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SHORT COMMUNICATION

Determination of the Enantiomeric Purity of Solriamfetol by High-Performance Liquid Chromatography in Polar Organic Mode Using Polysaccharide-Type Chiral Stationary Phases

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Abstract

Solriamfetol is a novel FDA approved enantiopure drug used for the treatment of excessive sleepiness associated with narcolepsy or obstructive sleep apnea. Only the active *R*-enantiomer is marketed, therefore, a method to determine the chiral purity is essential. Chiral separation of solriamfetol was performed by HPLC using polysaccharide-type chiral stationary phases in polar organic mode and described for the first time. Seven different polysaccharide-based chiral columns (Lux Amylose-1, Lux i-Amylose-1, Lux Amylose-2, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3 and Lux Cellulose-4) were employed for the separation of enantiomers using polar organic mode, with mobile phases consisting of 0.15% diethylamine in methanol (MeOH), 2-propanol (IPA) or acetonitrile (ACN). During the screening phase, the best results ($R_s > 5$, in less than 10 min) was obtained on a Lux Amylose-1 column with 0.15% diethylamine in methanol with an ideal elution order (the distomer eluted before the *R*-enantiomer). The effects of binary mobile phases on the resolutions and retention factors were also investigated containing different percentages of MeOH:ACN and MeOH:IPA. Using optimized parameters (Lux Amylose-1 column with 0.05% diethylamine in MeOH with 0.6 mL/min flow rate at 20 °C) high enantioresolution ($R_s = 5.3$) was achieved within 6 min. Thermodynamic analysis was performed and revealed an enthalpy-driven enantioseparation. The developed, isocratic HPLC method was validated according to current ICH guidelines and proved to be reliable, linear, precise and accurate for the determination of 0.1% *S*-enantiomer as a chiral impurity in *R*-solriamfetol.

Keywords Sunosi® · Chiral separation · Polar organic mode · Enantioselective HPLC · Polysaccharide-type chiral stationary phase

Introduction

Solriamfetol ((*R*)-2-amino-3-phenylpropylcarbamate, formerly known as JZP-110, Fig. 1) is a dopamine and norepinephrine reuptake inhibitor indicated in treating daytime sleepiness associated with narcolepsy or obstructive sleep apnea. Solriamfetol was given FDA approval in March 2019 and is currently sold under the brand name Sunosi®. The medication is a federally controlled substance because it has the potential to be abused [1]. Solriamfetol has a single asymmetric carbon, resulting in two enantiomers. The drug is marketed as a single enantiomeric agent; the formulation on the market contains only the active *R*-enantiomer, while the inactive *S*-enantiomer could be present as a chiral impurity [2]. Determination of chiral purity of single enantiomeric agents by suitable analytical methods is not only essential but also a regulatory requirement. Analytical

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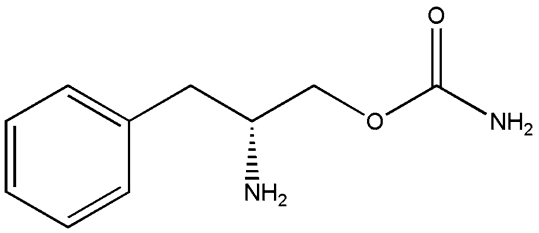

Fig. 1 The structure of solriamfetol. The active, *R*-enantiomer is on the market

characterization or determination of solriamfetol is scarcely described [3, 4], while there are no publications dealing with the chiral separation of solriamfetol enantiomers in the literature. Chiral separation can be achieved using different analytical approaches, among, which most certainly, liquid chromatography-based techniques are the most widely applied. Enantioseparations are based most often on a direct resolution on chiral stationary phases (CSPs). Polysaccharide-type CSPs, based on phenylcarbamate- or ester derivatives proved to be extremely popular, due to their versatility and multimodal nature, presenting applicability in normal-phase, reversed-phase and polar organic mobile phase modes [5, 6]. In polar organic mode only polar organic solvents, alcohol, acetonitrile (ACN) or their combinations are used as mobile phases, with or without different acidic and/or basic additives. Due to the several advantages of polar organic mobile phase mode it is widely used in chiral drug analysis [7, 8]. The present work describes the enantioseparation of solriamfetol using chiral liquid chromatography. To achieve a fast and efficient method, polysaccharide-based CSPs in the polar organic mode were investigated. Based on the obtained results, the developed method was optimized and validated according to ICH guidelines for the determination of the *S*-enantiomer as chiral impurity in *R*-solriamfetol.

