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Normal-phase high-performance liquid chromatographic separations using ethoxynonafluorobutane as hexane alternative I. Analytical and chiral applications

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Abstract

A novel, environmentally friendly, fluorinated solvent — ethoxynonafluorobutane — has been used to replace *n*-hexane in normal-phase HPLC applications. Fast gradients of methanol in ethoxynonafluorobutane on a cyano column have been successfully applied to the separation of steroids, benzodiazepines, NSAIDs, tricyclic antidepressants, β-adrenergic blocking agents and mixtures of purines and pyrimidines. Small amounts of triethylamine and trifluoroacetic acid added to such gradients significantly improved peak shape and column performance for basic and acidic solutes. Ethoxynonafluorobutane and its mixtures with methanol have also been demonstrated to have a unique selectivity in chiral HPLC applications.

Keywords: Mobile phase composition; Ethoxynonafluorobutane; Steroids; Benzodiazepines; Purines; Pyrimidines; β-Blockers; Nonsteroidal anti-inflammatory drugs

1. Introduction

Reversed-phase HPLC is a powerful tool to analyze and purify synthetic intermediates and compounds with pharmacological activity. The ability to retain molecules with vastly different hydrophobicity and the excellent selectivity of reversed-phase HPLC are routinely exploited every day by thousands of scientists throughout the world. Wide use of short HPLC columns and standard linear gradients of acetonitrile or methanol in water revolutionized combinatorial techniques and significantly increased overall productivity in medicinal chemistry.

Nevertheless, normal-phase chromatography (mostly based on the use of silica gel and organic solvents) remains the method of choice for an organic and/or medicinal chemist. It is a common practice to protect polar functions in the molecule (e.g., hydroxy, amino) with various protective groups, carry them through numerous synthetic steps and deprotect only at the final stages of the synthesis. Such intermediates, in many cases, are readily soluble in organic solvents and can be separated from starting materials and side products by normal-phase silica gel chromatography using gravity columns or low efficiency disposable cartridges filled with coarse particles.

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Among the disadvantages associated with bare silica, poor reproducibility and peak tailing for basic analytes are the most common. Gradient elution is not recommended due to accumulation of strongly retained polar modifiers on the top of chromatographic columns which may result in solvent demixing and associated performance loss for sample components with low retention [1].

Many drawbacks of traditional (silica- and alumina-based) normal-phase chromatography may be avoided by using polar bonded stationary phases (BSP) (amino, diol and cyano). Compounds with moderate to strong polarity can be separated on columns packed with these materials. Separations performed on BSP columns are quite reproducible because of much quicker column equilibration. The shape of the chromatographic peaks is less affected by the compound's basicity or acidity and can be corrected by adding volative additives (e.g., triethylamine and acetic acid). BPS columns can also be cleaned with strong polar solvents.

1,1,2-Trichloro-1,2,2-trifluoroethane (FC-113) has been demonstrated to be a superior mobile phase carrier solvent for normal-phase HPLC [2]. Its solvent strength (comparable with *n*-hexane), complete miscibility with all common polar modifiers and significantly higher solubilizing properties for many organic compounds suggest its use as a carrier solvent in liquid chromatography. Unfortunately, environmental considerations prevent this solvent from being widely employed as a hexane replacement in chromatography.

In our search for a simple and robust technique that can allow for quick and efficient normal-phase HPLC separations, we have investigated analytical aspects of high-speed, high-performance LC using cyanopropyl-bonded silica columns and gradients of methanol (and other solvents) in ethoxynonafluorobutane. We report here the applicability of such gradients for analytical separations of mixtures of steroids, benzodiazepines, non-steroidal anti-inflammatory agents, tricyclic antidepressants, purines and pyrimidines, and β-adrenergic blocking agents.

In addition, we describe the use of ethoxynonafluorobutane-based mobile phases for chiral normal-phase HPLC of racemic standards and the separation of enantiomers of a $5HT_{2C}$-active compound.

2. Experimental

All steroids, tricyclic antidepressants, β-blockers, NSAIDs, benzodiazepines, purines and pyrimidines, guaifenesine, Troger's base, *trans*-stilbene oxide, triphenylene and dimethyl phthalate used in this study were purchased from Aldrich–Sigma (St. Louis, MO, USA).

Ethoxynonafluorobutane was purchased as 3M NovecTM Engineered Fluid HFE-7200 from 3M Company (St. Paul, MN, USA). All other solvents were of HPLC grade and obtained from EM Science (Gibbstown, NJ, USA).

The 1050 and 1100 (Agilent Technologies) liquid chromatographs equipped with an autosampler, a thermostatted column compartment and a diode-array detector were used for analytical HPLC.

Normal-phase HPLC separations were carried out at 0.6 ml/min on a 0.2×10-cm Luna CN column (Phenomenex, Torrance, CA) packed with 3-μm particles. Linear gradients of solvent B in solvent A were used, solvent A being ethoxynonafluorobutane and solvent B — methanol or a mixture of methylene chloride–methanol (8:2); 0.1% of triethylamine (TEA) or trifluoroacetic acid (TFA) were added to both solvent A and B to reduce peak tailing for basic or acidic compounds. A Primesphere C_{18} (0.2×15 cm) column packed with 5-μm particles (Phenomenex) was used for reversed-phase HPLC.

Chiral HPLC was performed on Chiralcel OD and OJ and Chiralpak AD and AS columns (0.46×25 cm) packed with 10-μm particles (Chiral Technologies, Exton, PA, USA).

