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Research Article

Enantioseparation of solriamfetol and its major impurity phenylalaninol by capillary electrophoresis using sulfated gamma cyclodextrin

R-solriamfetol is a recently approved drug used for the treatment of excessive sleepiness associated with narcolepsy and sleep apnea. Herein, a capillary electrophoretic method was developed, enabling the simultaneous analysis of the API and its *S*-enantiomer in addition to the enantiomers of its major impurity phenylalaninol. Twenty-nine different cyclodextrins (CDs), including native, neutral, and charged ones were screened as potential chiral selectors, and the best results were obtained with sulfated CDs. Randomly sulfated-β-CD exhibited outstanding enantioresolution, the peaks of phenylalaninol enantiomers inserted between the two peaks of solriamfetol enantiomers, while sulfated-γ-CD (S-γ-CD) showed remarkable resolution values in a much shorter analysis time with the optimal enantiomer migration order. Among the single isomer sulfated CD derivatives, substituent dependent enantiomer migration order reversal could also be observed in the case of heptakis(6-*O*-sulfo)-β-CD (HS-β-CD) or heptakis(2,3-*O*-dimethyl-6-*O*-sulfo)-β-CD (HDMS-β-CD) with *R*-,*S*-solriamfetol, and heptakis(2,3-*O*-diacetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD) resulting *S*-,*R*-solriamfetol migration order. The sulfated-γ-CD system was chosen for method optimization applying orthogonal experimental design. The optimized method (45 mM Tris-acetate buffer, pH 4.5, 4 mM S-γ-CD, 21°C, +19.5 kV) was capable for the baseline separation of solriamfetol and phenylalaninol enantiomers within 7 min. The optimized method was validated according to the ICH guidelines and successfully applied for the analysis of pharmaceutical preparation (Sunosi® 75 mg tablet), thus it may serve as a routine procedure for the laboratories of regulatory authorities as well as in Pharmacopoeias.

Keywords:
Experimental design / NMR / Sulfated gamma-cyclodextrin / Sunosi / Validation
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Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

Solriamfetol (chemically known as *R*-2-amino-3-phenylpropylcarbamate hydrochloride, Fig. 1A), Sunosi®, is a novel norepinephrine-dopamine reuptake inhibitor drug

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Abbreviations: **6-(SB)$_7$-β-CD**, heptakis(6-*O*-sulfobutyl)-β-CD; **CE-β-CD**, carboxyethylated-β-CD; **CM-α-CD**, carboxymethylated-α-CD; **CM-β-CD**, carboxymethylated-β-CD; **CM-γ-CD**, carboxymethylated-γ-CD; **COSY**, correlation spectroscopy; **DIME-β-CD**, dimethylated-β-CD; **DS**, degree of substitution; **EMO**, enantiomer migration order; **HDAS-β-CD**, heptakis(2,3-*O*-diacetyl-6-*O*-sulfo)-β-CD; **HDMS-β-CD**, heptakis(2,3-*O*-dimethyl-6-*O*-sulfo)-β-CD; **HMBC**, heteronuclear multiple bond correlation; **HP-α-CD**, 2-hydroxypropylated-α-CD; **HP-β-CD**, 2-hydroxypropylated-β-CD; **HP-γ-CD**, 2-hydroxypropylated-γ-CD; **HSQC**, heteronuclear single quantum correlation; **HS-β-CD**, heptakis(6-*O*-sulfo)-β-CD; **ODMS-γ-CD**, octakis(2,3-*O*-dimethyl-6-*O*-sulfo)-γ-CD; **PLS**, partial least squares; **RAME-α-CD**, randomly methylated-α-CD; **RAME-β-CD**, randomly methylated-β-CD; **RAME-γ-CD**, randomly methylated-γ-CD; **ROESY**, rotating-frame overhauser effect spectroscopy; **SBE-α-CD**, sulfobutyl-ether-α-CD; **SBE-β-CD**, sulfobutyl-ether-β-CD; **SBE-γ-CD**, sulfobutyl-ether-γ-CD; **SP-β-CD**, sulfopropylated-β-CD; **SP-γ-CD**, sulfopropylated-γ-CD; **S-β-CD**, sulfated-β-CD; **S-γ-CD**, sulfated-γ-CD; **TRIME-α-CD**, permethylated-α-CD; **TRIME-β-CD**, permethylated-β-CD; **TRIME-γ-CD**, permethylated-γ-CD

Color online: See article online to view Figs. 1 and 2 in color.

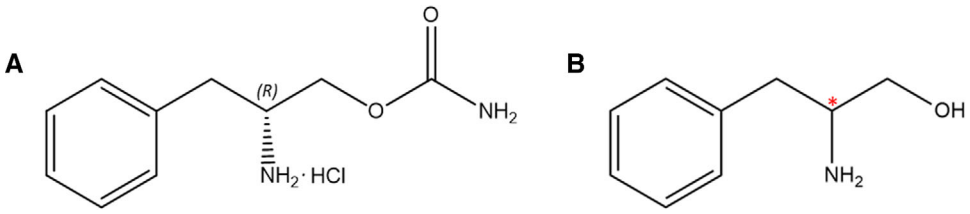

Figure 1. Chemical structure of the active *R*-solriamfetol (A) and racemic phenylalaninol (B).

used for the treatment of excessive sleepiness associated with narcolepsy and sleep apnea. Solriamfetol was approved in the United States in 2019 to improve wakefulness in adults and in January 2020 in the European Union [1]. Solriamfetol exhibits stereoisomerism due to the presence of a single chiral center (Fig. 1A). The drug is marketed as a single enantiomer API; the formulation contains only the active *R*-enantiomer, while the inactive *S*-enantiomer could be present as a chiral impurity.

