Journal of Chromatography B, 878 (2010) 2449–2452

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Determination of esmolol and metabolite enantiomers within human plasma using chiral column chromatography

Lei Fang, Crystal Bykowski-Jurkiewicz, Jeffrey G. Sarver, Paul W. Erhardt*

Center for Drug Design and Development, Department of Medicinal and Biological Chemistry, University of Toledo, College of Pharmacy, Toledo, OH, USA

ARTICLE INFO

Article history:
Received 21 April 2010
Accepted 13 July 2010
Available online 21 July 2010

Keywords:
Esmolol
Esmolol metabolite
Enantiomers
Chiral column chromatography
LC–MS/MS
HPLC

ABSTRACT

A high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed for the simultaneous determination of each of esmolol's enantiomers at the 25–1000 ng/ml concentrations observed in human plasma upon intravenous administration of this rapidly metabolized *beta*-adrenergic receptor blocking agent. Alternatively, a high performance liquid chromatography (HPLC) UV detection method has been developed for the simultaneous determination of each of the enantiomers for esmolol's metabolite which, in turn, achieve 2.5–50 μg/ml concentrations in human plasma. Utilizing chiral columns, these methods do not require a precolumn asymmetric derivatization step. Linearity in all cases was >0.99. Precision and accuracy at all but the lowest concentrations were within ±6% for the esmolol enantiomers and within ±2.5% for the esmolol metabolite enantiomers. These values should be suitable for performing thorough pharmacokinetic studies for all of the stereoisomers of this prototypical soft drug and its corresponding metabolite.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Unique among the family of drugs known as '*beta*-adrenergic receptor blockers', esmolol was designed to have a specific pharmacokinetic (PK) profile that is ideally suited for intravenous administration in critical care settings. Regarded as an ultra-short acting agent, it has a half-life of about 10 min because it undergoes rapid metabolism by blood esterases to an inactive acid metabolite (Fig. 1). This profile allows for moment-to-moment control of esmolol's activity by adjustment of its infusion drip-rate [1]. Esmolol and similarly behaving compounds from this niche class of therapeutic agents, have come to be called 'soft drugs' [2]. Representing one of the first clinically validated approaches toward such PK-driven drug design strategies [3], esmolol constitutes the prototypical soft drug structure. In addition, because prodrugs typically rely upon these same types of enzymes for rapid bioactivation, esmolol also has become a useful standard during PK assessments pertaining to ester hydrolyses of candidates for this more prevalent class of therapeutic agents [4].

Like nearly all of the marketed '*beta*-blockers', esmolol is administered as its racemate even though the (S)-enantiomer is known to be the active stereoisomer. A limited number of studies have focused on the metabolic disposition of the esmolol enantiomers [5,6], or upon the sterochemical outcomes of the racemic form by using asymmetric derivatization prior to chromatography [5,7,8]. Among these studies, none have deployed direct measurements by chiral column chromatography, and none have thoroughly accounted for the corresponding stereoisomeric metabolites even though the latter's residency times *in vivo* far out-last that of their enantiomeric parents. We are interested in delineating the specific stereochemical aspects for esmolol and its metabolite in relation to plasma protein binding in order to better understand their contribution to the overall PK profile displayed by this prototypical softdrug. As an initial step toward that goal, we report herein the development of the first stereospecific assay methods for the quantitative measurement of both the metabolite and parent compounds' enantiomers from human plasma by using chiral HPLC and LC–MS/MS, respectively.