Thin-layer chromatography (TLC) was done using Silica Gel 60 F_{254} precoated plates (EM Science).

3. Results and discussion

HPLC columns packed with Luna CN 3-μm particles were shown to possess excellent chromatographic properties. They reportedly maintain high efficiency at the elevated flow-rates, can be equilibrated quickly and, as a result, exhibit highly reproducible retention times under gradient elution conditions. High-speed gradients of methylene chloride and methanol in *n*-hexane with or without small

amounts of TEA were shown to provide excellent separation for a variety of classes of organic compounds [3].

3M NovecTM Engineered Fluid HFE-7200 is a new 3M specialty fluid that is designed to replace ozone-depleting and chlorinated materials in many applications [4]. It is used as a cold cleaner (movie film, wipe solvent), cleaning and rinsing agent for vapor degreasing, lubricant carrier, specialty solvent, dispersion medium, reaction medium and spray contact cleaner. This proprietary non-flammable fluid has zero ozone depletion potential, is practically non-irritating and non-toxic and does not have mutagenic properties. Its atmospheric lifetime is 0.8 years and it has been accepted for the commercial use by regulatory agencies in the US, Canada, Japan, Korea, Australia and Europe.

HFE-7200 is a mixture of two inseparable isomers (ethyl perfluoroisobutyl and ethyl perfluorobutyl ethers) with essentially identical properties. It is a clear, colorless liquid with a faint odor. We found it to be completely miscible with all common organic solvents, including DMSO, acetonitrile and methanol. According to the manufacturer, its non-volatile residue is 1.0 ppm maximum. The solvent's physical properties are listed in Table 1 along with properties of hexane and FC-113.

We studied chromatographic behavior of various classes of organic compounds on a short high-performance LC column packed with 3-μm particles of Luna CN (Phenomenex) and employing fast and shallow (~20 column volumes) gradients of methanol in HFE-7200.

3.1. Separation of steroids using cyanopropyl column and gradients of methanol in HFE-7200

A mixture of 12 steroids that included progesterone, 11-keto-progesterone, 11α-, 11β- 17α-, 20α-, 20β-hydroxy-progesterones, dexamethasone, corticosterone, hydrocortisone and 20α- and 20β-dihydrocortisols (**1–12**, respectively) was prepared to compare elution properties of *n*-hexane and HFE-7200. This model mixture contained components with the same steroidal skeleton and various numbers of polar (keto and hydroxy) groups, as well as several diastereomeric pairs and positional isomers. We found that almost all components were baseline resolved when a linear gradient of methanol in HFE-7200 was used (Fig. 1). The overall chromatographic performance of the system was comparable to the separation of the same mixture using a gradient of methylene chloride and methanol in *n*-hexane [3] (Fig. 2). Interestingly, when gradients of methyl *tert*.-butyl ether or a mixture of methylene chloride–methanol (8:2) in HFE-7200 were employed, the corresponding changes in chromatographic selectivity facilitated the separation of only 10 out of 12 components of the mixture of steroids.

We found this technique to be reproducible and robust; our observations were similar to those reported by Layne et al. [3]. From 10 consecutive runs

Table 1
Physical properties of fluorinated solvents and *n*-hexane

	HFE-7200[a]	FC-113[b]	*n*-Hexane[b]
Boiling point (°C)	76	47.6	68.7
Viscosity (cP)	0.61	0.71	0.313
Density (g/ml)	1.43	1.56	0.65
Molecular mass	264	187	195
UV cutoff (nm)	220	231	191.5
Flammable	No	No	Yes
Solubility of water in solvent (ppm)	92	90	100
Solvent strength on silica	~0.012[c]	0.02	0.01

[a] Data from manufacturer (see Ref. [4]).
[b] Data from Ref. [2].
[c] As evaluated by comparison of R_f for triphenylene on a silica TLC plate in *n*-hexane and HFE-7200.

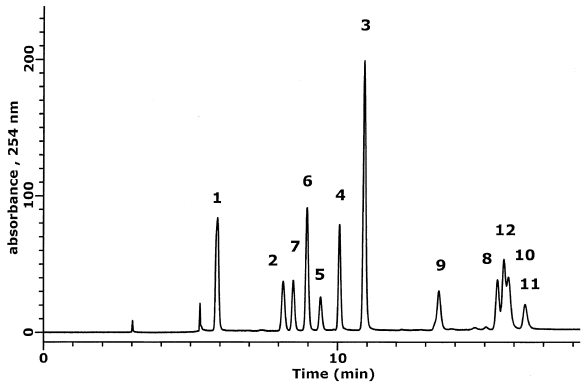


Fig. 1. Separation of steroids on a Luna CN column. Gradient of methanol in HFE-7200 from 0 to 20% in 20 min.

under conditions reported in Fig. 1 and column re-equilibration with ~5 column volumes after each run, the standard deviation for peaks 1, 7 and 12 (retention time values) did not exceed 3.7, 1.4 and 1.1%, respectively.

Having established good chromatographic performance of mobile phases comprising gradient elution with methanol in HFE-7200 on a cyano column, we investigated the applicability of this technique for normal-phase separation of other classes of pharmaceutically relevant compounds.

