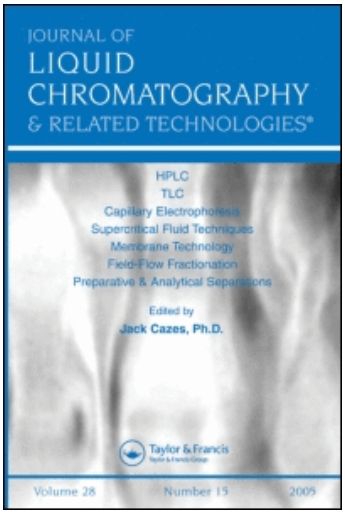


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DEVELOPMENT OF REVERSED-PHASE CHIRAL HPLC METHODS USING MASS SPECTROMETRY COMPATIBLE MOBILE PHASES

Kumar V. Penmetsa[a]; Christopher D. Reddick[a]; Saul W. Fink[a]; Brent L. Kleintop[a]; Gerald C. DiDonato[a]; Kevin J. Volk[a]; Steven E. Klohr[a]
[a] Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ, U.S.A.

Online publication date: 22 March 2000

To cite this Article Penmetsa, Kumar V. , Reddick, Christopher D. , Fink, Saul W. , Kleintop, Brent L. , DiDonato, Gerald C. , Volk, Kevin J. and Klohr, Steven E.(2000) 'DEVELOPMENT OF REVERSED-PHASE CHIRAL HPLC METHODS USING MASS SPECTROMETRY COMPATIBLE MOBILE PHASES', Journal of Liquid Chromatography & Related Technologies, 23: 6, 831 — 839
To link to this Article: DOI: 10.1081/JLC-100101492
URL: http://dx.doi.org/10.1081/JLC-100101492

J. LIQ. CHROM. & REL. TECHNOL., 23(6), 831–839 (2000)

DEVELOPMENT OF REVERSED-PHASE CHIRAL HPLC METHODS USING MASS SPECTROMETRY COMPATIBLE MOBILE PHASES

Kumar V. Penmetsa,[1,*] Christopher D. Reddick,[2] Saul W. Fink,[1]
Brent L. Kleintop,[2] Gerald C. DiDonato,[1] Kevin J. Volk,[2] Steven E. Klohr[3]

Analytical Research and Development
Bristol-Myers Squibb Pharmaceutical Research Institute
[1]New Brunswick, NJ 08903, USA
[2]Princeton, NJ 08540, USA
[3]Wallingford, CT 06492, USA

ABSTRACT

The majority of chiral HPLC separations are performed in the normal-phase mode using alcohol-modified hexane mobile phases. Normal-phase chiral HPLC methods are not routinely coupled with electrospray ionization mass spectrometry (ESI-MS) because of the mobile phase incompatibility. In this study, we investigated the use of ESI-MS compatible mobile phases for chiral HPLC methods. This would enable the sensitivity and selectivity of LC/MS to be applied to chiral HPLC analyses.

We used a commercially available reversed-phase chiral HPLC column (Chiralcel® OD-R) that permits the use of aqueous organic-modified mobile phases. This paper describes the development of direct, isocratic, and simple reversed-phase chiral HPLC methods for the separation of enantiomers of benzoin, indapamide, 2-phenylbutyric acid, 3-phenylbutyric acid, *trans*-2-phenylcyclopropane-1- carboxylic acid, verapamil hydrochloride, and pindolol.

In addition, we also demonstrate that the reversed-phase chiral HPLC methods developed in this study can be directly coupled with ESI-MS without any modifications. Examples of reversed-phase chiral high performance liquid chromatography-mass spectrometry (RP Chiral-LC/MS) methods are shown for indapamide and pindolol.