2. Materials and methods

2.1. Chemicals and biological media

Racemic (S)- and (R)-esmolol hydrochloride salts, and racemic (S)- and (R)-metabolite internal salts were synthesized in our lab according to published procedures [1,3,9,10]. Physical properties including optical rotations [11], NMR spectra, and elemental analyses were in accord with assigned structures. Chiral column chromatography established 98% or higher enantiomeric purity for all of the pure enantiomers. HPLC Grade methanol,

* Corresponding author at: Center for Drug Design and Development, University of Toledo, College of Pharmacy, 2801 West Bancroft Street, Toledo, OH 43606, USA. Tel.: +1 419 530 2167; fax: +1 419 530 7946.
E-mail address: paul.erhardt@utoledo.edu (P.W. Erhardt).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.07.006

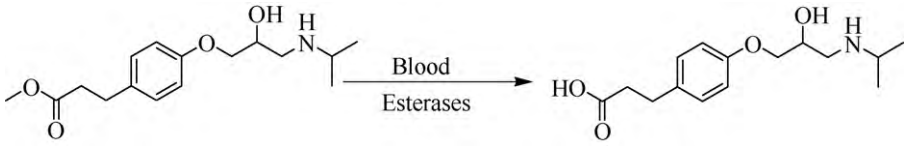

Fig. 1. Rapid hydrolysis of esmolol's ester to an inactive acid metabolite by blood esterases.

acetonitrile, ammonium trifluoroacetate (NH_4TFA) and sodium perchlorate ($NaClO_4$) were purchased from Aldrich (St. Louis, MO, USA). All other chemicals and reagents were purchased from common commercial sources and used without further purification. Human plasma was purchased from Innovative Research (Novi, MI, USA).

2.2. Instrumentation

Measurements of the esmolol stereoisomers were accomplished on an Alliance HT Model 2795 LC equipped with a quaternary pump, a degasser, an auto sampler/injector (syringe volume = 250 μl) and a column heater from Waters (Milford, MA, USA), coupled to a Quattro Micro (triple-quadrupole) MS/MS equipped with an ESCi Multi Mode Ionization source from Micromass (Manchester, UK). Measurements of the metabolite stereoisomers were accomplished on a Waters HPLC equipped with a Model 2695 Separation Module, a quaternary pump, a degasser, an auto sampler/injector (syringe volume = 100 μl), a column oven and a Model 2996 Photodiode Array Detector.

2.3. Chromatography conditions

The LC–MS/MS method utilized a 2.1 mm × 10 cm (5 μm) Astec Chirobiotic T Column purchased from Supelco (Bellefonte, PA, USA). The mobile phase was 0.1% NH_4TFA in methanol/methanol (10/90) with an isocratic elution at a flow rate of 0.3 ml/min for 14 min per run. The MS/MS was operated in the MRM mode with the following settings: dissolution gas (N_2) flow rate 700 l/h; source and dissolution gas temperatures 140 and 400 °C, respectively; ESI source tip (capillary) and extractor voltages 3 kV and 2 V, respectively; ion energies for MS1 and MS2 0.2 and 1.0 V, respectively; cone voltage 35 V; and, collision energy 27 V. The MRM transition was detected at 296.20 → 145.12. The HPLC method utilized a 2.1 mm × 15 cm (5 μm) Chiralcel OD-RH Column purchased from Chiral Technologies (West Chester, PA, USA). The mobile phase was 0.5 M $NaClO_4$ (pH = 3.0)/acetonitrile (87.5/12.5) with an isocratic elution at a flow rate of 0.08 ml/min for 40 min per run. Detector wavelength was set at 230 nm.

2.4. Sample preparation

Plasma samples (200 μl) intended for analysis of the esmolol stereoisomers were collected in 15 ml centrifuge tubes and immediately treated with 2 ml methylenechloride and 0.6 ml water. After centrifugation (Eppendorf 5810R) at 4000 rpm for 6 min, 500 μl of the methylenechloride layer was removed, evaporated to dryness under vacuum, and reconstituted in 100 μl MeOH. Separate plasma samples (200 μl) intended for analysis of the metabolite stereoisomers were similarly collected in 1.5 ml centrifuge tubes and immediately treated with 200 μl 6% perchloric acid. After centrifugation at 13,000 rpm for 5 min, 200 μl of the supernatant was removed and re-centrifuged for another 5 min. Both the LC–MS/MS analyses for esmolol and HPLC analyses for metabolite used 10 μl injection volumes.