3.2. Normal-phase separation of benzodiazepines

Benzodiazepines are widely prescribed drugs possessing anti-convulsant, sedative and tranquilizing properties [5]. The separation of benzodiazepines by HPLC and their monitoring in plasma and urine are usually performed using reversed-phase systems [6–11]. A number of normal-phase methods utilizing silica, cyano, amino, diol and thermally immobilized Carbowax 20M stationary phases and mixtures of hexane with ethyl acetate, isopropanol and ethanol have also been reported [12–14]. A technique utilizing a non-derivatized silica column and methanol with small amounts of perchloric and trifluoroacetic acid was successfully applied to separation of various benzodiazepines [7,15].

Traditional reversed-phase approach was initially tried for the separation of the mixture of diazepam, clobazam, clonazepam, lorazepam and alprazolam (**13–17**, respectively; Fig. 3). We found that four out of five components of the mixture could be successfully separated under isocratic conditions (Primesphere C_{18} 15×0.2-cm column, 0.4 ml/min flow-rate, 50% methanol and 30% acetonitrile in water with 0.1% formic acid) with (**16**) co-eluting with (**17**) and (**15**) in the case of methanol- and acetonitrile-based mobile phase, respectively.

We found that all five components of the same

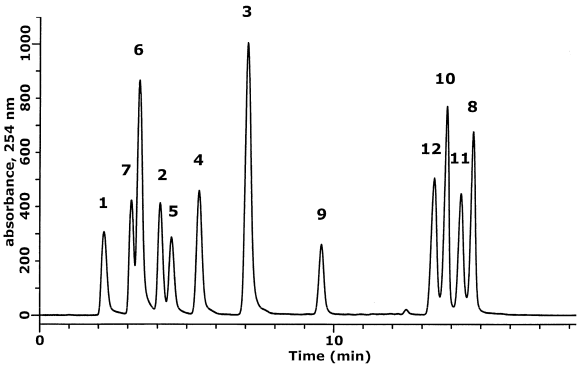


Fig. 2. Separation of steroids on a Luna CN column. Gradient of a mixture of methylene chloride–methanol (8:2) in *n*-hexane from 5 to 20% in 20 min.

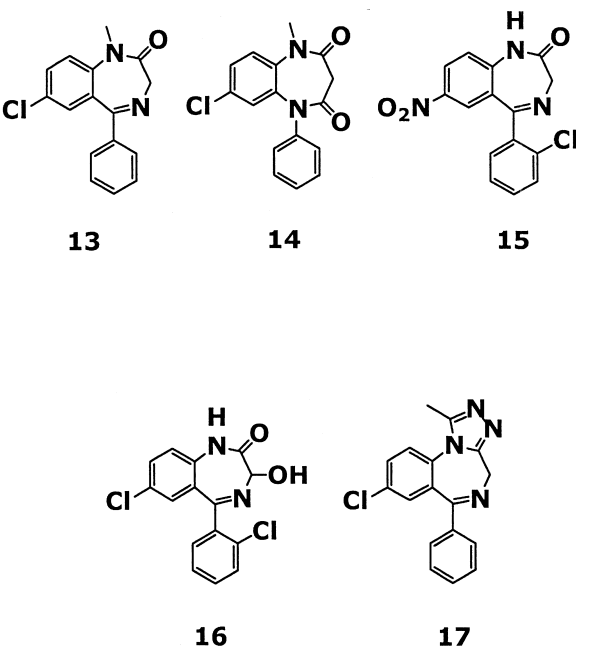


Fig. 3. Structures of benzodiazepines: diazepam, clobazam, clonazepam, lorazepam and alprazolam (**13**–**17**).

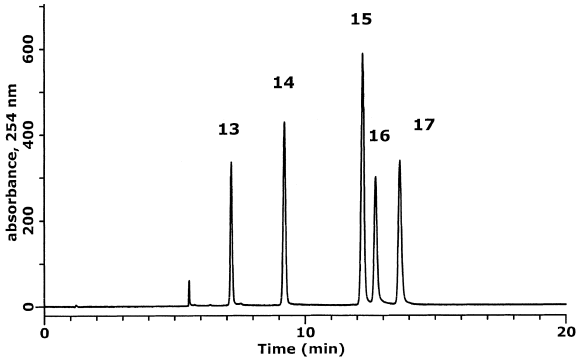


Fig. 4. Separation of benzodiazepines on a Luna CN column. Gradient of methanol in HFE-7200 from 0 to 20% in 20 min.

mixture could be nicely resolved when a gradient of methanol in HFE-7200 (Fig. 4) was employed. It seems reasonable to expect similar chromatographic conditions to simplify analysis of benzodiazepines in biological fluids, especially when liquid–liquid extraction is involved as a sample preparation procedure [11].

3.3. Separation of non-steroidal anti-inflammatory drugs using methanol gradients in HFE-7200

Reversed-phase HPLC is a major analytical tool for the separation and quantitation of non-steroidal anti-inflammatory drugs (NSAIDs) [16–18]. Underivatized silica and acidified mixtures of phosphate buffer and small amounts (5–10%) of acetonitrile have also been used to separate a mixture of NSAIDs and a possible retention mechanism of hydrogen bonding and quasi reversed-phase retention has been proposed [19].

