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Evaluation of ethoxynonafluorobutane as a safe and environmentally friendly solvent for chiral normal-phase LC-atmospheric pressure chemical ionization/electrospray ionization-mass spectrometry

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Abstract

Coupling normal-phase LC separation methods to atmospheric pressure ionization (API)-mass spectrometry (MS) for detection can be problematic because of the possible detonation hazard and because nonpolar solvents do not support ionization of the analyte. Unlike achiral separations, enantiomeric separations can be very sensitive to small changes in the separation environment. Thus, completely substituting the main mobile phase component of a normal-phase LC solvent for an environmentally friendly, nonflammable fluorocarbon-ether as a safe and effective solvent must be thoroughly evaluated before it can be recommended for enantioselective separations with API-MS detection. Ethoxynonafluorobutane (ENFB) was used as a normal-phase solvent for the enantioselective separation of 15 compounds on two macrocyclic glycopeptide chiral stationary phases (CSPs) and a new polymeric chiral stationary phase. The chromatographic figures of merit were compared between results obtained with the ENFB mobile phases and traditional heptane-based mobile phases. In addition, the limits of detection (LOD) using the API-MS compatible ENFB were examined, as well as flow rate sensitivities and compatibilities with common polar organic modifier. ENFB is a safe and effective solvent for enantioselective normal-phase/API-MS analyses.

Keywords: LC–MS; Enantiomeric separations; Green solvent; Teicoplanin; Vancomycin; Fluorocarbon solvent

1. Introduction

The chiral nature of enormous number of compounds contributes to their bioactivity and/or their various pharmaceutical/industrial uses. As a result, the Food and Drug Administration (FDA) has implemented policies for analyzing the enantiomers of chiral compounds [1]. Considerable research effort has been directed towards the optimization and validation of new, fast and feasible analytical methods for the determination of the chiral compounds of interest present in pharmaceutical formulations or in complex matrices such as the biological fluids. The vast majority of existing chiral separation techniques utilize high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection [2,3]. However, the limitations of UV detection, including poor sensitivity for non-UV absorbing compounds and lack of specificity, have motivated scientists to pursue other alternatives for enantioselective analysis. Mass spectrometry (MS) detection is such a candidate. Higher sensitivity, better detection limit and the ability to provide direct molecular weight information make mass spectrometry an ideal tool as an "information rich" detection method for enantioselective separations.

Practically, reverse-phase (RP) LC is the dominant separation mode in HPLC–MS analysis. This is, at least in part, due to the incompatibility between the usual normal-phase (NP) solvents such as *n*-hexane and *n*-heptane (Hep), and MS ionization sources, i.e., electrospray ionization (ESI) which can pose an explosion hazard [4]. Additionally, alkane solvents do not readily facilitate the formation of ions from ionization sources such as ESI [5]. Many enantioselective LC methods rely on bonded or coated chiral stationary phases (CSPs) and conventional normal-phase separation systems

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that utilize *n*-hexane or *n*-heptane mobile phases to achieve enantioselective separations. To overcome the problem of incompatibility between traditional normal-phase LC solvents and MS, a number of studies have employed post-column addition of MS-compatible polar organic or aqueous solvents [6–8]. Nevertheless, post-column addition can substantially reduce the sensitivity of an assay via dilution, which could be detrimental when the sample is limited. Also, massive post-column dilution can affect chromatographic resolution. Recently, a few reports have appeared which indicate that normal-phase solvents, such as hexane sometimes can be coupled with APCI-MS, with caution [9–11].

Recently, Kagan proposed the use of ethoxynonafluorobutane (ENFB), an environmentally friendly, fluorinated solvent, as an alternative to *n*-hexane for achiral normal-phase LC separations of various compounds, including steroids and benzodiazapines [12]. Separations with ENFB were found to be comparable to those where *n*-hexane was used as the main component of the mobile phase. In a follow-up communication, Kagan et al. [13] demonstrated the compatibility of ENFB for LC–APCI-MS using the same compounds. As expected, the detector response for non-polar compounds was stronger for ENFB mobile phases using APCI compared to reversed-phase mobile phase systems using ESI. For polar compounds, APCI and ESI ionization efficiencies were comparable [13]. Based on this NP–HPLC–APCI-MS method, they proposed a novel mass-directed NP preparative HPLC approach to auto-purify a wide variety of organic compounds [14]. This provided a practical alternative to the most commonly used preparative RP-HPLC approach.

Only a few examples of enantiomeric separations using normal-phase LC coupled with either ESI-MS [6,7] or APCI-MS [8–14] have been reported in the literature. As mentioned previously, post column addition of other MS friendly solvents (e.g., alcohols) was used to reduce the explosion hazard in most cases. Macrocyclic glycopeptide based chiral stationary phases, teicoplanin [15–18] and vancomycin [19,20] have been successfully used for the enantioselective separation of a variety of chiral compounds. The multi-modal capability of these stationary phases has enabled them to seamlessly integrate with LC–MS detection for reversed-phase and polar organic mode separations [16,17,20]. In addition to these modes, they can be used effectively for normal-phase chiral separations. In the following NP–HPLC–APCI-MS and NP–HPLC–ESI-MS studies, ethoxynonafluorbutane is directly substituted for *n*-heptane, without optimization of the chromatographic parameters, for the enantioselective separation of various compounds using macrocyclic glycopeptide stationary phases as well as a recently developed polymeric chiral stationary phase [21,22].

