Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 820 (2005) 1–8
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Chiral liquid chromatography-tandem mass spectrometric methods for stereoisomeric pharmaceutical determinations

Jiwen Chen, Walter A. Korfmacher, Yunsheng Hsieh *

Drug Metabolism and Pharmacokinetics Department, 2015 Galloping Hill Road, K-15-3700, Kenilworth, NJ 07033, USA

Received 5 October 2004; accepted 10 February 2005
Available online 19 April 2005

Abstract

The characterization of the drug metabolism and pharmacokinetic (DMPK) profiles of stereoisomers is a fundamental aspect of the drug discovery and development processes. Therefore, chiral drug bioassays are very important to pharmaceutical and biomedical researchers. The recent developments in chiral liquid chromatography coupled to atmospheric pressure ionization tandem mass spectrometry (LC-API-MS/MS) for the analysis of pharmaceuticals are reviewed. Various ionization techniques including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric photoionization (APPI) interfaced with chiral liquid chromatographic methods are described in terms of their ionization efficiencies, matrix effects and limitations. Examples were selected to demonstrate the applicability of these methods for enantioselective bioanalysis.

Keywords: Chiral separation; LC-MS/MS; Pharmaceuticals; Atmospheric pressure ionization; Tandem mass spectrometry; Drug discovery

Contents

1. Introduction

The need for the separation and development of enantiomers from the pharmacological, scientific and economic points of view was reviewed by Lindner et al. [1] of the University of Vienna in Austria. The knowledge of the pharmacokinetic characteristics of each of the enantiomeric pharmaceuticals and their absorption, distribution, metabolism and excretion (ADME) properties is essential for successful drug development. It is also important to explore the biological responses of new chemical entities (NCEs) with respect to stereochemistry as part of the lead characterization process [2]. According to the US Food and Drug Administration's (FDA) policy statement for the development of new stereoisomeric drugs, in order to evaluate the pharmacokinetics of a single enantiomer

* Corresponding author. Tel.: +1 908 7405385; fax: +1 908 7402916.
E-mail address: yunsheng.hsieh@spcorp.com (Y. Hsieh).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.02.012

or mixture of enantiomers, manufacturers should develop quantitative assays for the individual enantiomers in vivo samples early in the drug development process [3].

In the past, most applications of high-performance liquid chromatography (HPLC) to stereoisomer chromatography were developed using either UV detection or by using derivatization reagents containing fluorophores with a fluorescence detector. Currently, there is a trend toward converting these UV or fluorescence-based chiral HPLC methodologies into more sensitive mass spectrometry-based approaches without losing the enantioselectivity of the assay [4]. In addition, the specificity of tandem mass spectrometry (MS) assays generally means that less sample preparation is needed for most assays. Furthermore, HPLC combined with tandem mass spectrometry (HPLC-MS/MS) is particularly attractive for the simultaneous quantification of drug molecules and their metabolites in biological matrices. In this article, we review the current bioanalytical methods for the stereoisomeric determination of pharmaceuticals using chiral HPLC systems coupled with an atmospheric pressure ionization (API) source and a tandem mass spectrometer (MS/MS). The limitations and strategies for interfacing each of three most common API sources with either reversed-phase or normal phase solvents used in chiral separation are also discussed.

2. Chiral stationary phases

Two decades ago, most drugs with a chiral center were commercialized as racemates. Although chirality is a critical factor in the in vivo system, it was often ignored due to the difficulty of performing enantiomer separations. This situation has significantly changed due to the advent of chromatographic enantioseparation techniques and the availability of commercial chiral stationary phases (CSPs) such as polymeric-based [5,6], Pirkle-type [7,8] and bonded macrocyclic antibiotic [9] stationary phases. Polysaccharides derivatives (one of the polymeric-based classes) are currently the most popular chiral selector for enantioseparation of various compounds due to their versatility, durability and loading capacity. In contrast to polysaccharide CSPs, enantiomer discrimination mechanisms in Pirkle-type CSPs are well characterized and consequently their chromatographic behaviors are highly predicable. Pirkle-type columns appear to be more robust and to be able to provide better column reproducibility than polysaccharide CSPs [10]. Since both π–π interactions and hydrogen bonding are stronger under normal phase conditions for enantioseparation in these CSPs, the majority of chiral separations on polysaccharide and Prikle-type columns are typically performed using normal phase mode [11].

While normal phase chromatography is common for polysaccharide and Pirkle-type CSPs, macrocyclic and protein-based chiral selectors are suitable for reversed-phase conditions. The applications of a new class of macrocyclic-based CSPs, the macrocyclic antibiotics, were recently reviewed by Ward et al. [9]. The macrocyclic antibiotics including the ansamycins, the glycopeptides and the polypeptide antibiotic thiostrepton have gained popularity as chiral selectors in HPLC. These CSPs have broad selectivity because several distinctive enatiomer recognition mechanisms are present in close proximity.

