Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 248–251

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Development of liquid chromatographic enantiomer separation methods and validation for the estimation of (*R*)-enantiomer in eslicarbazepine acetate

Mahesh Kumar Mone[a,b,*], K.B. Chandrasekhar[b]

[a] *Drug Discovery Facility, Advinus Therapeutics, Quantum Towers, Plot-9, Rajiv Gandhi Infotech Park, Phase-I, Hinjewadi, Pune 411 057, India*
[b] *Department of Chemistry, Jawaharlal Nehru Technological University, College of Engineering, Anantapur 515 002, India*

ARTICLE INFO

Article history:
Received 10 June 2010
Received in revised form 28 July 2010
Accepted 10 August 2010
Available online 19 August 2010

Keywords:
Eslicarbazepine acetate
Licarbazepine
Enantiomeric separation
Chiral stationary phase
Polar organic solvent chromatography

ABSTRACT

Chiral separation method development was carried out for eslicarbazepine acetate and its (*R*)-enantiomer on diverse chiral stationary phases. Better chiral selectivity was observed on cellulose tris-(3,5-dichlorophenylcarbamate) immobilized column (Chiralpak IC-3). Under polar organic mode (POM), with 100% acetonitrile as mobile phase and 0.5 ml/min flow, a resolution close to three was achieved. With normal phase (NP) mobile phase consisting dichloromethane:ethanol (90:10, v/v) and 1.0 ml/min flow, a resolution close to six was achieved. Detection was done by UV at 220 and 240 nm respectively. Both the methods were found to be robust and were validated with respect to robustness, precision, linearity, limit of detection, limit of quantification and accuracy. The proposed methods are suitable for the accurate estimation of (*R*)-enantiomer in bulk drug samples up to 0.1% when a 1 mg/ml analyte test solution is chromatographed.

1. Introduction

Epilepsy is a common, chronic and complex neurological disorder resulting in to recurrent unprovoked seizures. Eslicarbazepine acetate (BIA 2-093), *S*-(−)-10-acetoxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide is a second generation drug to oxacarbazepine (OXC) and a third generation drug to carbamazepine (CBZ) antiepileptic drugs. It is structurally similar to CBZ and OXC but structurally different at the 10, 11 position. It is a prodrug to (*S*)-licarbazepine, which is a monohydroxy derivative which is also an active metabolite of OXC. This structural modification was made to improve efficacy and safety preventing formation of toxic epoxide metabolite. Eslicarbazepine acetate is solely metabolized to (*S*)-licarbazepine in the liver there by improving its efficacy [1–4]. Fig. 1 shows the structures of eslicarbazepine acetate (ESL) and its active metabolite eslicarbazepine.

Eslicarbazepine acetate has been extensively studied for its metabolism and pharmacokinetics. Since (*S*)-enantiomer alone shows distinct advantage over the (*R*)-enantiomer, its chirality, chiral inversion, pharmacokinetics and metabolism are of great interest for different research groups. Extensive research has been done in this regard leading to the development of bio-analytical chiral separation methods and a thorough understanding of pharmacokinetics and metabolism [5–7]. Undoubtedly, the chiral purity of ESL, when prepared as a bulk drug, for making formulation, should be high, with least possible content of (*R*)-enantiomer. To estimate the (*R*)-enantiomer content a robust, sensitive chiral analytical method is a prime requisite. To our knowledge there is no report of an enantiomeric separation method intended for this application. Our aim was to explore different chiral stationary phases (CSPs) and choose the CSP which provides the best possible separation of these enantiomers, to optimize the conditions and validate extensively with respect to various method validation parameters.

2. Experimental

2.1. Chemicals and materials

Eslicarbazepine acetate standard was procured from Sigma Aldrich, India. Ultra high pure water was obtained from in-house Millipore Milli Q water system. Acetonitrile and methanol were purchased from JT Baker, USA. *n*-Hexane and dichloromethane were procured from Merck, India. Ethyl alcohol was procured from Les alcools, Canada. *n*-Heptane was procured from Qualigens, India. Ammonium acetate (Fluka, Germany) and trifluoroacetic acid (TFA) were procured from Sigma Aldrich, India. Methyl t-butyl ether was procured from Spectrochem, India.