We found that a mixture of NSAIDs consisting of ibuprofen, fenoprofen, naproxen, tolmetin, indomethacin and indoprofen (**18–23**, respectively; Fig. 5) could be easily separated on a Luna cyano column using methanol gradient (from 2 to 10% in 20 min) in HFE-7200 in the presence of 0.1% TFA (Fig. 6). The elution order of the least (ibuprofen, **18**) and the most (indoprofen, **23**) retained components was opposite to the elution order exhibited by the same compounds under reversed-phase conditions [18]. Distorted peak shape of ibuprofen (**18**, Fig. 6) was due to the fact that it has been eluted in system's void volume. We also found that the

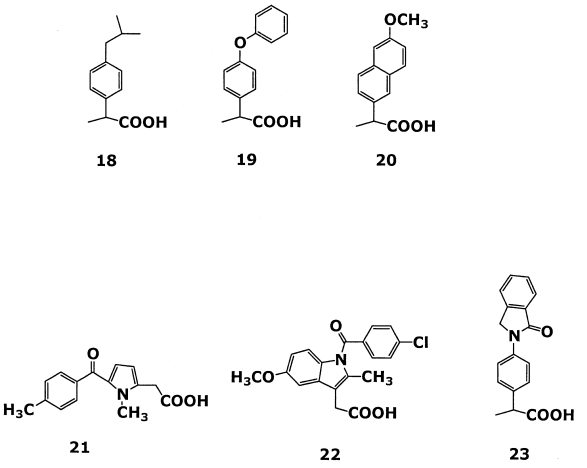


Fig. 5. Structures of NSAIDs: ibuprofen, fenoprofen, naproxen, tolmetin, indomethacin and indoprofen (**18**–**23**).

presence of TFA was essential to achieve good chromatographic performance and peak shape.

3.4. Normal-phase HPLC separation of tricyclic antidepressants

Tricyclic antidepressants (TACs) are commonly used for the treatment of depressive disorders and HPLC-based methods are widely employed to analyze them in biological fluids. It is highly desirable for a chromatographic method to be able to separate various TACs in a single run because of a lesser cost of maintenance in a clinical laboratory environment [20]. A number of such methods utilizing reversed-phase HPLC [20–24], ion-pairing techniques [25],

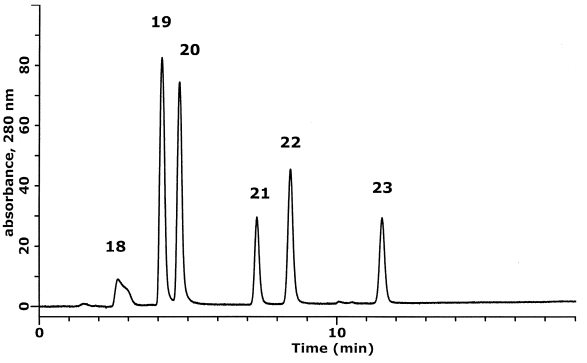


Fig. 6. Separation of NSAIDs on a Luna CN column. Gradient of methanol in HFE-7200 (0.1% TFA) from 2 to 10% in 20 min.

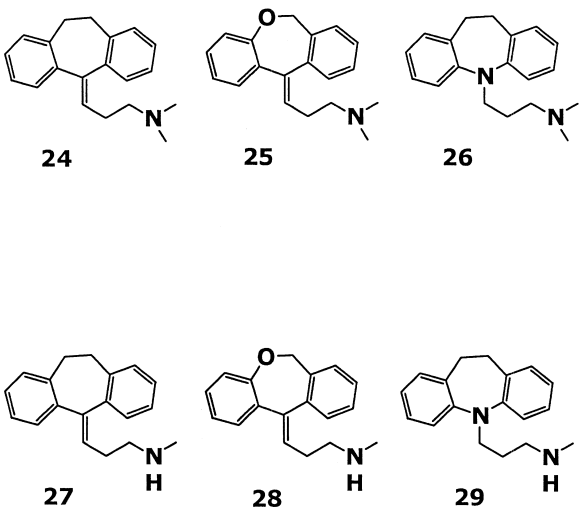


Fig. 7. Structures of tricyclic anti-depressants: amitriptyline, doxepine, imipramine, nortriptyline, nordoxepine and desipramine (**24**–**29**).

underivatized silica with aqueous methanol and butylamine [26] and perchloric acid with methanol [27] as well as a normal-phase approach using *n*-hexane–methanol–nonylamine and silica column [28] have been reported.

We found that a mixture of amitriptyline, doxepine, imipramine, nortriptyline, nordoxepine and desipramine (**24–29**, respectively; Fig. 7) can be separated on a Luna cyano column using a complex gradient of methanol in HFE-7200 in the presence of 0.1% TEA (Fig. 8). The elution order of amitriptyline (**24**), imipramine (**26**), nortriptyline (**27**) and desipramine (**29**) was opposite to the elution order of the same compounds under reversed-phase conditions as reported in Ref. [20]. The addition of small amounts of TEA to the mobile phase was necessary to achieve good peak shape for this mixture's basic components [3].

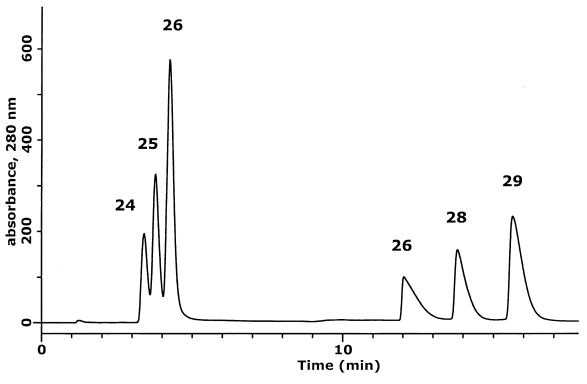


Fig. 8. Separation of tricyclic anti-depressants on a Luna CN column. Gradient of methanol in HFE-7200 (0.1% TEA): 1–3.7% (0–6 min), 3.7–50% (6–20 min).