Materials and Methods

Materials

The synthetic base materials for solriamfetol enantiomers, such as D-phenylalaninol (*R*-(+)-2-amino-3-phenyl-1-propanol), L-phenylalaninol (*S*-(−)-2-amino-3-phenyl-1-propanol), sodium cyanate and methansulfonic acid together with diethylamine (DEA) and triethylamine were purchased from Sigma-Aldrich, Hungary. (Budapest, Hungary). Gradient grade Methanol (MeOH), acetonitrile (ACN) and 2-propanol (IPA) were purchased from Merck (Darmstadt, Germany). Other chemicals (dichloromethane, sodium hydroxide, sodium sulfate) were all of the analytical reagent grade from Molar Chemicals (Budapest, Hungary). Solriamfetol enantiomers were synthetized based on a recent patent [9] with some minor modifications. The steps of the synthesis together with the nuclear magnetic resonance and mass spectrometric data are included in Supplementary Materials.

The employed chiral columns were of identical dimensions (150×4.6 mm, 5 µm average particle size) and were ordered from Phenomenex (Torrance, CA, USA): Lux Cellulose-1 [cellulose tris(3,5-dimethylphenylcarbamate)], Lux Cellulose-2 [cellulose tris(3-chloro-4-methylphenylcarbamate)], Lux Cellulose-3 [cellulose tris(4-methylbenzoate)], Lux Cellulose-4 [cellulose tris(4-chloro-3-methylphenylcarbamate)], Lux Amylose-1 [amylose tris(3,5-dimethylphenylcarbamate)], Lux i-Amylose-1 [amylose tris(3,5-dimethylphenylcarbamate)], immobilized and Lux Amylose-2 [amylose tris(5-chloro-2-methylphenylcarbamate).

HPLC Analysis

LC-UV experiments were carried out on a JASCO HPLC system (JASCO PU-2089 Plus binary gradient pump, AS-4050 autosampler, MD-2010 Plus diode array detector and CO2065 Plus column oven). The software used to operate the equipment and data processing was ChromNAV. UV detection was performed at 210 nm. MeOH was used as a sample solvent for the preparation of solutions throughout the study. During the preliminary experiments, a solution containing 300 µg mL^{-1} *R*-solriamfetol and 100 µg mL^{-1} *S*-solriamfetol was used. Based on the limit of quantitation (LOQ) value of *S*-solriamfetol, the final test solution of *R*-solriamfetol used for validation and method applicability testing was 8000 µg mL^{-1}. All impurity level percentages are reported relative to this concentration. Injection volume was 2 µL and three parallel measurements were performed in all cases.

Results and Discussion

Method Development

In the method scouting phase, separation of solriamfetol enantiomers was attempted on seven chiral polysaccharide-type stationary phases including amylose-based Lux Amylose-1, Lux i-Amylose-1 and Lux Amylose-2 as well as cellulose-based Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3 and Lux Cellulose-4 were evaluated in polar organic mode using a mobile phase consisting of 0.15% DEA in MeOH, ACN or IPA with 0.5 mL min^{-1} flow rate at room temperature. Differences of the chiral selectors and also of the mobile phases applied in our study, analyte retention, enantioselectivity or elution order are expected to differ. Based on a literature survey and also our previous studies

[10–12] MeOH, IPA and ACN can influence retention and chiral interactions in different ways, therefore, these three solvents were used as a neat solvent and later in different combination in our study. The chromatographic parameters, such as retention time of the second enantiomer, resolution and elution order are summarized in Table 1 using a mobile phase consisting of 0.15% DEA in MeOH, IPA and ACN.

