in Fig. 6. The time for the analysis of protriptyline and trimipramine was decreased from about 8.5 min per sample to 3.5 min per sample.

The CN and PFPP phases must provide reproducible results if these phases are to be accepted for routine use. As shown in Fig. 7, the peak shape, MS signal and retention times of sumatriptan on the CN and PFPP phases are consistent after over 8 h (> 2500 column volumes) of analyses.

Since the increase in the ESI-MS signal in this research is due to an improvement in the desolvation process in the interface, operation of the mass spectrometer in the MS/MS mode produces similar signal enhancements when the acetonitrile concentration in the mobile phase is increased.

4. Conclusions

The CN and PFPP phases offer significant advantages when compared with C18 or C8 phases for the HPLC/ESI/MS of basic drugs. By retaining polar, basic drugs with 90% acetonitrile in the mobile phase, the ESI-MS signal is enhanced by over a factor of 9 when compared with a C18 phase which required 6.0% acetonitrile for the analysis of a model solute, codeine. The improvement in signal is due to more efficient desolvation

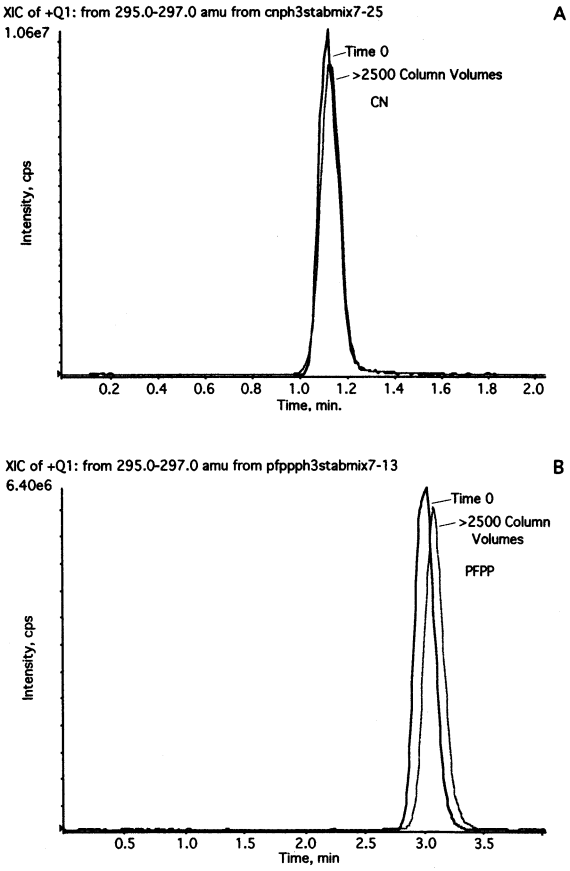


Fig. 7. Overlayed chromatograms to show the reproducibility of the CN (A) and PFPP (B) columns by the injection of 3.5 ng of sumatriptan after more than 2500 column volumes (> 8 h) of mobile phase.

in the ESI source with the use of higher concentrations of acetonitrile. Since the ESI-MS signal is enhanced when the CN and PFPP phases are used, these phases will be useful for the low-level detection of drugs, metabolites and impurities in various matrices. In addition, one mobile phase composition provided good retention and good peak shape of many different classes of basic drugs. All the basic drugs were eluted with retention times between 1 and 7 min. These general conditions are possible on both the CN and PFPP phases and offer advantages over C18 phases which often require adjustment of the mobile phase composition for each class of solutes. The use of one mobile phase composition leads to faster HPLC method development that is often a rate-limiting step in a HPLC/ESI/MS analysis. The CN and PFPP phases also show reproducible retention times and peak shape for over 8 h of analyses.

To determine the widespread application of the CN and PFPP phases, the use of such phases for assay validation of basic pharmaceuticals in various matrices is currently under investigation.

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