* Corresponding author at: Drug Discovery Facility, Advinus Therapeutics, Quantum Towers, Plot-9, Rajiv Gandhi Infotech Park, Phase-I, Hinjewadi, Pune 411 057, India, Advinus publication number: ADV-A-006. Tel.: +91 20 66539629; fax: +91 20 66539620.
E-mail addresses: mahesh.mone@advinus.com, maheshmone@yahoo.com (M.K. Mone).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.08.015

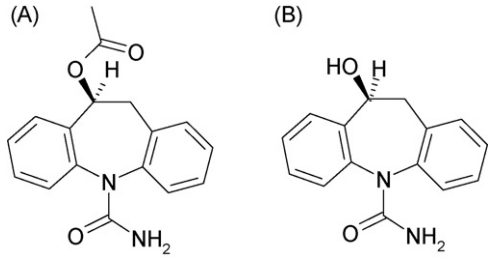

Fig. 1. Structures of (A) eslicarbazepine acetate and its active metabolite (B) (*S*)-licarbazepine.

2.2. Chiral stationary phases

Chiralcel OD-H, Chiralcel OJ-H with dimensions 250 mm × 4.6 mm, 5 μm, based on tris-(3,5-dimethylphenyl-carbamate of cellulose, tris-(4-methylbenzoate) of cellulose respectively, Chiralcel OJ-RH with dimensions 150 mm × 4.6 mm, 5 μm, based on tris-(4-methylbenzoate) of cellulose, Chiralpak IA-3, and Chiralpak IC-3 with dimensions 150 mm × 4.6 mm, 3 μm, based on tris-(3,5-dimethylphenyl carbamate) of amylose, tris-(3,5-dichlorophenyl carbamate) of cellulose respectively were procured from Daicel, Japan. R,R Whelk-O1 column with dimensions 250 mm × 4.6 mm, 5 μm, was procured from Regis Technologies, USA. Macrocyclic glycopeptides, Chirobiotic R (Ristocetin A), Chirobiotic V (Vancomycin), Chirobiotic T (Teicoplanin) and Chirobiotic TAG (Teicoplanin aglycone) with dimensions 100 mm × 4.6 mm and 250 mm × 4.6 mm, 5 μm, were procured from Astec Technologies, USA.

2.3. Instrumentation

HPLC separations were carried out using Agilent 1200 series HPLC systems equipped with diode array detector (G1315B), quaternary pump (G1311A), an on-line degasser (G1322A), auto-sampler (G1367B), with auto-sampler thermostat (G1330B) and a column thermostat (G1316A). Data acquisition and system suitability calculations were performed by Agilent chemstation software.

2.4. Chromatographic methods and conditions

The final separation methods were chosen with Chiralpak IC-3 column (150 mm × 4.6 mm, 3 μm, Daicel make). Two final methods were chosen and optimized. Both the methods were validated. The normal phase method (NP method) comprised of dichloromethane:ethanol (90:10, v/v) and 1.0 ml/min flow. Detection was by UV at 240 nm and injection volume was 20 μl. The polar organic solvent chromatographic method (POSC method) was with 100% acetonitrile as mobile phase at a flow rate of 0.5 ml/min. Detection was by UV at 220 nm and injection volume was 10 μl. Column was maintained at 25 °C for both the methods. Other chiral stationary phases mentioned in Section 2.2 were used to explore chiral selectivity with various mobile phases compatible to the respective CSP.

Table 2
Robustness results of chiral separation methods.

Parameters	Resolution values	
	NP method	POSC Method
Column temperature		
20 °C	6.05	3.02
25 °C	6.01	2.99
30 °C	5.53	2.94
Flow rate		
0.9 ml/min	5.69	3.12[a]
1.1 ml/min	5.45	2.83[b]
Ethanol content in mobile phase		
8%	5.99	NA
12%	5.03	NA

NA – not applicable.
[a] At 0.4 ml/min.
[b] At 0.6 ml/min.

2.5. Sample preparations

For method development, samples were prepared in their respective mobile phase related solvents, at a concentration of 1 mg/ml. For NP method validation, the samples were prepared in ethanol and for POSC method validation samples were prepared in acetonitrile. Stock solutions of (*R*)-enantiomer were prepared at a concentration of 100 μg/ml. For precision and recovery studies, final concentration of the analyte was fixed at 1 mg/ml.