3.5. Separation of purines and pyrimidines

Purines and pyrimidines are essential components of nucleic acids and their analysis in biological liquids plays an important role in the clinical field. Early separation techniques were based on an ion-exchange approach which exploited different basic or acidic dissociation constants of those compounds [29,30]. Later, reversed-phase HPLC became the technique of choice for the separation of purines and pyrimidines [31–37]. An ion-pair HPLC method using *n*-heptane sulfonate and a C_{18} column at neutral pH was successfully applied to resolve a mixture of several nucleic acid bases including a pair of those (cytosine and 5-methyl cytosine) which could not be separated in the absence of an ion-pairing agent [38]. Mixtures of dichloromethane, methanol and aqueous ammonium formate-formic acid solutions were also used with silica gel columns to separate purines and pyrimidines [39]. Recently, mixed partition–adsorption chromatographic systems and silica sorbents were shown to possess good selectivity and peak symmetry for a mixture of several purines and pyrimidines [40,41].

We found that a mixture of caffeine, theophylline, thymine, theobromine, uracil, adenine, methyl cytosine and cytosine (**30**–**37**, respectively; Fig. 9) could be nicely separated on a short cyano column when a gradient of methanol in HFE-7200 was employed in the presence of TEA (Fig. 10).

Almost all components of the mixture were baseline resolved and, interestingly, the elution order for caffeine (**30**), theophylline (**31**), thymine (**32**), uracil (**34**), adenine (**35**) and cytosine (**37**) corresponded to the elution order reported in [40].

Methyl cytosine (**36**) and cytosine (**37**) that were separated previously only by ion-pairing HPLC [38] or HPCE in the presence of sodium dodecyl sulfate [42]), were also successfully resolved with separation coefficient α approximately equal to 1.1. The

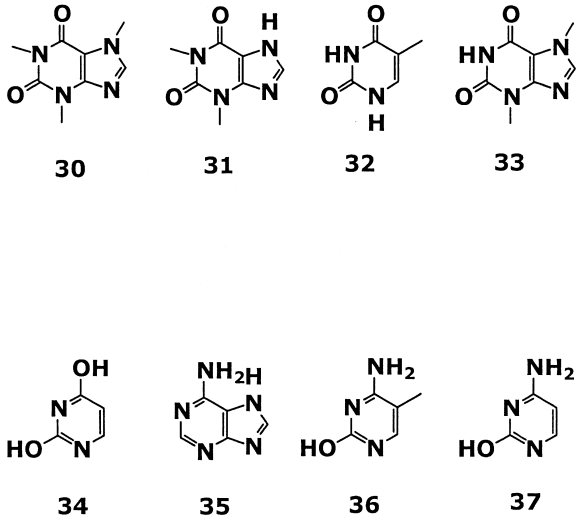


Fig. 9. Structures of purines and pyrimidines: caffeine, theophylline, thymine, theobromine, uracil, adenine, methyl cytosine and cytosine (**30**–**37**).

absence of TEA had a pronounced detrimental effect on the separation (Fig. 11).

3.6. β-Adrenergic blocking agents

β-Blockers comprise an important class of therapeutic agents and chromatographic methods play a major analytical role in their development, drug formulation, metabolism and therapeutic drug monitoring [43]. Their basic properties are defined by the secondary amine function and the aromatic group provides lipophilic character. A low level of residual

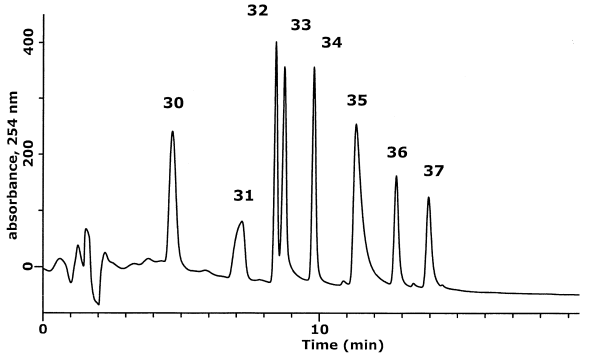


Fig. 10. Separation of purines and pyrimidines on a Luna CN column. Gradient of methanol in HFE-7200 (0.1% TEA) from 5 to 20% in 20 min.

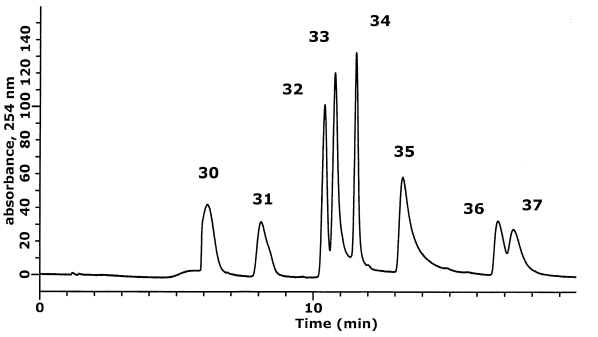


Fig. 11. Separation of purines and pyrimidines on a Luna CN column. Gradient of methanol in HFE-7200 (no TEA added) from 0 to 20% in 20 min.

silanols seems to be a requirement for the successful use of a reversed-phase HPLC column in β-blockers analysis [44]. Interactions between β-blockers (and other basic drugs) and residual silanols often result in a broadened peak shape which can be prevented by employing ion-pairing reagents or competing bases like TEA [45]. Underivatized silica dynamically modified with long-chain quaternary ammonium compounds [46] and used with methanol-rich high-pH buffers [47] or with acidic reversed-phase buffer systems [48] were also applied for the separation of β-blockers. Their chromatographic behavior on Ultrasphere CN columns using mixtures of *n*-hexane–isopropanol–*n*-butyl amine as a mobile phase was also correlated with their hydrophobicity (log *P*) [49].