INTRODUCTION

Chiral separation of pharmaceutical compounds continues to be an active area of research; optical purity determination is critical in cases where one enantiomer has undesirable physiological response or has toxic side effects. HPLC is the most widely used technique for separation of chiral pharmaceutical compounds. The majority of chiral HPLC separations are performed in the normal-phase mode with ultraviolet detection. Normal-phase chiral HPLC methods are not routinely coupled with ESI-MS because the high percent hexane mobile phases used in conjunction with the high voltage electrospray ion source presents a potential explosion hazard. Although, it is possible to couple normal-phase HPLC methods to a mass spectrometer, the methods typically require modifications.[1] Post-column addition of an organic solvent (e.g., 2-propanol) with water is needed to overcome the miscibility problem with hexane and to improve the electrospray ionization process. However, for standard and routine chiral HPLC-MS methods, it would be beneficial if the mobile phases used were readily compatible with the electrospray ionization without the need for post-column modifiers.

Chiral HPLC-MS methods would be especially useful for monitoring process intermediates during the synthesis of chiral drugs, in pharmacokinetic studies of single enantiomers or racemic mixtures, and for formulation studies to measure the potential rate of drug racemization. The use of a mass spectrometer (MS) offers the advantage of high sensitivity, specificity, and the ability to analyze a wide variety of drug compounds. In addition, MS also adds the ability to obtain molecular weight and structural information, which can be used to verify the presence of two enantiomeric compounds resolved on a chiral column.

Recently, a few reversed-phase chiral HPLC methods have been described for the separation of drug enantiomers.[2,3,4,5] These methods typically used a mixture of aqueous buffers (e.g., sodium perchlorate, phosphate and acetate) and organic solvents as the mobile phase. The purpose of this study was to develop direct, isocratic, and simple reversed-phase chiral HPLC methods using mass spectrometry compatible mobile phases without the use of buffers for separation of chiral compounds. Reversed-phase isocratic chiral HPLC separations using simple mobile phases (water/acetonitrile) and (water/acetoni-

trile/trifluoroacetic acid) are shown for a few neutral, acidic and basic compounds. In addition, the methods developed in this study can be directly coupled with ESI-MS without any modifications as shown here for indapamide and pindolol.

EXPERIMENTAL

Chemicals

(±) Benzoin, indapamide, (±) verapamil hydrochloride, and pindolol were obtained from Sigma Chemical Company (St. Louis, MO, USA). (±) 2-Phenylbutyric acid, 3-phenylbutyric acid and *trans*-2-phenylcyclopropnae-1-carboxylic acid were obtained from Fluka Chemical Corporation (St. Louis, MO, USA). Acetonitrile was obtained from EM Science (Gibbstown, NJ, USA). Trifluoroacetic acid was obtained from J. T. Baker (Phillipsburg, NJ, USA).

Apparatus

The chiral separations were performed on a Waters Alliance 2690 module, 996 Photodiode array detector and Millennium 2010 chromatography data system. The Chiralcel® OD-R column (Cellulose tris 3,5-dimethylphenyl carbamate), 4.6 x 250 mm i.d., 10-µm particle diameter was obtained from Chiral Technologies, Inc., (Exton. PA, USA). For the HPLC-MS experiments, the HPLC system was coupled to a Finnigan MAT TSQ 7000 mass spectrometer equipped with a Finnigan ESI source. Device control and data acquisition were achieved with Finnigan ICIS software (Rev. 8.3) run on a UNIX workstation.

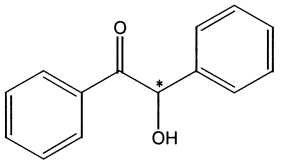
Preparation of Standard Solutions

The working standard solutions used for the HPLC-UV experiments were prepared at a concentration of 0.5 mg/mL in 50:50 acetonitrile:water. Working standards of indapamide and pindolol for the HPLC-ESI experiments were prepared in a similar manner. All standard solutions were stored at 4°C.