2.5. Calibration curves

Because esmolol is rapidly converted to its metabolite, its clinical plasma concentrations reside in the ng/ml range. This prompts the use of an LC–MS/MS method and construction of standard calibration curves for each of the enantiomers in human plasma from 25 to 1000 ng/ml. Alternatively, concentrations of esmolol's metabolite within human plasma reside in the μg/ml range, thus allowing for HPLC analysis with standard calibration curves constructed from 2.5 to 50 μg/ml. In all cases, parent solutions were prepared in triplicate, diluted to provide 7 and 6 concentration points for the respective esmolol and metabolite enantiomers, and then readied for analyses according to the sample preparation methods indicated above. Samples were injected in triplicate providing 9 data points at each concentration.

2.6. Recovery, precision and accuracy

Recoveries of the esmolol and metabolite enantiomers from plasma were evaluated at three concentrations within the linear calibration range by direct comparison to aqueous stock solutions having the same concentrations (theoretical values). Precision (coefficient of variation; CV %) and accuracy (%) parameters were similarly assessed. Experiments for the esmolol enantiomers were conducted at 25, 200 and 1000 ng/ml, and those for the metabolite enantiomers were at 2.5, 25 and 50 μg/ml. Although it was later determined that the esmolol hydrochloride salts produce slightly acidic stock solutions (pH ca. 5) that can afford stability for several days during storage at 2–4 °C, all of the development and validation studies, including those for the metabolite stereoisomers which no longer contain the potentially labile ester moiety, were performed by using freshly prepared standard solutions.

2.7. Data processing and statistical analysis

Data acquisition and processing was facilitated by MassLynx (version 4.01) and Empower 2 software. Data comparisons were performed using one-way ANOVA, with $p < 0.05$ considered to be statistically significant.

3. Results

Representative LC–MS/MS and HPLC chromatograms for racemic esmolol and metabolite within human plasma are shown in Fig. 2 Panels A and B, respectively. Although baseline separation was not achieved, the overlap between the stereoisomer peaks was minimal and allowed for reproducible integrations of each enantiomer. In both cases, follow-up studies with each of the pure enantiomers corresponded exactly with each of the two peaks observed for their racemic mixtures, thus providing defined retention times (min) of 10.3 and 11.4 for the respective (S)- and (R)-esmolol antipodes, and of 26.3 and 29.9 for the respective (S)- and (R)-metabolite antipodes. Calibration curves for racemic esmolol and metabolite within human plasma are shown in Fig. 3 Panels A and B, respectively. Studies with each of the pure enantiomers similarly provided two sets of linear relationships for quantification across ranges of 25–1000 ng/ml, and 2.5–50 μg/ml,