2. Experimental

2.1. Reagents and solvents

All racemic compounds were purchased from Sigma-Aldrich (St. Louis, MO), except phensuximide, 3a,4,5,6-tetrahydrosuccinimido-(3,4-b) acenaphthen-10-one that were donated by Astec (Whippany, NJ), and phenyl allyl sulfoxide, allyl methyl sulfoxide, 2-(allylsulfinyl)-ethanol and diphenylmethyl phenyl sulfoxide which were kindly donated by Prof. William Jenks of Iowa State University. Ethoxynonafluorobutane was purchased as Novec^{TM} Engineered Fluid HFE-7200 from 3M Co. (St. Paul, MN). Its physical properties are listed in Table 1 [23]. HPLC grade *n*-heptane, methanol (MeOH) and 2-propanol (IPA) were acquired from Fisher (Pittsburgh, PA). Hundred percent pure ethyl alcohol (EtOH) was purchased from Apper Alcohol (Shelbyville, KY). All compounds were dissolved in 100% IPA and diluted to 100 $\mu g\,ml^{-1}$ prior to injection.

2.2. HPLC and MS instrumentation

A HP 1050 HPLC system (Agilent Technologies, Palo Alto, CA) with a UV VWD detector, an auto sampler, and computer controlled Chem-station data processing software was used for chromatographic separations employing heptane and ethanol as the mobile phase. UV detection was carried out at 254 nm for all the compounds except for allyl methyl sulfoxide and 2-(allylsulfinyl)-ethanol which were detected at 220 nm.

Table 1
Selected properties of ENFB, *n*-hexane and *n*-heptane

	HFE-7200[a]	*n*-Hexane[b]	*n*-Heptane[b]
Formula	$C_4F_9OC_2H_5$	$CH_3(CH_2)_4CH_3$	$CH_3(CH_2)_5CH_3$
Molecular wt.	264	86	100
Boiling point (°C)	76	69	98.5
Freeze point (°C)	−138	−25	−3
Flash point (°C)	None	−22	−4
UV cutoff (nm)	220	191.5	198
Density (g/ml at 25 °C)	1.43	0.66	0.68
Vapor pressure (mmHg at 25 °C)	109	151	46
Viscosity (cps at 25 °C)	0.61	0.48	0.57
Surface tension (dynes/cm at 25 °C)	13.6	17.9	19.6

[a] Data from manufacturer (see ref. [19]).
[b] Data from http://www.sigmaaldrich.com except surface tension (see ref. [25]).

Two pumps (LC-10AD, Shimadzu, Kyoto, Japan), a Shimadzu mixer and a six-port injection valve equipped with a sample loop (5 μl, Rheodyne, Cotati, CA) coupled to a Thermo Finnigan (San Jose, CA) LCQ Advantage API ion-trap mass spectrometer with an APCI or ESI ion source was used for NP–HPLC–MS analyses. The entire flow from HPLC column was directed to the ion source. The MS was operated in positive ion mode using full scan mode first to identify the product ion which then can be monitored by selected ion monitoring (SIM) mode for each compound. Nitrogen (Praxair, Danbury, CT) was used as both sheath and auxiliary gases. Ultra-high purity helium (Linweld, Lincoln, NE) was used as the dampening gas in the ion trap.

2.3. Columns and mobile phases

Separations were carried out at room temperature on 250 mm × 4.6 mm i.d. Chirobiotic V or Chirobiotic T chiral columns from Astec (Whippany, NJ) or the SS-PCAP column (developed in-house) [22]. The SS-PCAP (250 mm × 4.6 mm i.d.) is a poly (*trans*-1,2-cyclohexanediamine acrylamide) stationary phase having a particle size of 5 μm and was obtained from Astec. For UV detection, the mobile phase only consisted of *n*-heptane and ethanol. For MS detection, the normal-phase mobile phase systems contained ENFB with ethanol, methanol, or IPA as the organic modifier. Mobile phase flow-rates were 1.0 ml min^{-1} unless otherwise noted.

2.4. Ionization and MS acquisition conditions

The column eluent was introduced directly into the APCI source operated under the following set of conditions: corona discharge current, 5.00 μA; sheath and auxiliary gases were 80 and 20 arbs (arbitrary units), respectively; vaporizer temperature, 400 °C; capillary temperature, 200 °C. For ESI mode, the operation conditions were: voltage, +4.50 KV; sheath and auxiliary gases were 50 and 40 arbs, respectively; capillary temperature, 300 °C. MS data were acquired using Xcalibur software Version 3.1 available from Thermo Finnigan.

3. Results and discussion

3.1. Using the MS-compatible normal-phase solvent, ENFB (HFE-7200)

Novec™ Engineered Fluid HFE-7200 (ENFB) was originally developed by 3 M Co. as a cleaning fluid, deposition solvent and heat transfer fluid [23]. HFE-7200 is an azeotropic mixture of ethyl nonafluoroisobutyl ether and ethyl nonafluorobutyl ether with similar properties (Fig. 1). The environmentally friendly properties of this solvent include zero ozone depletion potential and a low atmospheric lifetime of 0.77 years [23]. The boiling point and solvent strength of

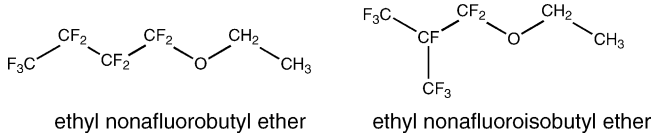

Fig. 1. Structure of ENFB (HFE-7200).