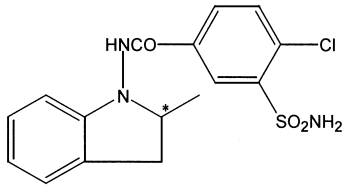
RESULTS AND DISCUSSION

Reversed-Phase Chiral HPLC Methods

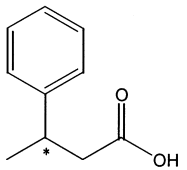
The structures of the compounds used in this investigation are shown in Figure 1. The chiral center is marked by an asterisk. In order to use mass spectrometry (MS) compatible mobile phases, a chiral stationary phase that allows



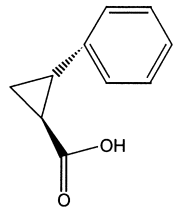
Benzoin



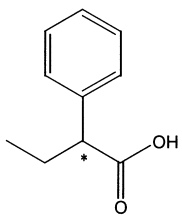
Indapamide



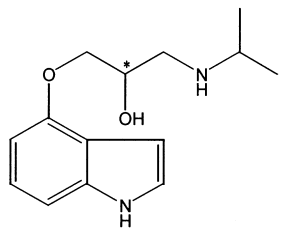
3-phenylbutyric acid



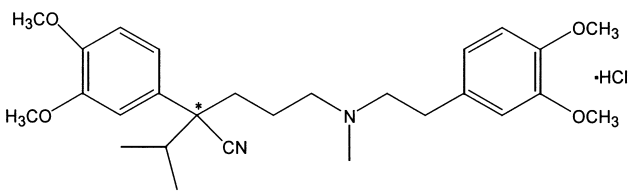
***trans*-2-phenylcyclopropanecarboxylic acid**



2-phenylbutyric acid



Pindolol



Verapamil Hydrochloride

Figure 1. Structures of compounds. The chiral center is marked by an asterisk.

the use of aqueous organic modified mobile phases is needed. Commercially available derivatives of cellulose and amylose HPLC columns are used extensively for analytical and preparative separations of a wide range of enantiomers. These columns can be obtained from Chiral Technologies, Inc., (Exton, PA, USA). Most of these columns are used in the normal-phase mode.

Recently, two reversed-phase cellulose-based chiral stationary phases (Chiralcel® OD-R and Chiralcel® OJ-R) have become commercially available. In this study, the use of Chiralcel® OD-R HPLC column was investigated for separation of a few racemic compounds. This column can be operated using 100% acetonitrile or 100% methanol and 20 to 100% water. In addition, the pH of the mobile phase should be between 2 to 7 for maximum column life. Neutral compounds were separated using a simple isocratic method with water/acetonitrile as the mobile phase. For the separation of acidic and basic compounds, 0.05% trifluoroacetic acid (TFA) was added to the mobile phase to suppress the ionization of the compounds. TFA is one of the most widely used mobile phase additives for analysis of charged compounds by HPLC-MS. Table 1 shows the reversed-phase chiral assay conditions for the test compounds.

Reversed-Phase Chiral HPLC/ESI-MS Experiments

To demonstrate the compatibility of reversed-phased chiral methods with ESI-MS, ion chromatograms were obtained for racemic solutions of indapamide and pindolol. Samples of both compounds were prepared at a concentration of 0.5 mg/mL in 50:50 water/acentonitrile and injected onto a Chiracel OD-R column for separation. The eluent (0.5 mL/min.) from each run was directed into the electrospray ionization source of a Finnigan MAT TSQ 7000 mass spectrometer. The ion source was operated in the positive ion mode with an ESI needle voltage of 4.5 kV. No post-column addition of organic modifiers was used to facilitate the electrospray process.

Initially, the mass spectrometer was set to monitor the protonated molecular ion $[M+H]^+$ for each compound. Figures 2A and 3A show the extracted ion chromatograms for the $[M+H]^+$ ion of indapamide and pindolol at m/z 366 and m/z 249, respectively. To confirm the enantiomeric separations, additional chromatographic runs were carried out for each compound with the mass spectrometer operated in the MS/MS mode.