2.6. Validation of the methods

System suitability and selectivity were checked by injecting 1 mg/ml racemic solution and also a spiked solution consisting 0.5% of the (*R*)-enantiomer in 1 mg/ml analyte solution. Separation factor was measured and monitored through out the validation process. To determine precision nine replicate injections of a spiked solution consisting 0.5% of (*R*)-enantiomer in 1 mg/ml eslicarbazepine acetate were made, retention time and areas were measured for which %RSD was calculated. Intermediate precision was also evaluated by making replicate injections for three consecutive days and measuring %RSD for retention times and areas ($n = 27$). LOD and LOQ were determined at a signal to noise ratio of 3 and 10 [8]. The precision at LOQ concentration was determined by injecting six replicates and measuring the %RSD of the area. Linearity of detector response was established by preparing seven calibration sample solutions starting with LOQ concentration, 900–10,000 ng/ml (900, 1000, 2000, 4000, 6000, 8000, 10,000 ng/ml). Each set of solutions was prepared in triplicate and was analyzed for three consecutive days. Sample concentration and peak areas were used to plot regression curves. The percent-

Table 1
Chiral selectivity results on various CSPs.

Column	Mobile phase	Retention time	Resolution
IC-3	100% acetonitrile, 0.5 ml/min	10.18, 11.77	2.99
IC-3	90:10, dichloromethane:ethanol, 1.0 ml/min	4.24, 6.77	6.01
IC-3	80:20, dichloromethane:methanol, 1.0 ml/min	2.77, 3.40	3.96
IC-3	100% THF, 0.5 ml/min	3.84, 3.96	0.67
Whelk-O1	20 mM ammonium acetate in ethanol, 0.5 ml/min	8.70, 9.34	1.80
OD-H	90:10 hexane:EtOH, 1.0 ml/min	18.05, 20.46	2.17
OJ-RH	50:40:10, 0.1%TFA in water:methanol: acetonitrile, 0.5 ml/min, 40 °C	9.72, 11.49	2.33
IA-3	100% acetonitrile, 0.5 ml/min	6.51, 6.73	0.65
IA-3	80:20, hexane:EtOH, 0.5 ml/min	6.34, 6.57	0.60
Teicoplanin	90:10, methyl t-butyl ether:EtOH (0.05% TEA), 1.5 ml/min	18.89, 21.23	0.73

Table 3
Validation parameters and results of chiral LC methods.

Validation parameters	Results	
	NP method	POSC Method
Precision (n = 9) (%RSD)		
Retention time (R-enantiomer)	0.04	0.12
Retention time (Eslicarbazepine acetate)	0.05	0.04
Peak area (R-enantiomer)	0.92	1.02
Peak area (Eslicarbazepine acetate)	0.23	0.10
Intermediate precision (n = 27)		
Retention time (R-enantiomer)	0.06	0.82
Retention time (Eslicarbazepine acetate)	0.07	0.29
Peak area (R-enantiomer)	1.01	1.36
Peak area (Eslicarbazepine acetate)	0.33	0.44
LOD-LOQ (R-Enantiomer)		
Limit of detection (ng/ml)	300	300
Limit of quantification (ng/ml)	900	1000
Precision at LOQ (%RSD) (n = 6)	1.83	1.80
Linearity (R-enantiomer)		
Calibration range (ng/ml)	900–10,000	1000–10,000
Calibration points	7	6
Correlation coefficient	0.9999	0.9999
Slope (%RSD)(n = 3, Inter-day)	0.71	1.84
Regression equation	$y = 18.206x - 0.6825$	$y = 41.474x - 7.2733$

age relative standard deviation of the slope and y-intercept of the calibration curve was calculated. For POSC method linearity was established from 1000 ng/ml (LOQ) to 10,000 ng/ml comprising six points. Standard addition and recovery experiments were conducted to establish accuracy of the analytical method for the quantitative estimation of (*R*)-enantiomer in presence of ESL. The study comprised of preparation of 1 mg/ml spiked solutions of ESL with 0.25% (0.25 μg/ml), 0.5% (0.50 μg/ml), 0.75% (0.75 μg/ml) concentrations of (*R*)-enantiomer. Each solution was prepared in triplicate and analyzed. The recovery of (*R*)-enantiomer was calculated from the slope and y-intercept of the calibration curve. To evaluate method robustness, experimental conditions were varied one at a time keeping others constant and the resolution between ESL and (*R*)-enantiomer was measured (Table 2). Column-to-column robustness was not investigated, as only one Chiralpak IC-3 column was used for this study. Solution stability at analyte concentration is ascertained by storing a tightly closed volumetric flask at room temperature and analyzing the same at 24 and 48 h.