Baseline separation was achieved only on Lux Amylose-1 and Lux i-Amylose-1 column, displaying the same amylose tris(3,5-dimethylphenylcarbamate) chiral selector. On both columns, the enantiomer elution order (EEO) was independent of the mobile phase employed: the distomer, *S*-solriamfetol eluted first, which is ideal for determination of enantiomeric purity. Partial enantioresolution was observed on Lux Cellulose-2 and Lux Cellulose-4 columns, where the EEO is opposite compared to amylose tris(3,5-dimethylphenylcarbamate) CSP, the *R*-enantiomer eluted first. In this work mobile-phase dependent elution order reversal was not observed. The best results were obtained on Lux Amylose-1 with a mobile phase consisting of 0.15% DEA in MeOH ($R_s = 5.65$, $t_2 = 6.01$). This setting was used as the starting point for the investigation of the effect of different chromatographic parameters on method performance. Changes in mobile phase composition can change the enantiorecognition mechanism and may improve resolution [13, 14]. Therefore, the effects of binary mobile phases on chromatographic parameters were also tested using different percentages of ACN and IPA in MeOH containing uniformly, 0.15% DEA. Figure 2 shows the effect of ACN and IPA content in MeOH on the retention and resolution of solriamfetol enantiomers using Lux Amylose-1 column.

In the case of ACN:MeOH mixtures U-shaped retention curve was registered, when plotting k_1 and k_2 versus the ACN content in the MeOH:ACN binary mobile phases (Fig. 2a). The retention reached minimum values at 50% ACN in MeOH. Resolution value reached a local minimum at 50% ACN and increased until 80% ACN content in

Table 1 Chromatographic data obtained during the scouting phase, in terms of resolution (R_s), enantiomer elution order and retention time of second-eluting enantiomer ($t_{r,2}$)

Column	Mobile phase with 0.15% DEA	R_s	Enantiomer elution order	$t_{r,2}$ (min)
Lux amylose-1	MeOH	5.65	$S<R$	6.01
	IPA	1.35	$S<R$	4.92
	ACN	2.89	$S<R$	11.2
Lux i-amylose-1	MeOH	1.52	$S<R$	4.40
	IPA	1.30	$S<R$	5.45
	ACN	1.23	$S<R$	7.21
Lux amylose-2	MeOH	–	–	4.44
	IPA	–	–	5.54
	ACN	–	–	10.15
Lux cellulose-1	MeOH	–	–	4.55
	IPA	–	–	6.00
	ACN	–	–	6.15
Lux cellulose-2	MeOH	–	–	4.37
	IPA	0.75	$R<S$	8.05
	ACN	0.56	$R<S$	12.12
Lux cellulose-3	MeOH	–	–	4.28
	IPA	–	–	5.27
	ACN	–	–	5.08
Lux cellulose-4	MeOH	–	–	4.31
	IPA	1.39	$R<S$	7.32
	ACN	1.16	$R<S$	7.42