In this mode, $[M+H]^+$ ion of each enantiomer is preferentially selected and fragmented via collisional induced dissociation (CID) with argon gas. The resulting MS/MS fragmentation spectrum serves as a structural template for the molecule. For a pair of enantiomers, the MS/MS fragmentation spectra are identical. It should be pointed out, however, that since the MS/MS spectra gen-

Table 1

Reversed-Phase Chiral HPLC-UV Assay for the Test Compounds: Chromatographic Parameters and Conditions

Compound	Retention Time(t_R) of Enantiomer 1 (min.)	Retention Time(t_R) of Enantiomer 2 (min.)	Resolution (R_s)
Benzoin[1]	24.06	28.67	4.78
Indapamide[1]	24.57	37.20	7.51
3-Phenylbutyric acid[2a]	12.35	13.20	1.78
Trans-2-phenylcylcopropane-1-carboxylic acid[2b]	13.18	14.09	1.81
2-Phenylbutyric acid[2c]	26.77	28.85	2.06
Pindolol[2d]	7.16	8.12	2.50
Verapamil hydrochloride[2c]	12.43	14.10	1.96

Mobile phase composition: [1]water/acetonitrile = (60/40). [2]0.05% TFA in water/ 0.05% TFA in acetonitrile; a = (60/40); b = (60/40); c = (70/30); d = (75/25); e = (70/30). Conditions: Chiralcel® ODR-R 4.6 x 250 mm i.d., 10 µm particle diameter; flow rate: 0.5 mL/min.; column temperature: 35°C; UV detection at 210 nm; injection volume: 5.0 µL of a 0.5 mg/mL standard solution.

erated for enantiomers are the same, MS/MS experiments alone cannot be used to differentiate individual enantiomers.

In these experiments, the argon collision gas pressure was adjusted to 2.0 mTorr and the collision energy for indapamide and pindolol was set to 15 eV and 22 eV, respectively. For indapamide, the two enantiomers at t_R = 23.6 min. and 35.3 min. in the extracted ion chromatogram produced a major product ion at m/z 132, resulting from cleavage of the central N-N bond (Figures 2B and 2C). The product ion spectra for the two enantiomers at t_R = 7.0 min. and 7.8

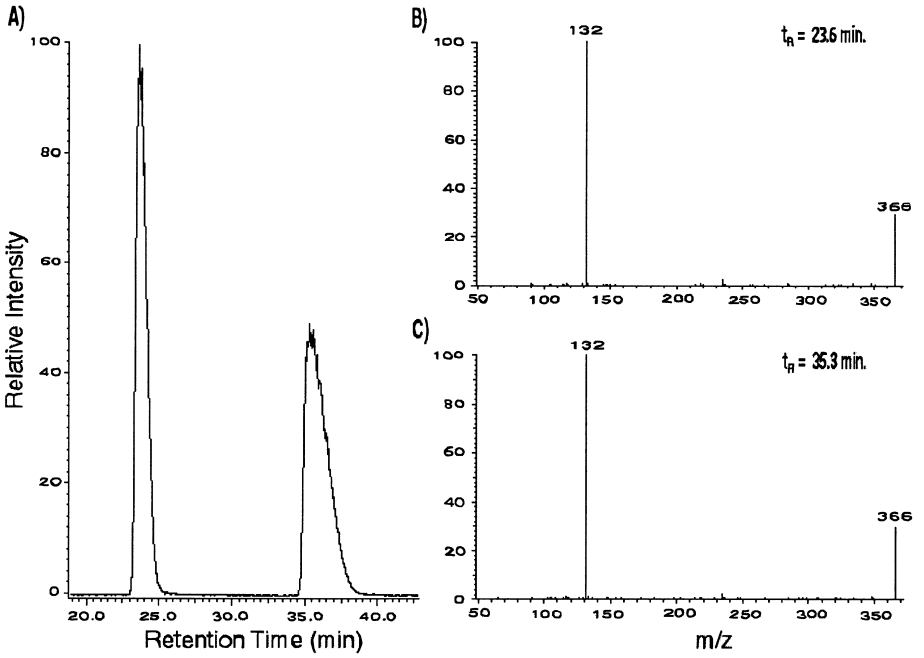


Figure 2. Reversed-phase chiral HPLC/ESI-MS separation of indapamide enantiomers. A) extracted ion chromatogram for $[M+H]^+$ ion at m/z 366. B) MS/MS product ion spectrum for enantiomer at t_R = 23.6 min. C) MS/MS product ion spectrum for enantiomer at t_R = 35.3 min.

min. in the extracted ion chromatogram for pindolol were also analogous (Figures 3B and 3C). For both compounds tested, comparisons of the respective MS/MS fragmentation spectra confirmed the enantiomeric separations.