3. Chiral HPLC-APCI-MS/MS methods

The exponential growth in HPLC-MS applications since the late 1980s is primarily due to the introduction of the concept of atmospheric pressure ionization (API) for the HPLC-MS interface because the earlier HPLC-MS interfacing techniques were not robust, were difficult to operate and had poor sensitivity. API is defined for all ionization techniques where the ions are generated at atmospheric pressure and includes atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI) and atmospheric pressure photoionization (APPI). The atmospheric pressure chemical ionization (APCI) source contains a long (∼13 cm) heated nebulization quartz tube (350–500 °C) that rapidly converts the column eluent into a gas–vapor mixture. Ionization of analytes is mainly induced by a corona discharge needle where the solvent vapor might also act as the reagent gas for chemical ionization reactions. In general, the APCI source is considered to be not suitable for normal phase conditions. The primary challenge is the possible explosion hazard when a high flow of flammable solvents such as hexane (typically 1 mL/min) is introduced into the APCI source at an elevated temperature in the presence of a corona discharge. To circumvent this problem, two approaches have been explored. The first approach is to lower the heated nebulizer temperature. Ceccato and co-authors have developed a normal phase HPLC-APCI-MS/MS method using a Chiralpak AD column for enantiomeric separation of tramadol and its metabolite in human plasma where the probe temperature was set to 250 °C [12]. The authors reported good linearity for each enantiomer from 0.5 to 250 ng/mL. In a similar approach achieved by Paanakker et al. [13], a method was developed for the determination of the enantiomers of Org 4428 in human plasma at a heated nebulizer temperature of 240 °C. The disadvantage of this approach is the incomplete desolvation of the column eluent, resulting in poorer sensitivity and peak tailing as a result of the lower nebulizer temperature.

In the second approach, a large aqueous make-up flow is adapted post-column to reduce the hexane concentration in the mobile phase prior to the APCI source. This setup has been utilized in coupling a polysaccharide CSP column (Chiralpak AD) to APCI-MS by Miller-Stein et al. [14]. A principle drawback of this approach is the dilution of the analyte. In addition, the resolution of the enatiomers might be sacrificed due to extra column band broadening effect. Based upon our experience, the use of a tightly sealed ion source with a small negative pressure and nitrogen as the nebulizer and auxiliary gas is also efficient in preventing the risk of explosion. Puente [2] reported neither explosions

nor lack of ionization under regular APCI operational conditions for normal phase chiral HPLC-MS/MS. Although tandem mass spectrometric detection provides little chiral discrimination, it still maintains greater selectivity over fluorescence detection for the endogenous interferences, metabolites and internal standards. For example, Alebic-Kolbah demonstrated that unlike MS/MS detection, fluorescence detection used in the verapamil-nor verapamil chiral assay required additional separation of (+)-glaucine (used as an internal standard) from the analytes, resulting in longer run times [11].

In contrast to normal phase solvents, polar organic mobile phases, consisting of methanol with some volatile modifier such as ammonium trifluoacetate, are compatible with APCI-MS. Consequently, there is no requirement for post-column addition or source temperature adjustments. For example, the success of developing a bioanalytical method for quantification of fluoxetine, an antidepressant, in human plasma was presented by Shen et al. [15]. Bakhitiar and Tse demonstrated base-line separations for eight pharmaceutical compounds with diverse structures using either a vancomycin (Chirobiotic V) column or a teicoplanin (Chirobiotic T) column [16]. The authors also made use of the inherent selectivity of tandem mass spectrometry to simultaneously quantify four pairs of pharmaceutical enatiomers within 10 min in a single assay. In addition to the ability to directly couple to the APCI source, advantages for performing reversed-phased chromatography include the ease of sample preparation and the possibility for direct plasma injection with minimal sample pretreatment [17–21]. For example, Xia et al. were able to combine a dual-column extraction system and a tandem mass spectrometer in sequence for the automated determination of R and S-propranolol in rat plasma [22]. The calibration curve was linear from 2 to 2000 ng/mL. The signal to noise ratio of each enantiomer for the lowest standard (2 ng/mL) was larger than 50 using only 25 μL of plasma (Fig. 1).