We found that a mixture of alprenolol, propranolol, acebutolol and atenolol (**38**–**41**, Fig. 12; log *P* values 3.63, 3.66, 2.31 and 0.75, respectively [49]) could be resolved on a short Luna CN column using a gradient of methanol in HFE-7200 with 0.1% TEA (Fig. 13). The elution order of the mixture's com-

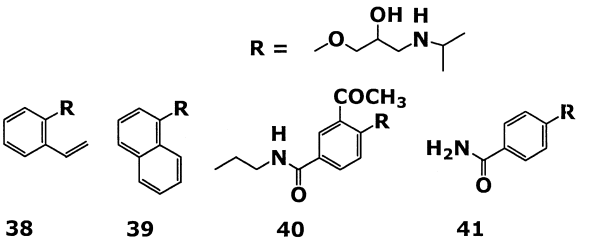


Fig. 12. Structures of β-blockers: alprenolol, propranolol, acetobutolol and atenolol (**38**–**41**).

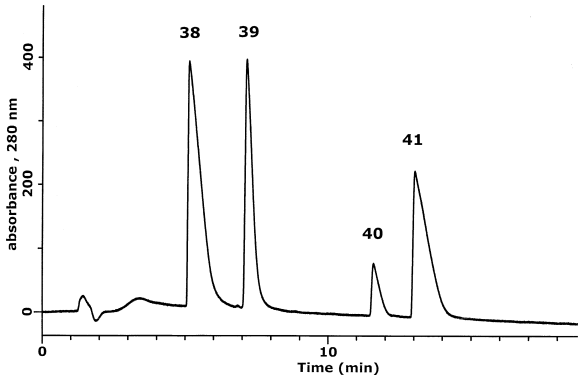


Fig. 13. Separation of β-blockers on a Luna CN column. Gradient of methanol in HFE-7200 (0.1% TEA) from 5 to 50% in 20 min.

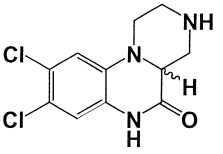
ponents roughly corresponded to their hydrophobicity with more lipophilic (**38** and **39**) compounds eluting earlier. We believe that the ability of this technique to cover a wide range of solute polarities in a single chromatographic run can be exploited as an alternative tool for bioanalysis of β-blockers.

3.7. Chiral HPLC separations with HFE-7200

Chiral HPLC is an indispensable tool to analyze potential drug candidates for chiral purity and to isolate enantiomers from racemic mixtures for biological testing. The vast majority of such work at this Wyeth–Ayerst facility is performed using chiral HPLC column packing materials based on wide-pore silica coated with derivatized polysaccharides (cellulose and amylose). Columns available commercially from Chiral Technologies (Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS) were capable in the past to resolve ~95% of the racemic mixtures produced by Discovery Research medicinal chemists. A wide variety of experimental conditions have been used to achieve adequate separation of enantiomers including mixtures of *n*-hexane with various alcohols as well as aqueous methanol and acetonitrile. Usually, a racemate is subjected to a number of standardized HPLC tests utilizing five different chiral columns and several mobile phases. After initial screening, a potential hit (successfully resolved enantiomers) is optimized and then scaled-up for preparative chiral HPLC.

We have investigated chiral HPLC separation of several common racemic mixtures routinely used for Daicel column testing with mobile phases in which *n*-hexane is replaced with HFE-7200 (Table 2) and methanol is used as an alternative to ethanol.

It is obvious that replacement of *n*-hexane with HFE-7200 was not detrimental to a columns' chiral selectivity which was actually enhanced in the case of Chiralpak AD and Chiralcel OJ columns. Encouraged by this finding, we studied the separation of enantiomers of WY-041594 synthesized previously and possessing 5-HT_{2C} activity [50]:



WY-041594

Enantiomers of WY-041594 were separated previously [50] on a Chiralpak AD column using ethanol with $\alpha \sim 1.77$ (Table 3). Addition of *n*-hexane to the ethanol resulted in increased retention for both racemates and decreased column selectivity. Replacement of *n*-hexane with a fluorinated solvent caused significant increase in retention for both

Table 2
Separation factors (α) of racemic standards on popular Daicel columns with *n*-hexane- and HFE-7200-based mobile phases[a]

	OD/ guaifenesine	AD/Troger's base	AS/Troger's base	OJ/t-stilbene oxide
EtOH	1.66	2.01	1.54	1.63
50% EtOH in *n*-hexane	2.24	2.18	1.62	1.54
50% EtOH in HFE-7200	1.85	2.17	1.65	1.61
50% MeOH in HFE-7200	1.66	2.5	1.61	1.7
gradient MeOH in HFE-7200[b]	1.31	1.89	1.42	1.41

[a] All columns (25×0.46 cm, supplied by Chiral Technologies) were operated at 1 ml/min and room temperature.
[b] Linear gradient from 10 to 100% in 20 min.