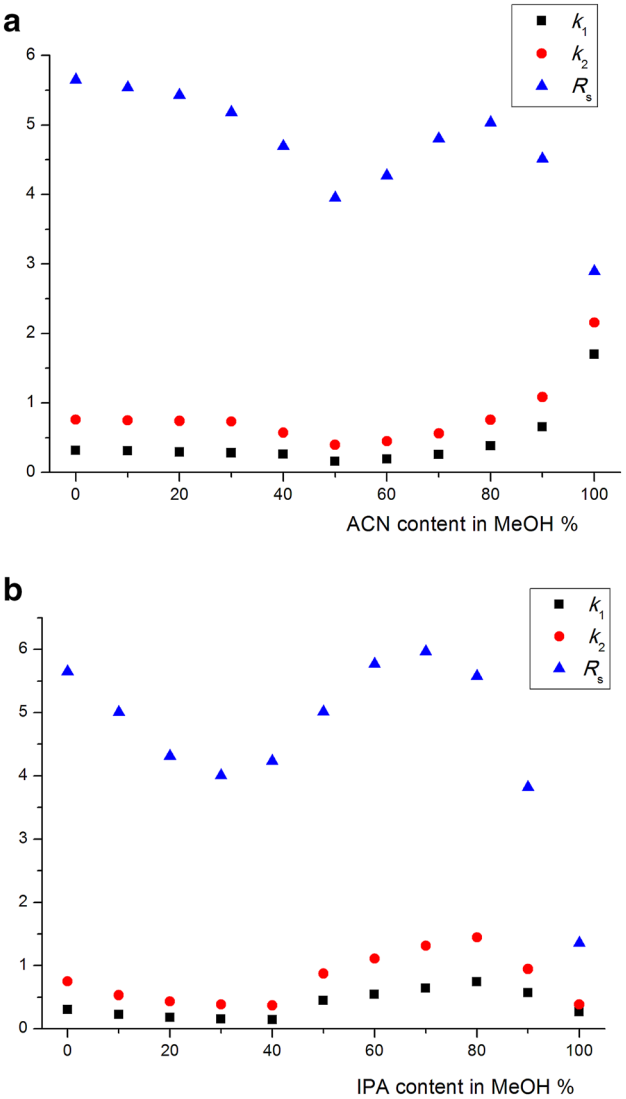

Fig. 2 Plots of the retention and resolution factors as a function of the ACN (**a**) and IPA content (**b**) in MeOH on Lux Amylose-1 column. (Chromatographic conditions: mobile phase 0.15% DEA in the indicated organic solvent composition, flow rate: 0.5 mL min^{-1}; column temperature: 20 °C; detection: UV at 210 nm)

MeOH; however, when the ACN content was above 80%, the resolution progressively decreased, and the lowest R_s value was observed in 100% ACN, where otherwise the highest retention factors were measured.

In the case of IPA:MeOH mixtures a very interesting retention profile was obtained with a local minima of retention factors at 40% and 100% IPA, as well as a local maxima at 0% and 80% IPA (Fig. 2b). One possible explanation of this phenomenon is that the CSP presents different stable secondary structures, which depend on the applied mobile phase composition. Similar assumptions were recently provided and thoroughly tested by Horváth and Németh, when trying to explain the observed hysteresis of retention and enantioselectivity on the same, coated amylose tris(3,5-dimethylphenylcarbamate) CSP in IPA:MeOH mixtures [13].

The highest resolution was observed on 70% IPA. Despite the interesting retention behavior described above in binary mixtures, a mobile phase consisting of 0.15% DEA in MeOH was chosen for, because of its simplicity, lower retention times and back-pressure as well as high resolution. As the basic additive of the mobile phase, apart from DEA, triethylamine was also tested at the same concentration level. A fundamental change in analysis time or selectivity was not observed, therefore, DEA was used as basic additive, throughout the study. Without the basic additive, no enantioseparation was observed.

Due to the very high resolution ($R_s > 5$) and appropriate retention time ($t_r < 10$ min) extensive optimization was not required. Different column temperatures, flow rate and DEA concentration were also tested.

Based on our investigation the optimized system is the following: Lux Amylose-1 column with 0.05% DEA in MeOH, 0.6 mL min^{-1} flow rate and 20 °C ($R_s = 5.3$, $t_r = 5.2$). A sample chromatogram obtained when applying the above-mentioned settings, on a sample containing 0.15% *S*-solriamfetol enantiomeric impurity in *R*-solriamfetol is presented in Fig. 3.

Experiments performed at different temperatures allowed us to calculate apparent thermodynamic parameters, based on the modified van't Hoff equation. Thermodynamic analysis revealed an enthalpy-driven enantioseparation. More details with the calculated thermodynamic parameters can be found in the Supplementary Materials.

Method Validation

Validation of the optimized HPLC method was performed according to the current ICH guideline [15]. Linearity, sensitivity (LOD, LOQ), accuracy and precision for the determination of *S*-enantiomer as a chiral impurity in the presence of the eutomeric *R*-solriamfetol were investigated as the main important parameter in the validation. Method sensitivity was evaluated for the determination of *S*-solriamfetol, by sequentially diluting sample solutions. The LOD of *S*-solriamfetol was determined as the concentration yielding a signal three times the baseline noise while LOQ was determined at 10:1 signal to noise ratio in the presence of the eutomer, *R*-solriamfetol solution. The LOQ was determined at 8 µg mL^{-1} (corresponding 0.1% impurity in 8000 µg mL^{-1} *R*-solriamfetol sample), while LOD of *S*-solriamfetol was 2.4 µg mL^{-1} (corresponding to