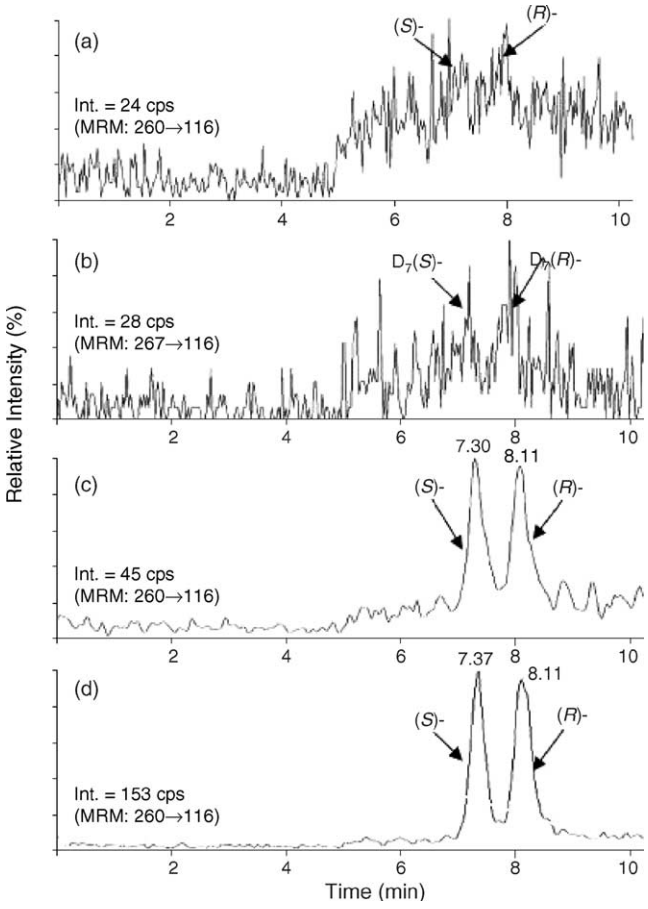

Fig. 1. Reconstructed selected reaction monitoring (SRM) chromatograms obtained by using the on-line dual-column extraction coupled with Chirobiotic T and LC-APCI-MS/MS system for (a) SRM channel for a double blank rat plasma; (b) SRM channel for D7-racemic-propranolol in a single blank of rat plasma; (c) racemic-propranolol at 0.5 ng/mL in rat plasma; (d) racemic-propranolol at 2 ng/mL in rat plasma [22].

4. Chiral HPLC-ESI-MS/MS methods

For the ESI, a fused-silica inner capillary and a stainless-steel outer capillary are used for introducing the sample and the nebulizing nitrogen gas, respectively. The solvent and analytes are ionized through the combined action of applying a high electric field (3–5 kV) and the pneumatic nebulization. These charged droplets shrink due to evaporation leading to the formation of highly charged microdroplets as they are directed toward the tandem mass spectrometer. Ions emitted from the microdroplet surface move into the gas-phase prior to mass spectrometric detection. Since ions are formed in solution, any solutes in the sample that can alter the surface concentration of analytes in the charged droplet will likely alter the response of the mass spectrometer. Direct coupling of a reversed-phase chiral column to the ESI is fairly straightforward, but some compromises might have to be made. For example, non-volatile modifiers in the mobile phase used for improving chiral selectivity may adversely affect the ion source performance.

Several examples of the utility of chiral HPLC-ESI-MS/MS can be found in the literature. Hedeland et al. [23] described the enanitoselective determination of verapamil and its desmethyl metabolite using α-acid glycoprotein column with HPLC-ESI-MS/MS. Other enantioselective determinations of pharmaceuticals such as methadone [24], propafenone [25], sotalol, oxprenolol, alprenolol, metoprolol, proprenolol, atenolol, norephedrine [26] and ibuprofen [27] using reversed-phase HPLC-ESI-MS assays are listed in Table 1. Most macrocyclic CSPs work well in reversed-phase and polar organic solvents. Enaotioselective quantification of chlorpheniramine was accomplished on a derivatized cyclodextrin column using an 85% aqueous mobile phase and the ESI source [28].

Normal phase solvents such as hexane are generally incompatible with the ESI source and also might raise a concern of an explosion hazard in the presence of the high voltage of

Table 1
Representative chiral HPLC-API-MS/MS methods for pharmaceuticals analysis