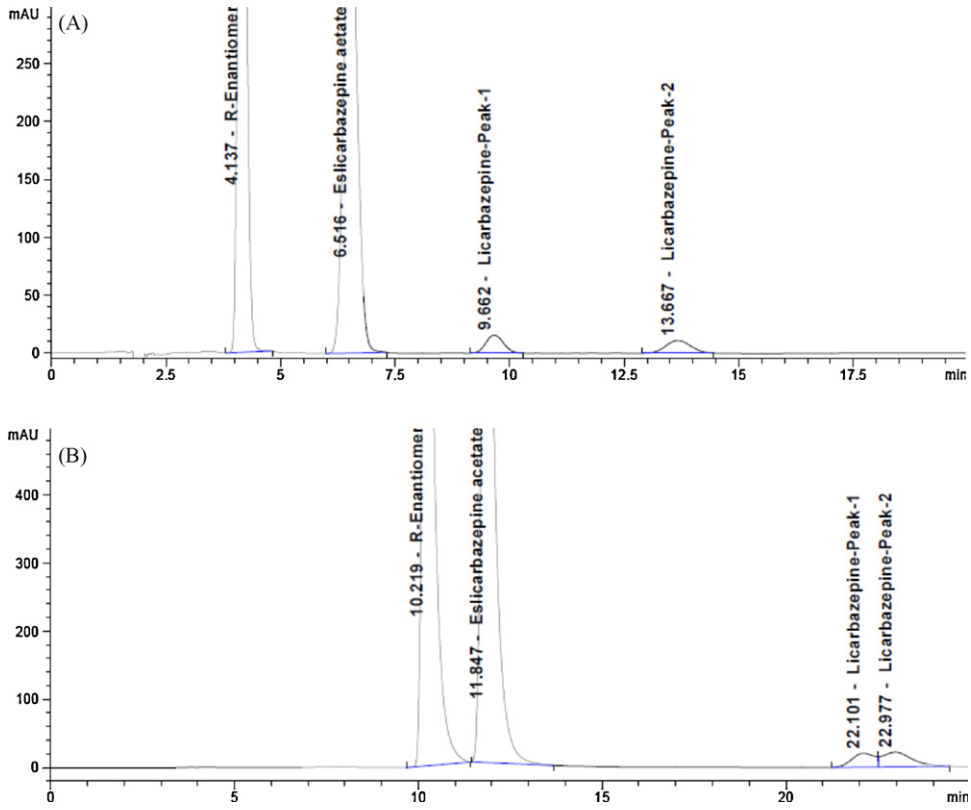

Fig. 2. Enantiomeric resolution of Eslicarbazepine acetate, its (*R*)-enantiomer and Licarbazepine on IC-3 column, under (A) normal phase (NP) conditions: Dichloromethane:EtOH, 90:10 (v/v), 1.0 ml/min, 20 μl injection (B) polar organic solvent chromatographic (POSC) conditions: acetonitrile, 100%, 0.5 ml/min, 10 μl injection.

3. Results and discussion

3.1. Chiral separation method development

Chiral separation development results on Chirobiotic-T, Whelk O1, OJ-H, OD-H and OJ-RH are summarized in Table 1. Chiralpak-IA-3, a polysaccharide immobilized column showed selectivity with 100% acetonitrile but resolution could not be improved beyond 0.7. Hexane, ethanol mobile phase too showed a similar trend with IA-3. Chiralpak-IC-3, another polysaccharide immobilized column containing chlorine showed similar selectivity with hexane, ethanol and hexane, IPA combinations. Drastic improvement in resolution was observed with dichloromethane, alcohol (ethanol and methanol) combinations. Even though dichloromethane, methanol combination displayed highest selectivity, this combination gave tailing at the base of the peak. Hence dichloromethane, ethanol mobile phase was chosen for optimization leading to a resolution of six. The same CSP showed considerable improvement in resolution with 100% acetonitrile which was optimized to a resolution of 3. The normal phase method showed baseline separation with the 10-hydroxy derivative eslicarbazepine enantiomers. Since these two methods belong to two different chromatographic mobile phase groups (NP and POSC) both were chosen for validation. Interestingly using basic or acidic additives did not show much effect either on resolution or peak shapes.