HFE-7200 are similar to those of *n*-hexane [12]. The viscosity and UV cutoff are slightly lower for *n*-hexane. Nevertheless, HFE-7200 has no flashpoint and low flammability, which makes it ideal for use with atmospheric pressure ionization sources (APCI and ESI) with MS detection. A comparison of the physicochemical properties of HFE-7200 (ENFB) with those of *n*-hexane and *n*-heptane are given in Table 1. According to the manufacture (see Section 2), it is completely compatible with Teflon, Peek, and Tygon tubing [23], allowing its use with most LC systems. However, we found that two small parts of our LC system were dissolved and/or damaged by ENFB. They are: the degas tubing of the Thermo Finnigan Surveyor LC pump and pressure sensor membrane on the Shimadzu LD-10A pump. They are both made from halogen containing polymers. These materials should be replaced when using ENFB containing mobile phases.

It is well-known that even small, seemingly insignificant changes in the mobile phase can adversely affect the selectivity of enantiomeric LC separations [20]. Indeed, changes in separation conditions that result in only small changes in routine achiral LC can totally negate or greatly diminish some enantiomeric separations. Consequently, the effect of substitution of a fluorocarbon ether solvent (ENFB) for the *n*-hexane/*n*-heptane component in an enantioselective normal-phase LC separation must be thoroughly evaluated for variety of compounds before it can be recommended as a viable alternative mobile phase. The analytes used in this study are shown in Fig. 2. All compounds were analyzed using the full scan mode in order to first pick up the appropriate *m*/*z* values for use in the selected ion monitoring mode. The $[M+H]^+$ ion was monitored in the SIM mode for each compound with the exception of diphenylmethyl phenyl sulfoxide. This particular compound fragments, as shown in Fig. 2, so that the 167 *m*/*z* was monitored.

The chromatographic separation parameters for *n*-heptane mobile phases versus ENFB substituted mobile phases are listed in Table 2. A majority of the compounds tested had slightly smaller resolutions (R_s) but similar selectivities (α) when ENFB was substituted for *n*-heptane as the main component of the mobile phase without optimization. Better resolutions could be achieved by altering the mobile phase composition. Nonetheless, all compounds studied yielded lower peak efficiencies (*N*) when ENFB-based mobile phases were used with MS detection (Table 2). The possible causes for this include: (a) extra-column band broadening as a result of interfacing with the MS detector (which occurs regardless of the mobile phase used), and/or (b) the higher viscosity of ENFB which can produce less efficient separations at higher flow rates as a result of poorer mass transfer of the analytes.

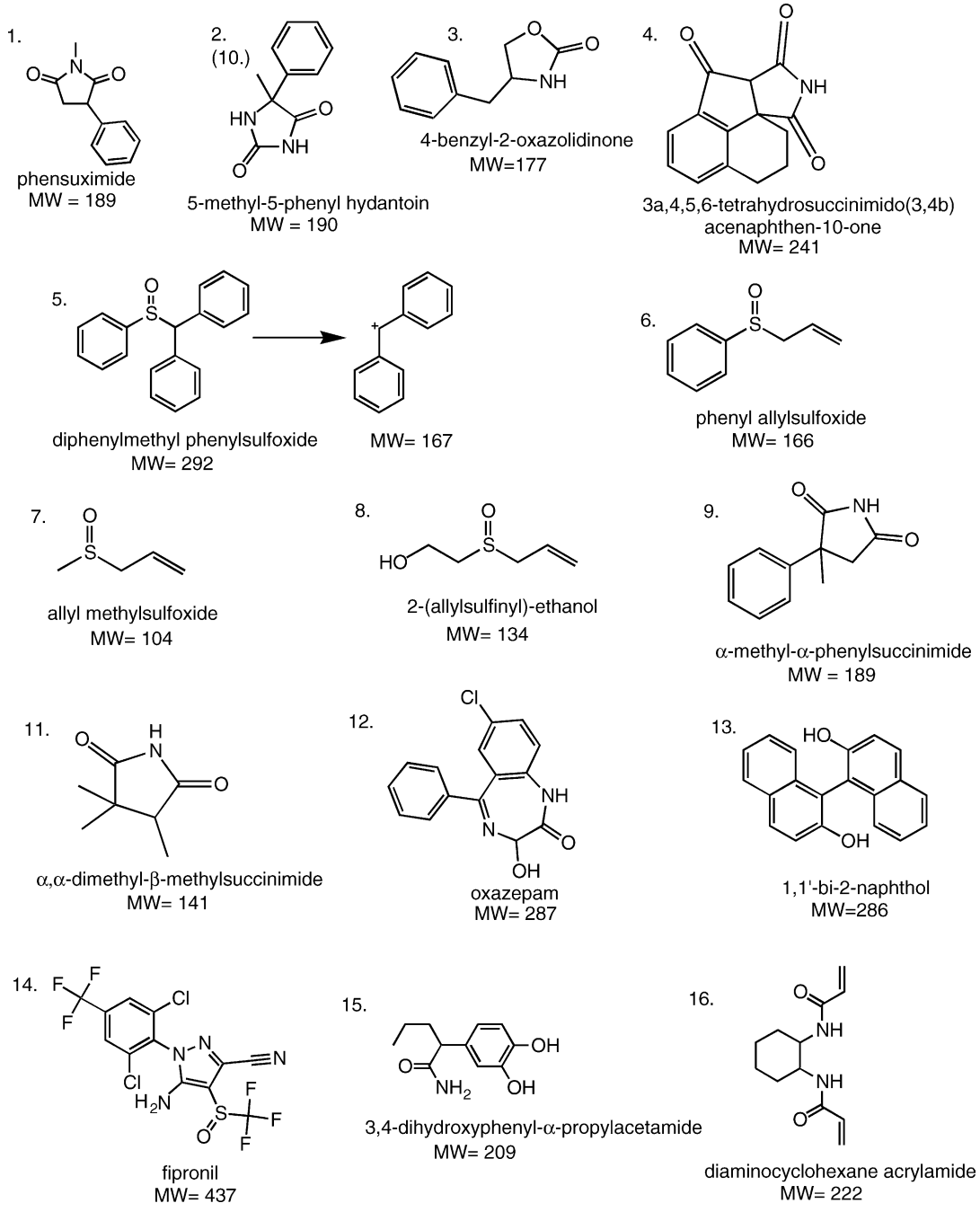

Fig. 2. Structures and molecular weights for compounds analyzed.