Pharmaceutical	Chiral column	API interface	Ionization mode	Limit of quantitation	Reference
Abuterol	Chiralbiotic T	ESI	Positive	0.025 ng/mL	[37]
Abuterol	Chirobiotic T	APCI	Positive	0.5 ng/mL	[38]
Amlopine	Chiral AGP	APCI	Positive	0.1 ng/mL	[47]
Azelastine	Chiralpak AD	ESI	Positive	40 ng/mL	[46]
Baclofen	Crownpak CR	APCI	Positive	0.15 ng/mL	[39]
Chlorpheniramine	Cyclobond I 2000	ESI	Positive	0.13 ng/mL	[28]
Felodipine	Chiralcel OJ-R	ESI	Positive	0.1 ng/mL	[30]
Fluoxetine	Chirobiotic V	APCI	Positive	2 ng/mL	[15]
Ibuprofen	Chiralpak AD-RH	ESI	Positive	12 ng/mL	[27]
Ketamine	Chiral AGP	ESI	Positive	1 ng/mL	[40]
Ketoprofen	Chirex 3005	ESI	Positive	0.05 ng/mL	[45]
Lercanidipine	Chiralpak AD	ESI	Positive	0.025 ng/ml	[44]
Methadone	Chiral AGP	ESI	Positive	5 ng/mL	[24]
Methylphenidate	Chiralbiotic V	APCI	Positive	0.2 ng/mL	[42]
Omeprazole	Chiralpak AD	ESI	Positive	9.6 pmol/L	[29]
Phenprocoumon	Chira-Grom-2	ESI	Positive	12.5 ng/mL	[43]
Pindolol	Chiral DRUG	ESI	Positive	0.25 ng/mL	[41]
Propafenone	Chiral AGP	ESI	Positive	20 ng/mL	[25]
Propranolol	Chirobiotic T	APCI	Positive	2 ng/mL	[22]
Terazosin	Chiralpak AD	ESI	Positive	0.0625 ng/mL	[31]
Tramadol	Chiralpak AD	APCI	Positive	0.5 ng/mL	[12]
Sotalol	Teicoplanin	ESI	Positive	4 ng/mL	[26]
Verapamil	Chrial AGP	ESI	Positive	0.1 ng/mL	[23]

the electrospray needle. In order to resolve these difficulties, post-column addition of MS-compatible solvents has been employed for the direct coupling of normal phase solvents in HPLC-ESI-MS systems. The applicability of normal phase HPLC to ESI-MS assays for the enatioselective determinations of omeprazole, an antiulcer drug [29], and felodipine (Fig. 2), an antihypertensive drug [30], in human plasma have been reported by Stenhoff et al. [29] and Lindmark et al. [30], respectively. Zavitsanos et al. [31] confirmed that the normal phase HPLC-ESI/MS results of terazosin enantiomers concentrations in human plasma are correlated with the HPLC-fluorescence results.

5. Chiral HPLC-APPI-MS/MS methods

The APPI source (similar to the APCI source) vaporizes the sample with a heated nebulizer (300–500 °C) to generate a dense cloud of gas-phase analytes. Ionization uses a photoionization lamp to emit 10-eV photons to form dopant radical cations (Fig. 3). The protonation reaction of a given analyte M involving the dopant and solvent molecules in positive ion mode is proposed as follows:

$$[\text{Dopant}] + h\nu \rightarrow [\text{Dopant}]^+ + \text{e}^-$$

$$[\text{Dopant}]^+ + n[\text{Solvent}] \rightarrow [\text{Dopant-H}] + [(\text{Solvent})_n + \text{H}]^+$$

$$[(\text{Solvent})_n + \text{H}]^+ + \text{M} \rightarrow n[\text{Solvent}] + [\text{M} + \text{H}]^+$$

Toluene (IE = 8.83 eV) is frequently chosen as the preferred dopant solvent due to its weak toxicity. Proton transfer

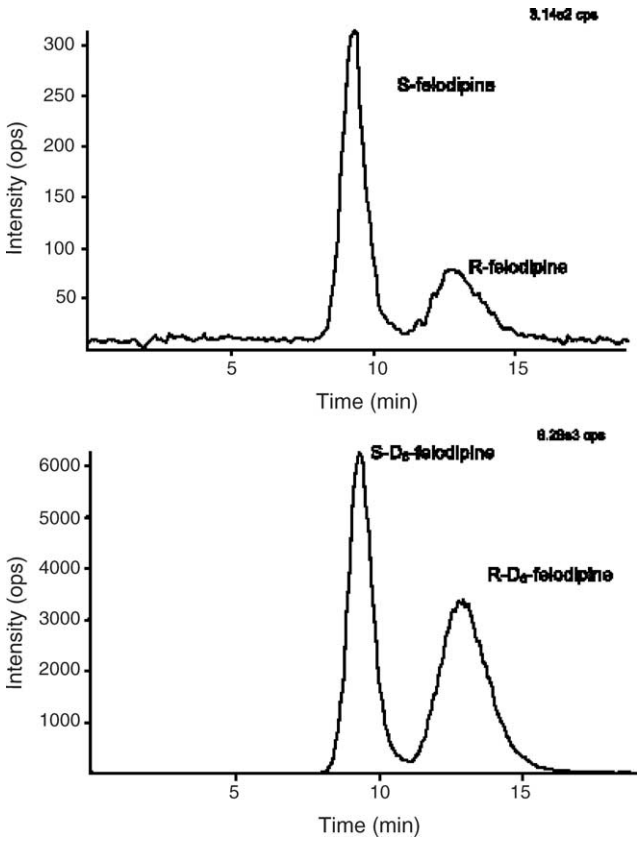

Fig. 2. Reconstructed SRM chromatograms of a study human plasma sample containing 0.7 nmol/L of S-felodipine and 0.3 nmol/L of R-felodipine [30].