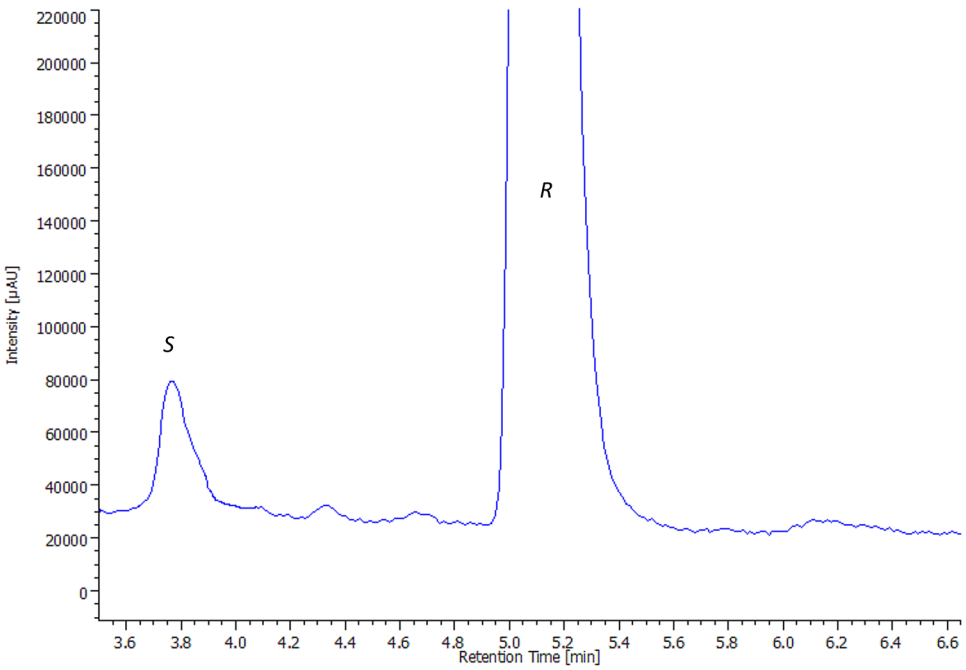

Fig. 3 Representative HPLC chromatogram of a solution containing 8000 µg mL^{-1} solriamfetol spiked with 12 µg mL^{-1} *S*-enantiomer (0.15%). Experimental conditions: Column, Lux Amylose-1 (150×4.6 mm I.D.); mobile phase, 0.05% DEA in MeOH; flow rate 0.6 mL min^{-1}, temperature, 20 °C, detection UV at 210 nm

0.03% impurity in 8000 µg mL^{-1} *R*-solriamfetol sample). Calibration curve was constructed at seven different concentration levels between 8 and 160 µg mL^{-1} corresponding to 0.1–2.0% enantiomeric impurity in 8000 µg mL^{-1} *R*-solriamfetol solution. Standard solution at each concentration was prepared in triplicate and injected once. Calibration plot was constructed by plotting peak areas against corresponding concentrations. According to the regression analysis, a linear relationship was found with the following equation $y = 0.0144x + 0.0002$ ($r^2 = 0.9995$) where y represents the peak area, x represents the concentration of *S*-solriamfetol (in mg mL^{-1}). Intraday and interday precision (repeatability based on RSD % of peak area) as well as intraday and interday accuracy expressed as recovery percentage were estimated at three levels of the impurities, i.e. 0.1%, 0.45% and 1.0% in the presence of 8000 µg mL^{-1} *R*-solriamfetol, respectively. RSD values of intraday and interday precision were below 2.8% and accuracy between ± 3%.

Concluding Remarks

Polar organic mode on polysaccharide-based CSPs proved to be an excellent choice for the rapid and highly efficient chiral separation of solriamfetol enantiomers. Using 0.05% DEA in MeOH as mobile phase, on the amylose tris(3,5-dimethylphenylcarbamate) based Lux Amylose-1 column, chiral separation of enantiomers of solriamfetol, a novel FDA approved drug, could be achieved with high resolution, in less than 6 min under isocratic condition. Thermodynamic analysis of the optimized method revealed a strong enthalpic control of the enantioseparation process. The effects of binary mobile phases on resolution and retention factors were also investigated in different ratios of MeOH:ACN and MeOH:IPA as mobile phases. In the case of ACN:MeOH mixtures U-shaped retention pattern was observed, while for IPA:MeOH mixtures retention profiles presented two minima. These findings can most probably be explained by various types of interactions that take place when using different polar organic mobile phase compositions and probably also conformational changes in the higher-order structure of the chiral selector. The method performance was tested in an ICH guide-based validation process and proved to be linear, accurate and precise for the determination of *S*-solriamfetol as an enantiomeric impurity in the approved eutomeric, *R*-solriamfetol.

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Compliance with Ethical Standards

Conflict of interest The authors have declared no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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