Table 2 also shows that all the compounds can be detected by APCI-MS, while three of them failed to be detected by ESI-MS. This is because that ESI is a softer ionization source than APCI, which sometimes limits its use when coupled with a normal-phase LC separation.

For comparison purposes, the enantiomeric separations of 5-methyl-5-phenylhydantoin, 3a,4,5,6-tetrahydrosuccinimido-(3,4-b)acenaphthen-10-one, and fipronil using ENFB (with APCI-MS detection) or *n*-heptane (with UV detection) are shown in Fig. 3. With similar volume ratios of *n*-heptane or ENFB to modifier, the peak shapes and retention times are comparable regardless of which stationary phase was utilized. The results clearly demonstrated that in most cases ENFB can be substituted for *n*-heptane with minimal effects on chromatographic retention while the other chromatographic parameters can be optimized by altering the composition of the mobile phase accordingly. A more detailed examination of the effects of substitutions of ENFB for *n*-heptane in enantioselective normal-phase LC separations follows.

Table 2
Comparison of chromatographic parameters for heptane versus ethoxynonafluorobutane substituted mobile phases

#	Compound name	Original M.P. (UV, 254 nm [a])	Flow rate (ml/min)	Chromatographic parameters				HFE-7200 M.P.[b] (APCI)	Chromatographic Parameters				HFE-7200 M.P.[b] (ESI)	Chromatographic parameters			
				k'_1	R_s	N_1	α		k'_1	R_s	N_1	α		k'_1	R_s	N_1	α
Chirobiotic V																	
1	Phensuximide	70:30 Hep:EtOH	0.5	2.15	1.56	8500	1.11	95:5 HFE:EtOH	5.47	1.41	1200	1.15	90:10 HFE:EtOH	2.16	1.29	3700	1.13
2[c]	5-Methyl-5-phenylhydantoin	100% EtOH	1.5	1.00	2.48	2600	1.61	100% EtOH	0.88	1.60	600	1.58	N/D				
3	4-Benzyl-2-oxazolidinone	70:30 Hep:EtOH	1	2.98	2.55	6500	1.20	70:30 HFE:EtOH	2.18	1.63	1500	1.30	75:25 HFE:EtOH	2.87	2.74	4000	1.27
4	3a,4,5,6-Tetrahydrosuccinimide(3,4-b) acenaphthen-10-one	75:25 Hep:EtOH	1	5.88	1.50	4500	1.14	75:25 HFE:EtOH	4.63	1.44	2300	1.17	75:25 HFE: EtOH	4.70	1.40	2400	1.15
5	Diphenylmethyl phenyl sulfoxide	90:10 Hep:EtOH	1	2.10	1.28	7400	1.09	90:10 HFE:EtOH	3.86	1.56	2900	1.16	90:10 HFE:EtOH	3.80	1.60	3000	1.14
Chirobiotic T																	
6	Phenyl allyl sulfoxide	90:10 Hep:EtOH	1	5.98	1.73	6900	1.11	95:5 HFE:EtOH	8.75	1.22	3400	1.10	90:10 HFE:EtOH	3.58	0.83	1450	1.11
7	Allyl methyl sulfoxide	75:25 Hep:EtOH	1	6.96	1.89	5300	1.13	75:25 HFE:EtOH	5.40	1.44	3600	1.13	75:25 HFE:EtOH	5.30	1.39	3400	1.13
8	2-(Allylsulfinyl)-ethanol	75:25 Hep:EtOH	1	6.81	2.96	5800	1.23	75:25 HFE:EtOH	7.53	1.58	1800	1.17	75:25 HFE:EtOH	7.42	1.55	1600	1.19
9	α-Methyl-α-phenyl succinimide	50:50 Hep:EtOH	1	0.95	1.44	4600	1.25	60:40 HFE:EtOH	1.75	1.60	1900	1.23	60:40 HFE:EtOH	1.77	1.64	1700	1.30
10[c]	5-Methyl-5-phenylhydantoin	50:50 Hep:EtOH	1	2.65	4.81	1000	3.01	50:50 HFE:EtOH	3.62	4.38	400	2.49	N/D				
11	α,α-Dimethyl-β-methylsuccinimide	70:30 Hep:EtOH	1	1.48	1.18	7800	1.10	95:5 HFE:EtOH	6.00	1.13	4900	1.10	N/D				
SS-PCAP																	
12	Oxazepam	50:50 Hep:EtOH	1.5	5.28	3.62	1800	1.51	40:60 HFE:EtOH	5.60	2.24	1600	1.45	40:60 HFE:EtOH	5.50	2.45	800	1.53
13	1,1′-Bi-2-naphthol	50:50 Hep:EtOH	1	5.16	3.00	3300	1.29	50:50 HFE:EtOH	3.29	2.59	2600	1.32	N/D				
14	Fipronil	80:20 Hep:EtOH	1	2.32	2.86	4800	1.21	80:20 HFE:EtOH	2.68	1.69	1100	1.25	80:20 HFE:EtOH	2.75	2.47	3500	1.30
15	3,4-Dihydroxyphenyl-α-propylacetamide	50:50 Hep:EtOH	1	3.73	1.39	2800	1.17	60:40 HFE:EtOH	9.70	1.39	1100	1.24	60:40 HFE:EtOH	9.80	1.30	900	1.22
16	Diaminocyclohexane acrylamide	90:10 Hep:EtOH	1	0.88	2.24	5000	1.32	90:10 HFE:EtOH	2.76	1.62	1400	1.35	90:10 HFE:EtOH	2.65	1.63	1200	1.40