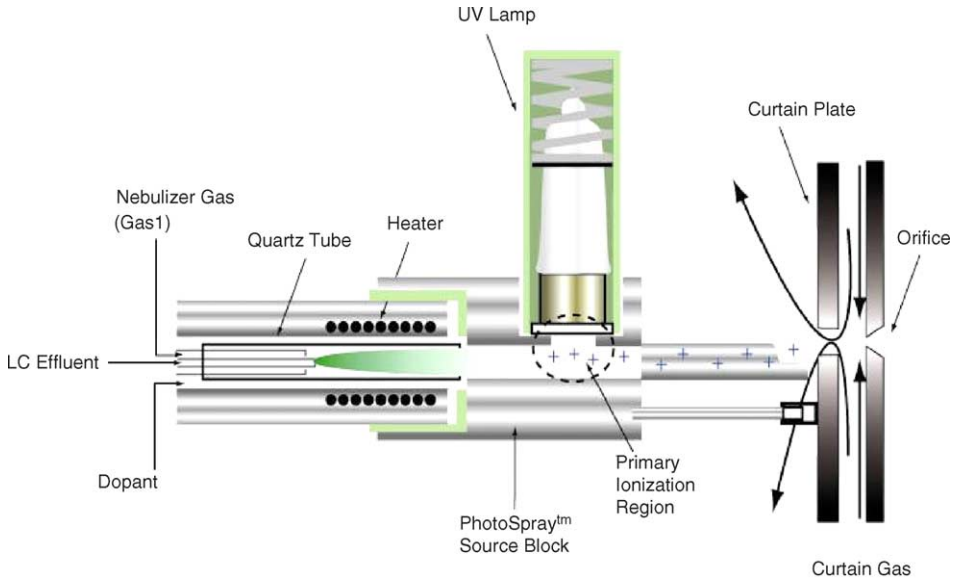

Fig. 3. Schematic diagram of an atmospheric pressure photoionization (APPI) source.

reaction occurs only if the proton affinity (PA) of the solvent is greater than that of the dopant and the PA of analyte is larger than that of the solvent [32]. Unlike the ESI and the APCI sources, the APPI source is compatible with both reversed-phase and normal phase conditions without the safety concerns of a potential explosion.

The schematics of an APPI source used in our laboratory with a Chiralcel OD-H column for the determination of propanolol mixtures [32] is shown in Fig. 3. The chiral HPLC-APPI/MS/MS method was tested again for two stereoisometric drug discovery compounds containing a hydroxyl group in an asymmetric center. Interestingly, we observed that there was no significant effect on ionization efficiency of the test compounds under normal phase conditions (ethanol/hexane = 20/80) with or without the addition of dopant. This may be explained due to "self-doping" effect where hexane (IE = 10.13 eV) solvents behave as dopant molecules to generate the protonated solvent molecules through proton transfer from the solvent mixture or its impurities [33]. Therefore, the normal phase HPLC-APPI-MS/MS system required neither introduction of dopant solution nor post-column addition of MS-compatible solvents.

Except for CSPs, many other variables such as additives, pH and composition of mobile phases will also have a strong impact on analyte retention times and selection factors. Effects of mobile phase composition, buffer concentration and pH values on the retention and the enantioselectivity of propranolol enantiomers using a β-cyclodextrin bonded CSP were investigated by Ching et al. [34]. These HPLC parameters frequently influence the ionization efficiency of analytes depending upon the API interfaces chosen [35]. As an example, Fig. 4 shows that the APPI signals of both R- and S-propranolol are associated with the ratios of ethanol in the isooctane mobile phase solvent [32].

6. Matrix effects

The possibility of "matrix effects" is a well-documented phenomenon in LC-MS/MS bioanalysis. Co-eluting matrix components may suppress analyte signals in a number of ways, thereby compromising the quality of the quantification data [32]. Different types of ionization interfaces exhibit susceptibility to matrix effects to different extents. Compared to the ESI source, the APCI source tends to be less susceptible to matrix effects. One aspect that should be considered as part of method development is the potential for matrix ionization suppression effects when the retention time window for the two enantiomers is more than 25% of the total chromatographic run time. As an example, in the enantioseparation of felodipine in plasma, the retention

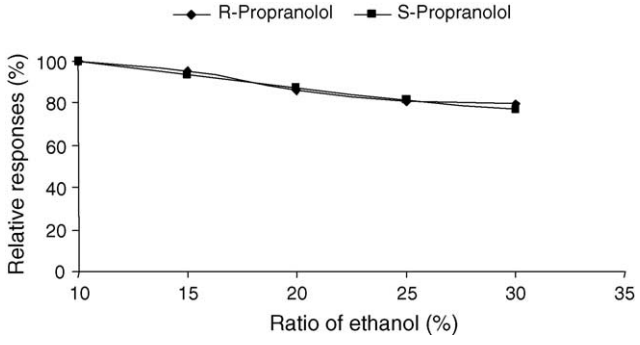

Fig. 4. Relative APPI responses of R-propranolol and S-propranolol as a function of the solvent composition.