3.2. Validation results of the method

System suitability chromatograms showed clear baseline separation among eslicarbazepine acetate, its (*R*)-enantiomer and licarbazepine at 20 μl and 10 μl injection volumes in NP and POSC modes (Fig. 2). Robustness study results showed no significant deviation in resolution values from the final optimized results (Table 2). Intermediate precision results are in agreement with individual repeatability studies indicating the overall stability and reproducibility of the method. Recovery studies of NP method conducted at three concentration levels in triplicate showed recovery ranging from 95.2 to 102.6% with an average recovery of 99.8%. Recovery studies of POSC method conducted at three concentration levels in triplicate showed recovery ranging from 94.7 to 100.0% with an average recovery of 97.1%. Solutions stability studies showed no signs of chiral inversion or degradation. Method validation results are summarized in Table 3.

4. Conclusions

Two simple and rapid chiral separation methods were developed with two different mobile phase conditions (NP and POSC) on the same column and optimized. The latest chiral column technology of immobilized polysaccharides, with 3 μm particles has been applied. This column technology assures better stability and longer life than the coated polysaccharide versions. Both the methods were validated extensively yielding satisfactory results with all the validation parameters. Hence these two methods can be applied for the accurate quantitative estimation of the (*R*)-enantiomer of eslicarbazepine acetate. Method choice can be made as per the convenience of the analyst, compatibility with the detection system or the sample nature. Usage of chlorinated solvents can be avoided by choosing the POSC method.

Acknowledgements

M.K. Mone would like to thank Drs. Rashmi Barbhaiya and Kasim Mookhtiar for permitting to carry out research work towards Ph.D.

References

[1] L. Almeida, P. Soares-da-Silva, Eslicarbazepine Acetate (BIA 2-093), Neurotherapeutics: J. Am. Soc. Exp. NeuroTherapeutics 4 (2007) 88–96.
[2] A. Parada, P. Soares-da-Silva, The novel anticonvulsant BIA 2-093 inhibits transmitter release during opening of voltage-gated sodium channels: a comparison with carbamazepine and oxcarbazepine, Neurochem. Int. 40 (2002) 435–440.
[3] M. Bialer, H.S. White, Key factors in the discovery and development of new antiepileptic drugs, Nat. Rev. Drug Disc. 9 (2010) 68–82.
[4] M. Bialer, S.I. Johannessen, H.J. Kupferberg, R.H. Levy, E. Perucca, T. Tomson, Progress report on new antiepileptic drugs: a summary of the Seventh Eilat Conference (EILAT VII), Epilepsy Res. 61 (2004) 1–48.
[5] D. Hainzl, A. Parada, P. Soares-da-Silva, Metabolism of two new antiepileptic drugs and their principal metabolites S(+)- and R(−)-10,11-dihydro-10-hydroxy carbamazepine, Epilepsy Res. 44 (2001) 197–206.
[6] L. Almeida, A. Falcão, J. Maia, D. Mazur, M. Gellert, P. Soares-da-Silva, Single-dose and steady-state pharmacokinetics of eslicarbazepine acetate (BIA 2-093) in healthy elderly and young subjects, J. Clin. Pharmacol. 45 (2005) 1062–1066.
[7] G. Alves, I. Figueiredo, M. Castel-Branco, A. Loureiro, A. Falco, M. Caramona, Simultaneous and enantioselective liquid chromatographic determination of eslicarbazepine acetate, S-licarbazepine, R-licarbazepine and oxcarbazepine in mouse tissue samples using ultraviolet detection, Anal. Chim. Acta 596 (2007) 132–140.
[8] IFPMA, ICH draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology Federal Register, vol. 60, IFPMA, Switzerland, 1995, pp. 11260–11262.