$k'_1 = (t_1 - t_0)/t_0$; $N_1 = 16(t_1/w_1)^2$; $R_s = 2(t_2 - t_1)/(w_1 + w_2)$; $\alpha = (t_2 - t_0)/(t_1 - t_0)$ where t_2 and t_1 are the retention times and w_2 and w_1 are the baseline peak widths of the second and first peak, respectively, and where t_0 is dead time. N/D means not detected.

[a] #7 and #8 were detected at 220 nm.

[b] All flow rates were 1.0 ml/min for mobile phases containing HFE-7200.

[c] Same compound has been used in these two separations under different conditions.

3.2. Limits of detection (LOD) for APCI-MS and ESI-MS versus UV detection using heptane and ENFB containing mobile phases

The limits of detection (LOD) for two selected compounds using four methods were investigated. For MS, the compounds were detected by SIM at their corresponding *m*/*z* values listed in Table 3, whereas compounds #5 and #16 were detected at UV wavelength of 254 and 220 nm, respectively. Each compound was injected at concentrations of 0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10.0, 50.0 and 100.0 μg/ml. Table 3 lists the LOD and linearity for LC–UV detection under two mobile phase compositions (either heptane or ENFB mobile phases) and the for LC–APCI-MS and LC–ESI-MS detection. For