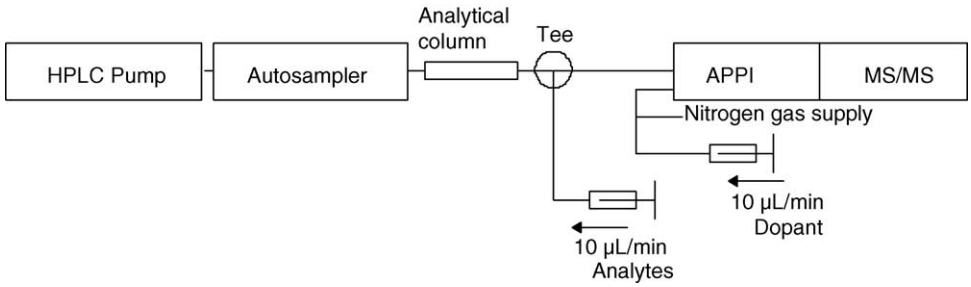

Fig. 5. Schematic diagram of post-column infusion technique with atmospheric pressure photoionization source.

time window for the two enantiomers was approximately 8 min (Fig. 2), accounting for 40% of the total LC run time (20 min). Maintaining nearly half of the chromatogram matrix-interference free, while achieving sufficient enatioseparation is no trivial task. In light of this, both the APCI and the APPI sources are likely to take a more prominent role in chiral bioanalysis due to their lower sensitivities to matrix effects.

The post-column infusion technique is an easy and effective way to evaluate the matrix ionization suppression issue HPLC-MS systems [32]. In general, the ESI source is more vulnerable than the APCI source to matrix ionization suppression from biological matrices. The schematic diagram of the post-column infusion system used in our laboratory for the matrix effect studies on the APPI source is shown in Fig. 5. In practice, test compounds are continuously infused into the Peek® tubing in between the analytical column and the mass spectrometer through a "tee" using a Harvard Apparatus Model 2400 (South Natick, MA, USA) syringe pump. Either a protein precipitation extract of blank rat plasma or mobile phase B (10 μL) was injected into the analytical column for comparison of ionization responses. Effluent from the analytical column mixed with the infused compounds and entered the API interface. The infusion HPLC-APPI/MS/MS chromatograms of the test compound after either a 10-μL injection of mobile phase or rat plasma extract is shown in Fig. 6. The differences in the infusion chromatograms between the mobile phase injection and the rat plasma extract injection are considered to be caused by the matrix ion suppression effects due to plasma sample extract constituents eluting from the column. Fig. 6 indicates the degree of loss of ion responses and the length of times required for the ion signals to