CONCLUSION

The enantiomeric pairs of compounds investigated in this study have been baseline resolved using simple, isocratic, reversed-phase chiral HPLC methods on a Chiralcel® OD-R column. The use of water/acetonitrile and water/acetonitrile/TFA mobile phases enabled direct coupling of the chiral methods to an electrospray mass spectrometer without any modifications. Examples of reversed-phase chiral HPLC/ESI-MS and MS/MS analysis were shown for indapamide and pindolol, demonstrating that the numerous advantages of

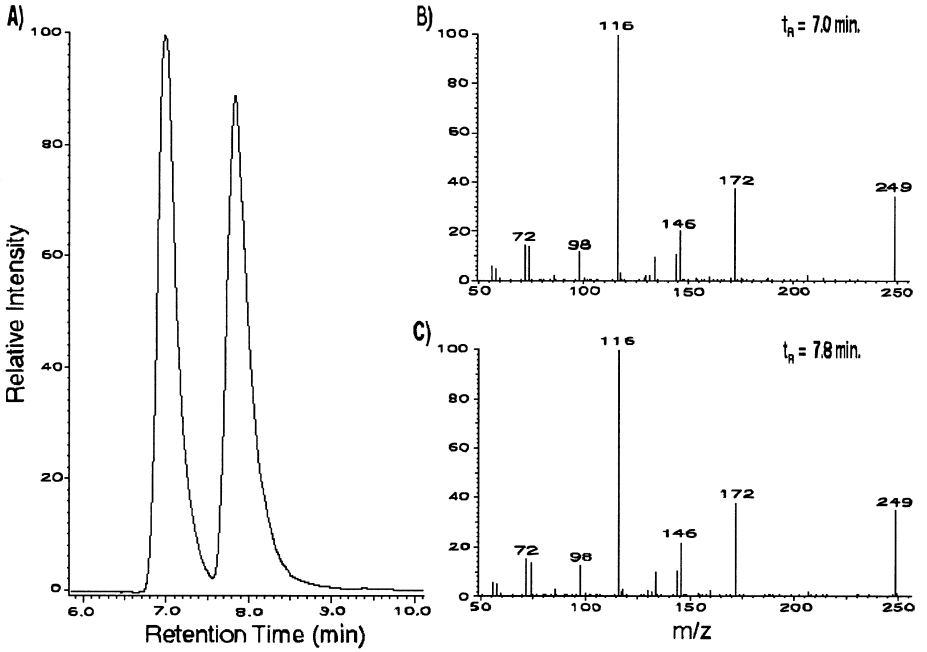


Figure 3. Reversed-phase chiral HPLC/ESI-MS separation of pindolol enantiomers. A) extracted ion chromatogram for $[M+H]^+$ ion at m/z 249. B) MS/MS product ion spectrum for enantiomer at t_R = 7.0 min. C) MS/MS product ion spectrum for enantiomer at t_R = 7.8 min.

LC/MS can also be realized with chiral HPLC separations. This technology could also be applied to the enantiomeric analysis of mixtures where the desired enantiomers can be identified amongst synthetic impurities and degradants. In addition, chiral LC/MS based methods can be used for high-throughput analysis of combinatorial libraries.

REFERENCES

1. T. Alebic-Kolbah, A. Paul Zavitsanos, J. Chromatogr., A, **759**, 65-77 (1997).

2. M. Tanaka, H. Yamazaki, H. Hakusui, Chirality, **7**, 612-615 (1995).

3. J. G. Ning, J. Chromatogr., A, **805**, 309-314 (1998).

4. H. Y. Aboul-Enein, V. Serignese, Biomed. Chromatogr., **8**, 22-25 (1994).

5. C. Rustichelli, V. Ferioli, G. Gamberini, Chromatographia, **44**, 477-483 (1997).

Received March 24, 1999 Author's Revisions October 12, 1999
Accepted August 16, 1999 Manuscript 5021