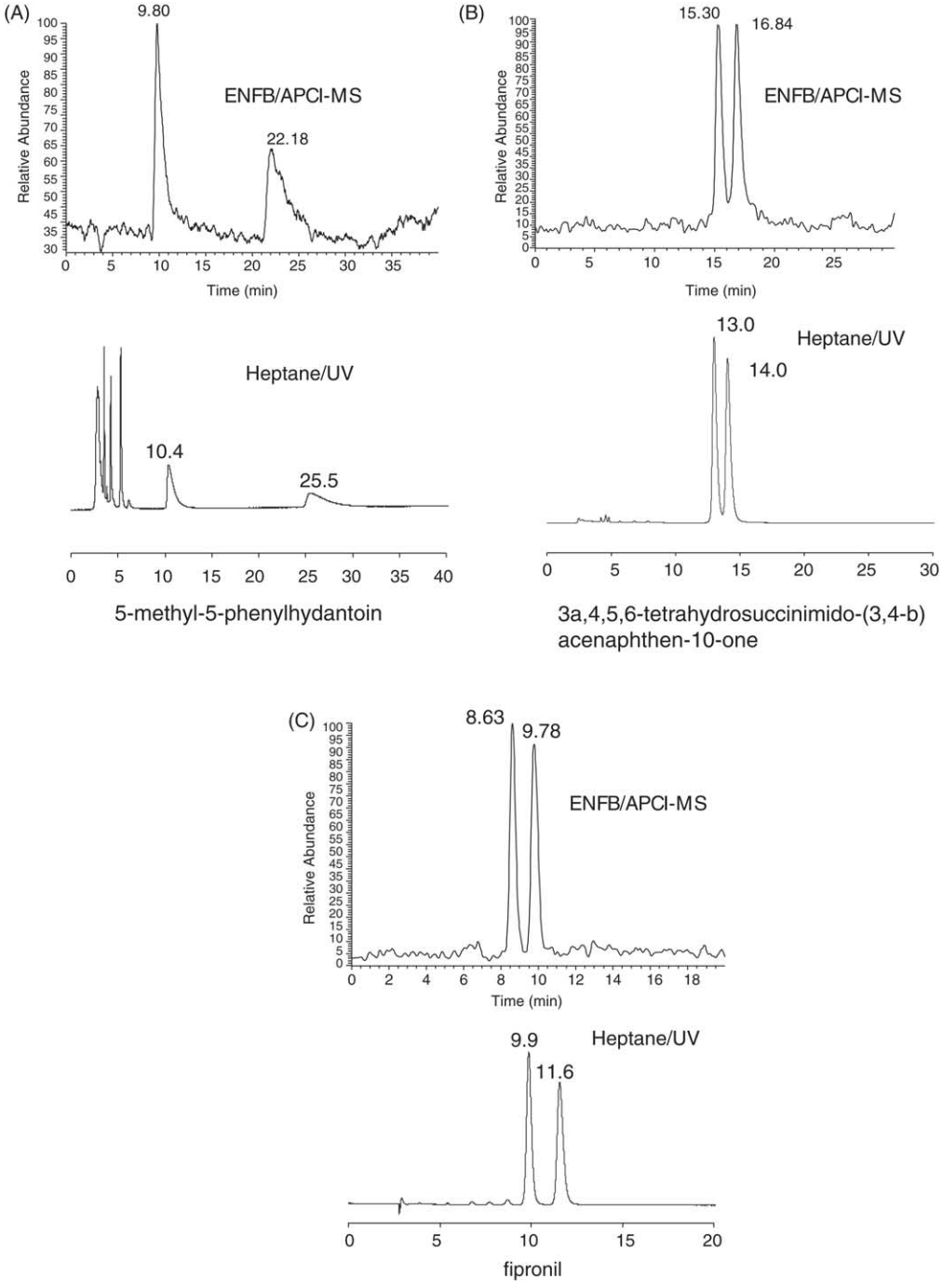

Fig. 3. Examples of ENFB-substituted and *n*-heptane mobile phase chiral separations of selected compounds using ENFB with APCI-MS detection (top panel) and *n*-heptane with UV (254 nm) detection (bottom panel). (A) 5-methyl-5-phenylhydantoin enantiomers separated on the Chirobiotic T stationary phase. (B) 3a,4,5,6-tetrahydrosuccinimido-(3,4-b) acenaphthene-10-one enantiomers separated on the Chirobiotic V stationary phase. (C) fipronil enantiomers separated on the SS-PCAP stationary phase.

Table 3
Limits of detection for selected compounds

Compound[a]	SIM (*m/z*)	Column	LC–UV–heptane			LC–UV–ENFB[b]			LC–APCI-MS			LC–ESI-MS		
			Linearity	r^2	LOD[c]	Linearity	r^2	LOD[c]	Linearity	r^2	LOD[c]	Linearity	r^2	LOD[c]
#5	167	V	$y = 2.1x + 44$	0.985	1 μg/ml	$y = 2.07x + 43$	0.986	1 μg/ml	$y = 3.4E{+}05x + 1E{+}6$	0.998	1 μg/ml	$y = 3E{+}06x + 1E{+}06$	0.996	50 ng/ml
#16	223	SS-P-cap	$y = 3.85x + 100$	0.99	1 μg/ml	$y = 4.1x - 3.7$	1	1 μg/ml	$y = 3E{+}06x - 827829$	0.999	500 ng/ml	$y = 2E{+}06x + 1E{+}07$	0.988	500 ng/ml

[a] For each compound, see Fig. 2 for the name and the structure. Separation conditions are listed in Table 2 except for #16, UV detection was carried out at 220 nm.
[b] Separations were done with mobile phase composition of 90% ENFB and 10% EtOH under flow rate of 1 ml/min.
[c] LOD, limit of detection based on signal to noise ratio = 3.

diphenylmethyl phenyl sulfoxide, the LOD is similar for both UV and APCI-MS detection but is 20 fold lower for ESI-MS. For diaminocyclohexane acrylamide, the LOD is slightly lower for MS over UV detection. And ESI-MS has a slightly lower sensitivity but comparable detection limits to APCI-MS. For both compounds, the sensitivity (as defined by IUPAC as the slope of the dose response curve [24]) is comparable when using UV detection regardless of the choice of mobile phase solvent. For MS detection, the sensitivity varies for different compounds when using APCI versus ESI.

The experimental results suggested that APCI-MS offers comparable detection to the common UV approach for compounds with strong chromophores, such as diphenylmethyl phenyl sulfoxide. For this particular compound, which is particularly easy to thermally decompose to ions following the path depicted in Fig. 2, ESI-MS provided much better detection performance (lower LOD and higher sensitivity) than the other approaches in this study (Table 3). Furthermore, the low surface tension of ENFB [23] allows facile desolvation of ions, which may enhance the ionization efficiencies for the compounds analyzed.

3.3. *Effect of flow-rate and sensitivity for APCI and ESI-MS detection*

MS detector response is proportional to the total number of ions being detected per unit time, making it a mass flow-dependent detector [25]. Therefore, it is possible that

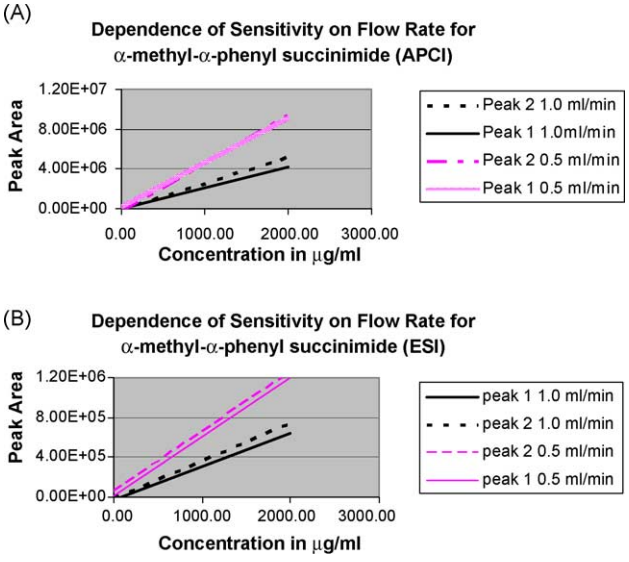

Fig. 4. Dependence of sensitivity on flow rate for α-methyl-α-phenyl succinimide using the Chirobiotic T stationary phase. Peaks 1 and 2 are the first and second eluting peaks, respectively. (A) APCI-MS detection: linearity of peak 1 curve for 0.5 ml min^{-1} flow rate, $y = 4526x + 139452$, $r^2 = 0.986$; linearity of peak 1 curve for 1.0 ml min^{-1} flow rate, $y = 2052x + 58451$, $r^2 = 0.999$. (B) ESI-MS detection: linearity of peak 1 curve for 0.5 ml min^{-1} flow rate, $y = 593x + 20269$, $r^2 = 0.999$; linearity of peak 1 curve for 1.0 ml min^{-1} flow rate, $y = 332x - 23565$, $r^2 = 0.994$. Linearities of peak 2 were similar to those of peak 1 for both flow rates with each ionization mode.