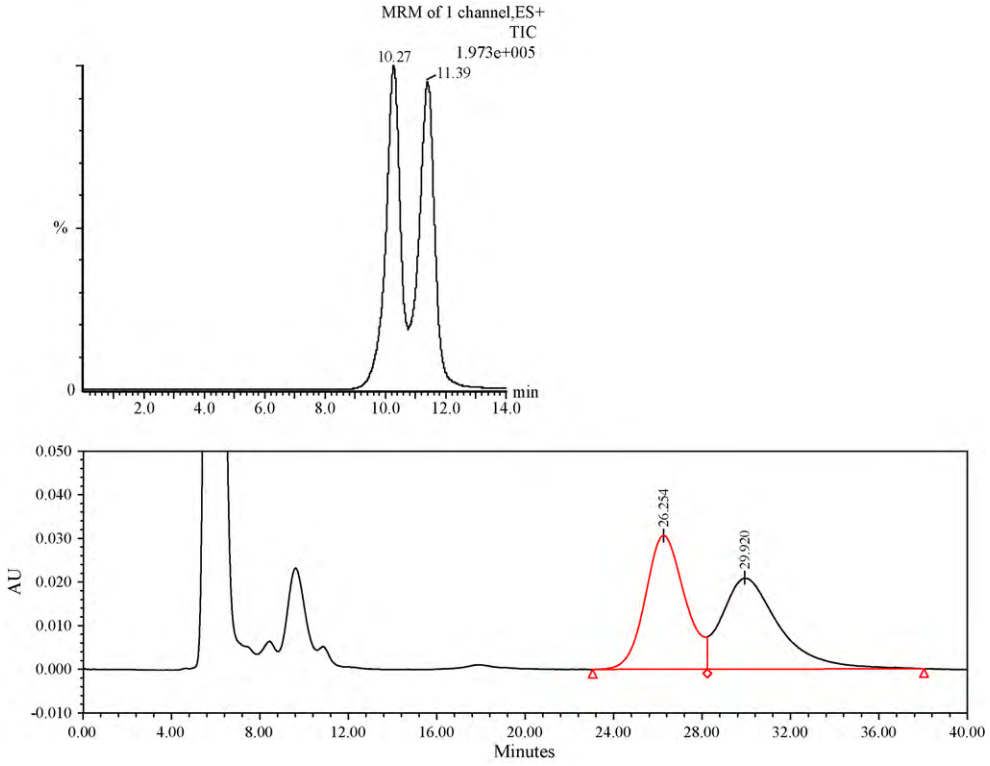

Fig. 2. Chiral column chromatograms for racemic esmolol and its metabolite in human plasma at 1 and 50 μg/ml, respectively. The top panel depicts the LC–MS/MS method with peaks at ca. 10.3 and 11.4 min for the respective (S)- and (R)-esmolol enantiomers using an Astec Chirobiotic T Column. The lower panel depicts the HPLC method with peaks at ca. 26.3 and 29.9 min for the respective (S)- and (R)-metabolite enantiomers using a Chiralcel OD-RH Column.

for the esmolol and metabolite stereoisomers, respectively. As in the racemate studies, data for the pair of enantiomers within each set of compounds was again statistically super-imposable. R^2 values of 0.997 and 0.998 were derived for (S)- and (R)-esmolol, while identical 0.999 values were derived for both isomers of the metabolite's enantiomeric pair.

Recovery, precision and accuracy data is recorded in Tables 1 and 2. Mean ± SD recovery values for (S)- and (R)-esmolol from human plasma ranged from 97.8 ± 6.4 to 101.6 ± 5.3%, and 94.4 ± 3.7 to 107.3 ± 0.4%, respectively. Intra- and inter-day precision and accuracy for the esmolol enantiomers were within ±6% at all but the lowest concentration. Recovery values for (S)- and (R)-metabolite ranged from 97.1 ± 9.8 to 108.4 ± 4.5%, and 98.1 ± 1.5 to 110.4 ± 6.0%, respectively. Intra- and inter-day precision and accuracy for the esmolol metabolite enantiomers were within ±2.5% at all but the lowest concentration.

4. Discussion and conclusion

While enantioselective HPLC bioassays for several other *beta*-blockers have utilized chiral stationary [12,13] and mobile [14,15] phases, methods pertaining to esmolol [5,7,8] have relied upon initial asymmetric derivatization to form diastereomeric analytes which are then differentiated by standard column chromatographic techniques [16–20]. In general, the latter have proven to be most effective when the asymmetric adduct also contains a chromophore