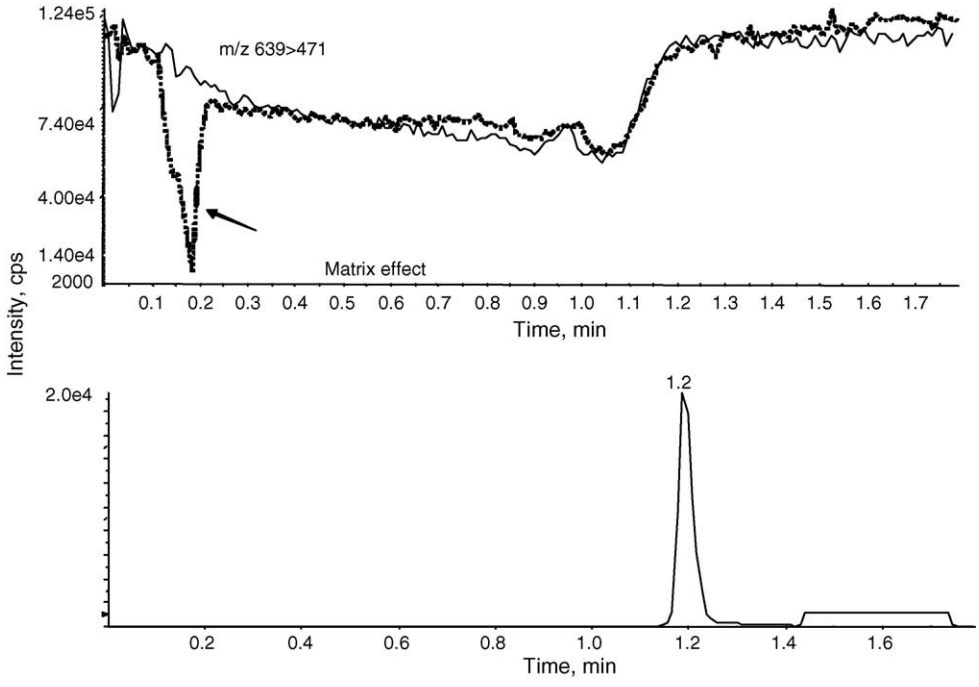

Fig. 6. (Top) The reconstructed infusion HPLC-APPI-MS/MS chromatograms of lonafarnib following mobile phase (solid line) and blank plasma precipitation extract injections (dot line). The region showing lower responses indicated the area of matrix ionization suppression. (Bottom) Representative reconstructed HPLC-APPI-MS/MS chromatogram of lonafarnib from standard rat plasma [32].

return to its pre-sample injection sensitivity; in this example, there is no significant matrix effect observed after 0.3 min.

7. Conclusions and future perspectives

MS/MS detection can be used not only for the determination of the dosed compound, but also for the complete resolution of the parent drugs from their metabolites (based on their unique MS/MS detection differences), thus often avoiding the need for additional separation or sample clean-up procedures for this purpose. The detection limits required for pharmacokinetic studies are achievable by using the more sensitive detection systems based on API sources and tandem mass spectrometry. In addition, hyphenation of the high-resolving power of HPLC to a tandem mass spectrometer (MS/MS) provides straightforward method development capabilities with excellent analytical linearity, sensitivity and selectivity for monitoring drug compounds. In most cases, HPLC-MS/MS methods demonstrate little or no chromatographic interference from endogenous components in complex biological samples. This makes it possible to shorten chromatographic times and therefore to allow higher sample throughput. Direct coupling of the chiral columns to an appropriate API source as part of the API-MS/MS system will continue to be popular. The analytical throughput of chiral LC-MS/MS is mainly dependent on the resolution efficiency of CSPs. Chiral LC-MS/MS per sample cycle times will likely remain long until higher efficiency CSPs becomes available.

Fast chromatography approaches such as micro-column technologies with smaller particle size and monolitic silica column methods [36] might make high-speed chiral separation possible without a noticeable effect on chromatographic resolution. Staggered injection parallel HPLC systems make use of blank chromatographic windows to provide a boost in throughput. For example, an increase in sample throughput by a factor of three can be achieved by staggering injections onto the four columns, allowing the mass spectrometer to continuously monitor the chromatographic window of interest [36]. Another attractive solution to the sample throughput problem is the use of supercritical fluid chromatography (SFC), where higher column efficiency is obtained with substantially lower mobile phase viscosity. Many chiral separations performed in the normal phase HPLC mode could potentially be replaced with SFC with shorter analysis time. In addition, the mobile phases used in SFC are compatible with most API sources.

Advancements in column technology have made chiral separation routine. More and more assays now have direct coupling of various chiral columns with an API source without compromising the detector sensitivity or the LC resolving power. With the increasing popularity of CSPs running in the polar organic mode, it is believed that this trend will continue. Finally, because the APPI source is amenable to both reversed-phase and normal phase solvents, it will be more widely employed for the determination of non-polar enantiomers in the future.