flow rate can greatly affect both sensitivity and response in MS detection [26–28]. Previously, our group reported that ESI-MS sensitivity gained nearly an order of magnitude when the flow rate (reversed-phase mode) was decreased from 0.8 to 0.4 ml/min for leucine [20]. To evaluate the dependence of sensitivity on flow rate for the new ENFB mobile phase, standards of α-methyl-α-phenyl succinimide were separated on the Chirobiotic T using flow rates of 1.0 and 0.5 ml min^{-1}. Both APCI-MS and ESI-MS detection were utilized. The dose response curves are shown in Fig. 4. Peaks 1 and 2 are the first and second eluting enantiomers, respectively. The sensitivity at the lower flow rate was slightly less than two-fold higher than that of at higher flow rate for both APCI-MS and ESI-MS. The observed sensitivity difference is insignificant compared to that observed previously for reversed-phase separations [20]. Clearly, flow rate has less of an impact on sensitivity in the current study. Due to the nature of the solvents used in normal phase separations, evaporation in the ionization source is much more efficient compared to the reversed-phase solvents. This may explain the similar sensitivities achieved at the different flow rates.

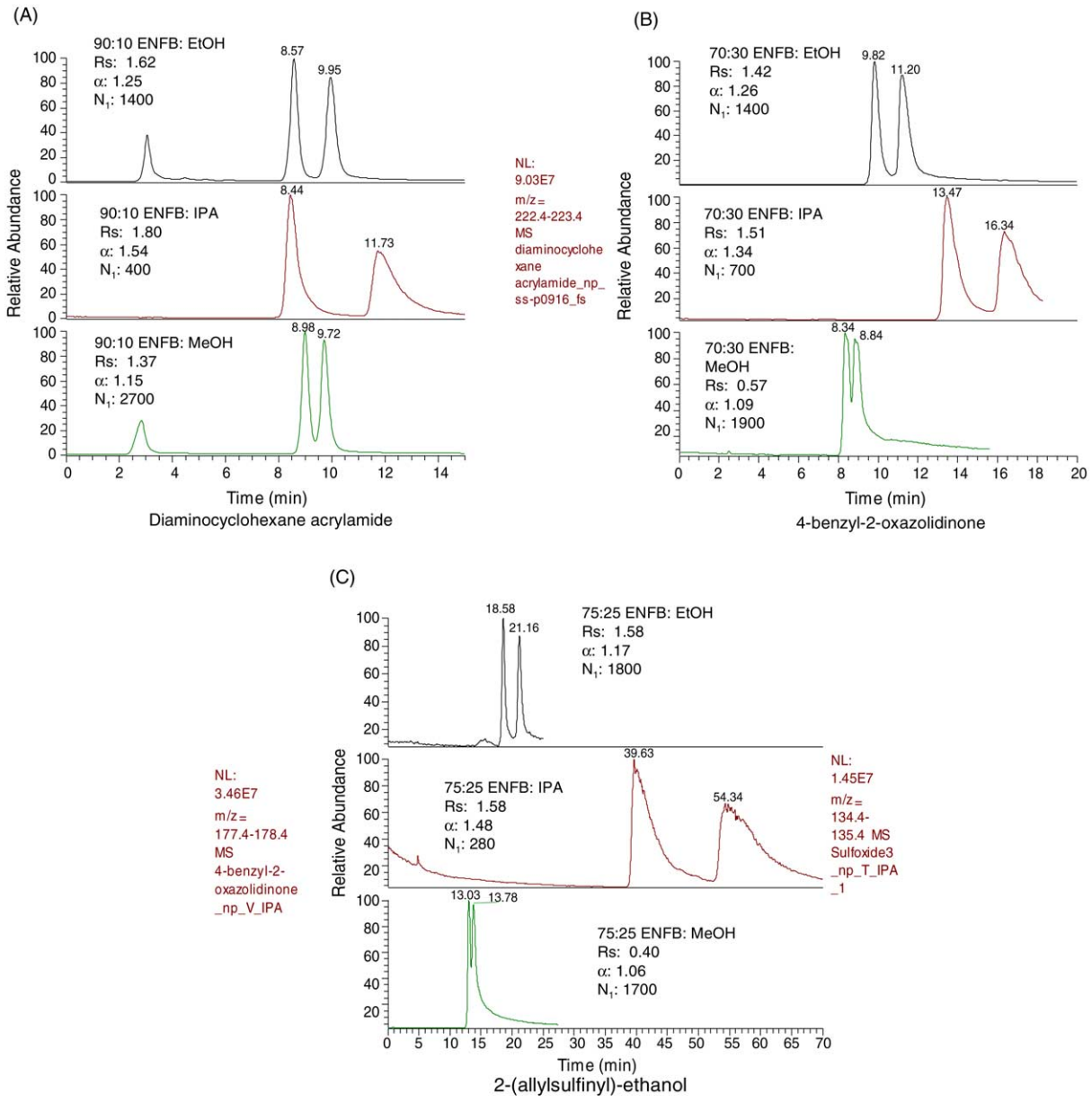

Fig. 5. Effect of organic modifier on chromatographic parameters using EtOH (top panel), IPA (middle panel) or MeOH (bottom panel) as the organic modifier. (A) diaminocyclohexane acrylamide enantiomers separated on the SS-PCAP stationary phase, (B) 4-benzyl-2-oxazolidinone enantiomers separated on the Chirobiotic V stationary phase, (C) 2-(allylsulfinyl)-ethanol enantiomers separated on the Chirobiotic T stationary phase. R_s, resolution; α, selectivity; N_1, peak efficiency for the first eluting peak. All flow rates were 1.0 ml min^{-1}.