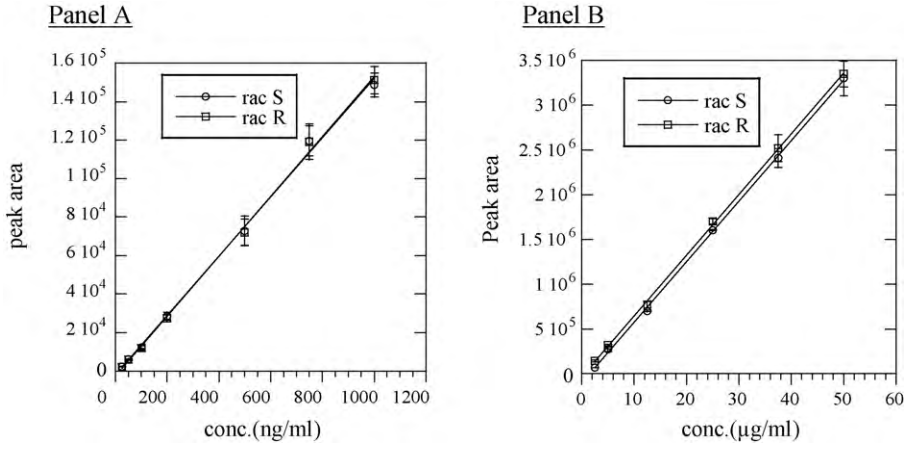

Fig. 3. Standard calibration curves in human plasma for racemic esmolol (25–1000 ng/ml) using the LC–MS/MS method (Panel A) and for its metabolite (2.5–50 μg/ml) using the HPLC method (Panel B). Error bars reflect SD for each point of multiple determinations. Note that each figure is being submitted as its own e-file according to the instructions. Tables 1 and 2 are provided immediately below.

Table 1
Recovery studies data (converted to % recovery), and inter-day assay performance studies data listed as experimental recoveries (recovery), precision (CV %) and accuracy (%), for (S)- and (R)-esmolol enantiomers in human plasma.

Conc (ng/ml)	% recovery[a]		Inter-day (S)			Inter-day (R)		
	(S)	(R)	Recovery[a]	CV %	%	Recovery[a]	CV %	%
25	101.6 ± 5.3	107.3 ± 0.4	24.2 ± 2.6	10.6	96.8	23.3 ± 3.7	15.8	93.4
200	102.3 ± 0.7	94.4 ± 3.7	199.4 ± 2.8	1.4	99.7	202.3 ± 12.2	6.0	101.2
1000	97.8 ± 6.4	99.8 ± 0.7	977.0 ± 31.8	3.2	97.7	996.6 ± 5.7	0.6	99.7

[a] Mean ± SD.

Table 2
Recovery studies data (converted to % recovery), and inter-day assay performance studies data listed as experimental recoveries (recovery), precision (CV %) and accuracy (%), for esmolol's (S)- and (R)-metabolite enantiomers in human plasma.

Conc (μg/ml)	% recovery[a]		Inter-day (S)			Inter-day (R)		
	(S)	(R)	Recovery[a]	CV %	%	Recovery[a]	CV %	%
2.5	97.1 ± 9.8	98.1 ± 1.5	2.6 ± 0.2	6.1	103.1	2.7 ± 0.3	10.7	106.5
25	105.0 ± 1.7	108.6 ± 0.3	25.0 ± 0.4	1.8	100.2	24.5 ± 0.6	2.5	97.9
50	108.4 ± 4.5	110.4 ± 6.0	49.9 ± 0.4	0.8	99.7	51.2 ± 1.0	1.9	102.3

[a] Mean ± SD.

that can enhance detection sensitivity [20]. In addition to requiring an extra step during sample processing, however, subtle variations of the biological matrix can potentially effect the derivatization reaction to a greater extent than they may have upon a direct determination procedure. Thus, the precision of the derivatization methods can sometimes be compromised [20]. Among previous studies involving the esmolol enantiomers, definitive accuracy and precision data has been conveyed only by Tang et al. [8]. They report an LOD at 3 ng/ml, linearity (>0.99) across a concentration range from 35 ng/ml to 12 μg/ml with precision and accuracy (RSO) near 14% at the low end and ca. 1% at the high end. Average recoveries were about 95% from human plasma. These investigators did not include the esmolol metabolites as part of their studies.