References

[1] N. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[2] M.L. de la Puente, J. Chromatogr. A 1055 (2004) 55.
[3] http://www.fda.gov/cder/guidance/stereo.htm.
[4] M.J. Desai, D.W. Armstrong, J. Chromatogr. A 1035 (2004) 203.
[5] C. Yamamoto, Y. Okamoto, Methods Mol. Biol. 243 (2004) 173.
[6] Y. Yashima, J. Chromatogr. A 906 (2001) 105.
[7] M. Hyun, Y. Cho, Methods Mol. Biol. 243 (2004) 197.
[8] W.H. Pirkle, C. Welch, J. Chromatogr. A 683 (1994) 353.
[9] T. Ward, A.B. Farris, J. Chromtogr. A 906 (2001) 73.
[10] K. Tachibana, A. Ohnishi, J. Chromatogr. A 906 (2001) 127.
[11] T. Alebic-Kolbah, A.P. Zavitsanos, J. Chromatogr. A 759 (1997) 65.
[12] A. Ceccato, F. Vanderbist, J.-Y. Pabst, B. Streel, J. Chromatogr. B 748 (2000) 65.
[13] J.E. Paanakker, J. de Jong, J.M.S.L. Thio, H.J.M. van Hal, J. Pharm. Biomed. Anal. 16 (1998) 981.
[14] C. Miller-Stein, C. Fernandez-Metzler, J. Chromatogr. A 964 (2002) 161.
[15] Z. Shen, S. Wang, R. Bakhtiar, Rapid Commun. Mass Spectrom. 16 (2002) 332.
[16] R. Bakhtiar, F. Tse, Rapid Commun. Mass Spectrom. 14 (2000) 1128.
[17] Y. Hsieh, J. Brisson, K. Ng, R. White, W. Korfmacher, Analyst 126 (2001) 2139.
[18] Y. Hsieh, J. Brisson, G. Wang, K. Ng, W. Korfmacher, J. Pharm. Biomed. Anal. 27 (2002) 285.
[19] Y. Hsieh, M. Bryant, J. Brisson, K. Ng, W. Korfmacher, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 767 (2002) 353.
[20] Y. Hsieh, K. Ng, W. Korfmacher, Am. Pharm. Rev. 5 (2002) 88.
[21] Y. Hsieh, M. Bryant, G. Gruela, W. Korfmacher, Rapid Commun. Mass Spectrom. 14 (2000) 1384.
[22] Y. Xia, R. Bakhtiar, R. Franklin, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 788 (2003) 317.
[23] M. Hedeland, E. Fredriksson, H. Lennernas, U. Bondesson, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 804 (2004) 303.
[24] H. Liang, R.L. Foltz, M. Meng, P. Bennett, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 806 (2004) 191.
[25] D. Zhong, X. Chen, J. Chromatogr. B Biomed. Sci. Appl. 721 (1999) 67.
[26] E. Badaloni, I.D. Acquarica, F. Gasparrini, S. Lalli, D. Misiti, F. Pazzucconi, C.R. Sirtori, J. Chromatogr. B Biomed. Sci. Appl. 796 (2003) 45.
[27] P.S. Bonato, M.P.F.M. Del Lama, R. de Carvalho, J. Chromatogr. B Biomed. Sci. Appl. 796 (2003) 413.
[28] K.M. Fried, A.E. Young, S. Usdin Yasuda, I.W. Wainer, J. Pharm. Biomed. Anal. 27 (2002) 479.
[29] H. Stenhoff, A. Blomqvist, P.O. Lagerstrom, J. Chromatogr. B Biomed. Sci. Appl. 734 (1999) 191.
[30] B. Lindmark, M. Ahnoff, B.A. Persson, J. Pharm. Biomed. Anal. 27 (2002) 489.
[31] A.P. Zavitsanos, T. Alebic-Kolbah, J Chromatogr. A 794 (1998) 45.
[32] Y. Hsieh, in: W. Korfmacher (Ed.), Using Mass Spectrometry for Drug Metabolism Studies, CRC press, Boca Raton, FL, 2004.
[33] G. Wang, Y. Hsieh, W. Korfmacher, Anal. Chem. 77 (2005) 541.
[34] C.B. Ching, P. Fu, S.C. Ng, Y.K. Xu, J Chromatogr. A 898 (2000) 53.
[35] J. Rauha, H. Vuorela, R. Kostiainen, Rapid Commun. Mass Spectrom. 36 (2001) 1269.
[36] Y. Hsieh, K. Cheng, W. Korfmacher, Current Drug Metabolism, 2005, submitted for publication.

[37] K.B. Joyce, A.E. Jones, R.J. Scott, R.A. Biddlecombe, S. Pleasance, Rapid Commun. Mass Spectrom. 23 (1998) 1899.
[38] G.A. Jacobson, F.V. Chong, N.W. Davies, J. Pharm. Biomed. Anal. 31 (2003) 1237.
[39] R. Goda, N. Murayama, Y. Fuyimaki, K. Sudo, J. Chromatogr. B 801 (2004) 257.
[40] M.E.R. Rosas, S. Patel, I.W. Wainer, J Chromatogr. B 794 (2003) 99.
[41] A. Motoyama, A. Suzuki, O. Shirota, R. Namba, J. Pharm. Biomed. Anal. 28 (2002) 97.
[42] R. Bakhtiar, L. Ramos, F. Tse, Rapid Commun. Mass Spectrom. 16 (2002) 81.
[43] B. Kammerer, R. Kahlich, M. Ufer, S. Laufer, C.H. Gleiter, Rapid Commun. Mass Spectrom. 18 (4) (2004) 458.
[44] V.A. Jabor, E.B. Coelho, D.R. Ifa, P.S. Bonato, N.A. dos Santos, V.L. Lanchote, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 796 (2003) 429.
[45] T.H. Eichhold, R.E. Bailey, S.L. Tanguay, S.H. Hoke, J. Mass Spectrom. 35 (2000) 504.
[46] U. Heinemann, G. Blaschke, N. Knebel, J. Chromatogr. B 793 (2003) 38.
[47] B. Streel, C. Laine, C. Zimmer, R. Sibenaler, A. Ceccato, J. Biochem. Biophys. Methods 54 (2002) 357.