3.4. Effect of modifier on chromatographic parameters

Since ENFB is completely miscible with a variety of solvents including methanol, ethanol and 2-propanol, ENFB containing mobile phases can provide greater flexibility in method development compared to conventional normal-phase solvents (i.e., *n*-hexane and *n*-heptane). However, the type of organic modifier can directly affect the chromatographic parameters of chiral separations. Fig. 5 shows examples of three compounds separated on different stationary phases using ethanol, 2-propanol (IPA), and methanol as the organic modifier, respectively. Methanol provided the highest peak efficiencies, but the worst resolutions, for the three compounds. In contrast, IPA led to the exact opposite trend, i.e., the lowest efficiencies and the highest resolutions. With peak efficiencies over 1400 theoretical plates, moderate selectivities, and baseline or near baseline resolutions, the use of ethanol as the organic modifier was often the best compromise.

3.5. Effect of modifier on APCI-MS sensitivity

Besides chromatographic efficiency, resolution, and selectivity, the type of organic modifier can affect APCI-MS or ESI-MS sensitivity. The effect of modifier on MS sensitivity was tested for 5-methyl-5-phenylhydantoin using APCI-MS.

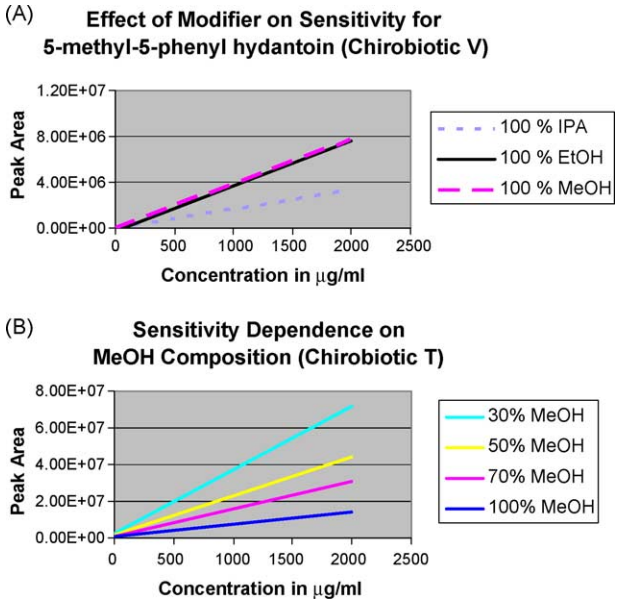

Fig. 6. (A) Effect of modifier on APCI-MS sensitivity for 5-methyl-5-phenylhydantoin using the Chirobiotic V stationary phase. All separations were carried out without ENFB using 100% organic modifier. Linearity of EtOH curve, $y = 3987.4x - 328021$, $r^2 = 0.9909$; linearity of MeOH curve, $y = 3886.8x + 17482$, $r^2 = 0.9905$; linearity of IPA curve, $y = 1706.9x - 61573$, $r^2 = 0.9900$. (B) APCI-MS sensitivity dependence on the methanol composition in the mobile phase for the same compound using Chirobiotic T stationary phase. All separations were carried out with ENFB, and MeOH as the organic modifier. The volume ratio of MeOH in the mobile phase is indicated in the figure.

This compound was chosen because it could be separated by the Chirobiotic V and Chirobiotic T columns using different compositions of modifier with ENFB. The dose response curves for 100% ethanol, methanol, and IPA using Chirobiotic V column are shown in Fig. 6(A), in which the response for the first eluting peak was charted for all three modifiers. The sensitivities for methanol and ethanol were nearly identical; both curves had slopes of approximately 4000. The sensitivity of IPA, however, was clearly much lower than that of the other two modifiers (<50%). While methanol and ethanol have similar surface tension, the surface tension of IPA is greater [29]. The desolvation efficiencies of IPA < methanol ≅ ethanol may contribute to the difference observed for MS sensitivity. Fig. 6(B) shows the dose response curves from 100% methanol to 30% methanol using Chirobiotic T column. In all four cases, very good separations ($R_s > 2.0$) have been achieved. The sensitivity of APCI-MS increases with decreasing amount of methanol in the mobile phase. The same trend was observed when ethanol was used as the modifier from 100%, 90%, and 70% to 50%. The results indicate that the sensitivity of MS detection can be optimized by changing the amount of alcohol in the normal-phase mobile phase.

4. Conclusions

In this study, ethoxynonafluorobutane was found to be a viable alternative to classic normal-phase solvents (*n*-hexane or *n*-heptane) for normal-phase enantiomeric separations. Its chemical characteristics, such as having no flashpoint and low flammability, made it especially attractive for use with API-MS detection. ENFB substituted mobile phases provided comparable selectivities for all the compounds tested, although resolutions and peak efficiencies were somewhat lower than *n*-heptane containing mobile phase methods. APCI-MS appears to be a more suitable detection method than ESI-MS for most of the small analytes in this study, because of better ionization efficiencies which lead to better sensitivities. The limits of detection and sensitivities for ENFB/APCI-MS detected compounds were either comparable to or better than those of *n*-heptane/UV detection. The miscibility of ENFB with most common organic solvents made it suitable for method development. Ethanol, as a compromise organic modifier, was found to provide better selectivities than methanol and better efficiencies than IPA mobile phase modifiers. Additionally, methanol and ethanol afforded better sensitivities for APCI-MS than IPA as an organic modifier. The amount of modifier in mobile phase greatly changes MS sensitivity.

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