The direct methods reported herein avoid the potential limitations associated with the derivatization procedures while allowing for more convenient sample preparation. They provide ready stereochemical assays for quantitative determinations of both the esmolol and metabolite enantiomers. Seeking to increase our overall experience with chiral columns, two of the latter were purchased from different vendors and separately deployed in each of the assay methods. The Chirobiotic T column was recommended for esmolol and we selected the Chiralcel OD-RH column as an interesting, alternative chiral reverse-phase system for use with the highly polar metabolite. Both types of columns proved to be satisfactory. No attempts were made to switch either of these columns from one method to the other. Examination of various internal standards during initial method development did not enhance assay performance parameters in either case. Upon optimization of each method, the levels of detection, accuracy and precision are similar to those reported by Tang et al. and should be quite suitable for performing stereochemically defined PK studies of this unique *beta*-blocker and its metabolite. Such studies will add to those for the racemic drug wherein the aforementioned concentration and half-life data have already become well established in humans [1,3,4].

Acknowledgements

The authors thank Dr. Jared Anderson (University of Toledo Chemistry Department) for his useful discussions about chiral chromatography, and the Global Cardiovascular Innovation Center (Cleveland Clinic Foundation) for partial funding of this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.07.006.

References

[1] P.W. Erhardt, Chron. Drug Discov. 3 (1993) 191.
[2] N. Bodor, P. Buchwald, Med. Res. Rev. 20 (2000) 58.
[3] P.W. Erhardt, in: J. Fischer, C.R. Ganellin (Eds.), Analogue-based Drug Discovery, Wiley-VCH, Weinheim, 2006, p. 233.
[4] P. Erhardt, R. Khupse, J. Sarver, J. Trendel, in: D. Abraham (Ed.), Burger's Medicinal Chemistry, Drug Discovery and Development, John Wiley & Sons, Hoboken, 2010, p. 103.
[5] C.Y. Quon, K. Mai, G. Patil, H.F. Stampfli, Drug Metab. Dispos. 16 (1988) 425.
[6] M. Okamura, M. Kumagai, Y. Murasaki, T. Ohkura, Y. Miyamto, Y. Kawai, T. Tamura, Y. Takariki, H. Tomisawa, Xenobio. Metab. Dispos. 16 (2001) 427.
[7] T. Toyo'oka, M. Toriumi, Y. Ishii, Biomed. Chromatogr. 15 (2001) 56.
[8] Y.-H. Tang, Y. He, T.-W. Yao, S. Zeng, J. Biochem. Biophys. Methods 59 (2004) 159.
[9] P.W. Erhardt, C.M. Woo, W.G. Anderson, R.J. Gorczynski, J. Med. Chem. 25 (1982) 1408.
[10] C.A. Zhang, P.W. Erhardt, Synth. Commun. (submitted for publication).
[11] G. Patil, K.H. Mai, Int. Pat. WO 88/01614 (1988) 1.
[12] T. Fornstedt, A.M. Hesseyren, M. Johansson, Chirality 9 (1997) 329.
[13] G. Lamprecht, T. Kraushofer, K. Stoschitzky, W. Lindner, J. Chromatogr. B 740 (2000) 219.
[14] G.H. Xie, D.J. Skanchy, J.F. Stobaugh, Biomed. Chromatogr. 11 (1997) 193.
[15] G. Bazylak, H.Y. Aboul-Enein, Liq. Chromatogr. Relat. Technol. 22 (1999) 1171.
[16] A.J. Sedman, J. Gal, J. Chromatogr.: Biomed. Appl. 278 (1983) 199.
[17] O.P. Kleiderning, W. Linder, Chromatographia 44 (1997) 465.
[18] X. Li, T.W. Yao, S. Zeng, J. Chromatogr. B 742 (2000) 433.
[19] Q.F. Tao, S. Jeng, J. Biochem. Biophys. Methods 54 (2002) 103.
[20] J. Bojarski, J. Biochem. Biophys. Methods 54 (2002) 197.