Table 3
Retention of enantiomers of WY-041594 on Chiralpak AD column (flow-rate 1 ml/min)

	Retention time (min)	Retention time (min)	Separation factor (α)
EtOH	5.21	7.15	1.77
25% EtOH in *n*-hexane	11.33	16.29	1.57
50% EtOH in *n*-hexane	6.24	8.05	1.51
50% EtOH in HFE-7200	16.49	33.20	2.21
50% MeOH in HFE-7200	16.48	37.90	2.55
MeOH	5.62	9.10	2.19

enantiomers and improved selectivity, which was even further enhanced when ethanol has been replaced with methanol (Fig. 14). Clearly, the changes in chiral selectivity when *n*-hexane is substituted with HFE-7200 and the consequent use of methanol (which is completely miscible with HFE-7200) expand the number of tools available to a chromatographer facing a difficult chiral separation.

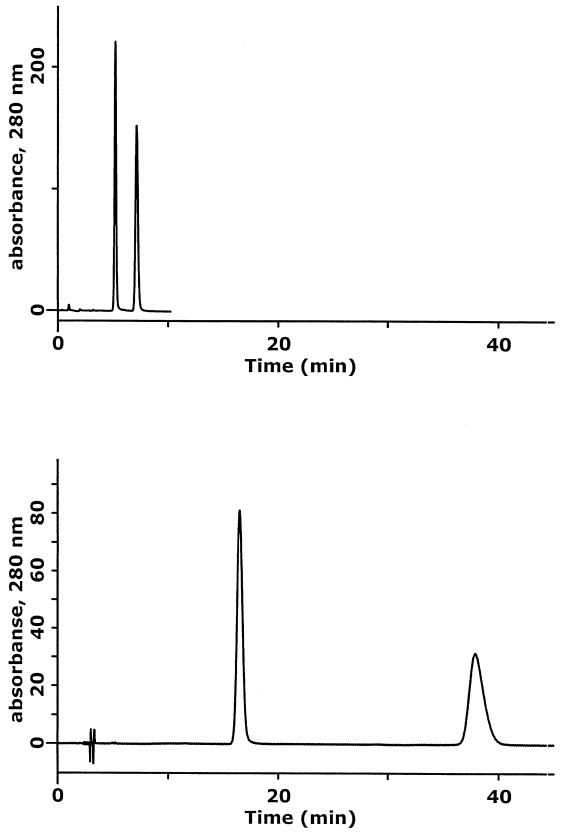


Fig. 14. Separation of WY-041594 enantiomers at room temperature. AD Chiralpak column (25×0.46 cm); flow-rate, 1 ml/min; mobile phase, ethanol (top trace), 50% methanol in HFE-7200 (bottom trace).

3.8. HFE-7200 regeneration in the lab

Despite its novelty, the use of HFE-7200 proved to be fairly reasonable from an economic point of view. Its cost is significantly lower than the cost of FC-113 and its mixtures with other solvents can be recycled on a centralized basis (returned to 3M Company via Safety-Kleen in the US). We found that HFE-7200 can be easily recovered in the laboratory from mixtures with methanol by washing them once with equal volumes of water, separation of the layers and quick distillation. Small amounts of TEA or TFA were eliminated by using acidified or slightly basic water for extraction. The effectiveness of methanol removal was monitored using chromatographic mobility of triphenylene and dimethyl phthalate on a thin-layer silica gel plate. These compounds have R_f 0.37 and 0.01, respectively, in fresh HFE-7200. Addition of small amounts of methanol (0.5%) to HFE-7200 results in R_f increase to 0.8 and 0.16, respectively.

4. Conclusions

Ethoxynonafluorobutane (HFE-7200) was successfully used to substitute *n*-hexane in a number of chromatographic applications based on normal-phase HPLC on a Luna cyanopropyl column. Gradients of methanol in HFE-7200 were found to be an effective separation tool for a number of diverse classes of biologically active compounds including steroids, benzodiazepines, NSAIDs, tricyclic antidepressants, purines and pyrimidines and β-adrenergic blockers.

The use of such gradients allows one to separate components with vastly different polarity in a single chromatographic run. In addition, we demonstrated that this fluorinated solvent may provide unique selectivities for performing chiral HPLC separations on polysaccharide-based columns. HFE-7200 is a non-toxic, environmentally friendly and economically sound solvent for normal-phase HPLC and we certainly hope that chromatographers will embrace it as one of many tools available to them.

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References

[1] L.R. Snyder, J.J. Kirkland, J.L. Glajch, in: Practical HPLC Method Development, 2nd edition, Wiley-Interscience, New York, NY, 1997, p. 287.
[2] J.L. Glajch, J.J. Kirkland, W.G. Schindel, Anal. Chem. 54 (1982) 1276.
[3] J. Layne, F. Ahmed, I. Rustamov, T. Farcas, T. Hanai. Poster PB5/19, Book of Abstracts, Volume II, 23rd International Symposium on High Performance Liquid Phase Separations and Related Techniques HPLC'99.
[4] http://www.3m.com/market/industrial/fluids/library/prodinfo/7200/7200.html
[5] M. Linnoila, in: E. Coster (Ed.), The Benzodiazepines — From Molecular Biology To Clinical Practice, Raven Press, New York, 1983, p. 267.
[6] W.R. Dixon, J. Earley, E. Postma, J. Pharm Sci. 64 (1976) 231.
[7] R. Gill, B. Law, J.P. Gibbs, J. Chromatogr. 356 (1986) 37.
[8] K. Valco, S. Olajos, T. Cserhati, J. Chromatogr. 499 (1990) 361.
[9] C. Lacroix, F. Wojciechowski, P. Danger, J. Chromatogr. 617 (1993) 285.
[10] M.A. Zilli, G. Nisi, J. Chromatogr. 378 (1986) 492.
[11] M. Rizzo, G. De Sarro, M. Zappala, A. Chimirri, J. Chromatogr. 731 (1999) 207.
[12] M.C. Pietrogrande, F. Dondi, G. Blo, P.A. Borea, C. Bighi, J. Liq. Chromatogr. 11 (1988) 1313.
[13] R. Mariot, L. Zangirolami, J. Chromatogr. B 677 (1996) 190.
[14] I. Choma, A.L. Dawidowicz, R. Lodkowski, J. Chromatogr. 600 (1992) 109.
[15] R.J. Flanagan, G.C.A. Storey, R.K. Bhamra, J. Chromatogr. 247 (1982) 15.
[16] A. Hercegova, J. Polonsky, Pharmazie 54 (1999) 479.
[17] T.D. Wilson, J. Liq. Chromatogr. 9 (1986) 2309.
[18] H.M. Stevens, R. Gill, J. Chromatogr. 370 (1989) 39.
[19] B.M. Lampert, J.T. Stewart, J. Chromatogr. 504 (1990) 381.
[20] M.P. Segatti, G. Nisi, F. Grossi, M. Mangiarotti, J. Chromatogr. 536 (1991) 319.
[21] S. Haertter, C. Hiemke, J. Chromatogr. 578 (1992) 273.
[22] P.P. Rop, T. Conquy, F. Gouezo, A. Vial, F. Grimaldi, J. Chromatogr. 375 (1986) 339.
[23] J.S. Kiel, R.K. Abramson, S.L. Morgan, J. Liq. Chromatogr. 6 (1983) 2761.
[24] I.M. McIntyre, C.V. King, S. Skafidis, O.H. Drummer, J. Chromatogr. 621 (1993) 215.
[25] C.T. Hung, R.B. Taylor, N. Paterson, J. Pharm. Biomed. Anal. 1 (1983) 73.
[26] B.A. Bidlingmeyer, J.K. Del Rios, J. Korpl, Anal. Chem. 54 (1982) 442.
[27] J.D. Blakesley, C.G. House, J. Spencer-Peet, D.C.F. Wood, Ann. Clin. Biochem. 23 (1986) 552.
[28] J.-H. Yan, J.W. Hubbard, G. McKay, K.K. Midha, J. Chromatogr. B 691 (1997) 131.
[29] P.R. Brown, S. Bobick, F.L. Hanley, J. Chromatogr. 99 (1974) 587.
[30] A. Floridi, C.A. Palmerini, C.J. Fini, J. Chromatogr. 138 (1977) 203.
[31] M. Zakaria, P.R. Brown, J. Chromatogr. 226 (1981) 267.
[32] R.A. Hartwick, S.P. Assenza, P.R. Brown, J. Chromatogr. 186 (1979) 647.
[33] A.A. Miller, J.A. Benvenuto, T.L. Loo, J. Chromatogr. 228 (1982) 165.
[34] J. Zhao, B. Todd, G.H. Fleet, J. Chromatogr. B 673 (1994) 167.
[35] M. Ryba, J. Chromatogr. 219 (1981) 245.
[36] M. Zakaria, P.R. Brown, J. Chromatogr. 225 (1983) 151.
[37] C. Yi, J.L. Faschihg, P.R. Brown, J. Chromatogr. 339 (1985) 75.
[38] M. Ehrlich, K. Ehrlich, J. Chromatogr. Sci. 17 (1979) 531.
[39] M. Ryba, J. Beranek, J. Chromatogr. 211 (1981) 337.
[40] H. Kazoka, J. Chromatogr. A 874 (2000) 45.
[41] H. Kazoka, V. Shatz, J. Chromatogr. A 732 (1996) 231.
[42] M.F. Fraga, R. Rodriguez, M.J. Canal, Electrophoresis 21 (2000) 2290.
[43] C.L. Davies, J. Chromatogr. 531 (1990) 131.
[44] J.A. De Shutter, P. De Moerloose, J. Chromatogr. 437 (1988) 83.
[45] M. De Smet, D.L. Massart, J. Chromatogr. 410 (1987) 77.
[46] S.H. Hansen, P. Helboe, M. Thomsen, Trends Anal. Chem. 7 (1988) 389.
[47] R. Gill, M.D. Osselton, R.M. Smith, T.G. Hurdley, J. Chromatogr. 323 (1985) 191.
[48] H. Richardson, B.A. Bidlingmeyer, J. Pharm. Sci. 73 (1984) 1480.
[49] T. Hamoir, D.L. Massart, J. Chromatogr. B 673 (1994) 1.
[50] G. Welmaker, J.A. Nelson, J.E. Sabalski, A. Sabb, J.R. Potoski, D. Graziano, M. Kagan, J. Coupet, J. Dunlop, H. Mazandarani, S. Rozenweig-Lipson, S. Sukoff, Y. Zhang, Bioorg. Med. Chem. Lett. 10 (2000) 1991.