Nowadays, the focus of interest is on the development of enantioselective syntheses and fast, sensitive, robust, and inexpensive methods to control the occurrence of enantiomeric contamination required by Pharmacopoeias and regulatory authorities. Besides HPLC, capillary electrophoresis offers an environmentally friendly alternative method for chiral separations, due to its low cost, flexible method development, and short analysis time. The successful enantioseparation in CE requires the application of chiral selectors, among which cyclodextrins (CDs) represent an eminent class thanks to the wide variety of structurally diverse (neutral and charged) derivatives, low UV cut-off, and relatively low price.

Apart from our recently published HPLC method utilizing polysaccharide-type chiral stationary phases there are no other published methods for the enantioseparation of solriamfetol [2]. While *S*-solriamfetol is the enantiomeric impurity of the API, phenylalaninol enantiomers (Fig. 1B) can be present either as residual starting material of the synthesis and/or as degradation product of solriamfetol. Phenylalaninol has been analyzed in several chiral methods [3–6], however its chiral capillary electrophoresis literature is limited to crown ether selectors [7]. To date, there is no available data on its CD-based enantioseparation. The existing chiral HPLC method lacks the enantioselectivity toward phenylalaninol and those enantiomers co-elute with the API. This work aims to develop, optimize, and validate an enantioselective CE method for the simultaneous determination of *R*-solriamfetol impurities (*S*-solriamfetol, *S*- and *R*-phenylalaninol).

2 Materials and methods

2.1 Materials

R- and *S*-solriamfetol were synthesized in our laboratory according to a recent patent [8]. D-Phenylalaninol hereinafter (*R*-(+)-2-amino-3-phenyl-1-propanol) and L-phenylalaninol hereinafter (*S*-(−)-2-amino-3-phenyl-1-propanol) were purchased from Sigma–Aldrich, Hungary (Budapest, Hungary).

All native CDs (α-, β-, and γ-CD) and their derivatives with various degrees of substitution: randomly methylated-α-CD DS∼11 (RAME-α-CD), randomly methylated-β-CD DS∼12 (RAME-β-CD), randomly methylated-γ-CD DS∼12 (RAME-γ-CD), dimethylated-β-CD DS∼14 (DIME-β-CD), permethylated-α-CD (TRIME-α-CD), permethylated-β-CD (TRIME-β-CD), permethylated-γ-CD (TRIME-γ-CD), 2-hydroxypropylated-α-CD DS∼3 (HP-α-CD), 2-hydroxypropylated-β-CD DS∼4.5 (HP-β-CD), and 2-hydroxypropylated-γ-CD DS∼3.2 (HP-γ-CD), carboxymethylated-α-CD DS∼3.5 (CM-α-CD), carboxymethylated-β-CD DS∼3 (CM-β-CD), carboxy methylated-γ-CD DS∼4 (CM-γ-CD), carboxyethylated-β-CD DS∼3 (CE-β-CD), sulfobutyl-ether-α-CD DS∼4 (SBE-α-CD), sulfobutyl-ether-β-CD DS∼6.3 (SBE-β-CD), sulfobutyl-ether-γ-CD DS∼4 (SBE-γ-CD), sulfopropylated-β-CD DS∼4 (SP-β-CD), sulfopropylated-γ-CD DS∼2 (SP-γ-CD), sulfated-β-CD DS∼13 (S-β-CD), sulfated-γ-CD DS∼14 (S-γ-CD), heptakis(6-*O*-sulfo)-β-CD (HS-β-CD), heptakis(2,3-*O*-dimethyl-6-*O*-sulfo)-β-CD (HDMS-β-CD), heptakis(2,3-*O*-diacetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD), octakis(2,3-*O*-dimethyl-6-*O*-sulfo)-γ-CD (ODMS-γ-CD), and heptakis(6-*O*-sulfobutyl)-β-CD (6-$(SB)_7$-β-CD) were products of CycloLab Ltd. (Budapest, Hungary).

D_2O (99.9% D) and CD_3COOD (99.5% D) were products of Merck (Darmstadt, Germany) and Cambridge Isotope Laboratories, Inc. (Tewksbury, USA), respectively. Acetic acid, Tris, NaOH, and methanol (Sigma-Aldrich, Budapest, Hungary) used for the preparation of buffer solutions, rinsing solutions, or applied as sample solvent were of analytical grade. All reagents were used without further purification. Bidistilled Millipore water was used throughout this study.

2.2 Capillary electrophoresis

The CE experiments were performed on an HP ^{3D}CE and on an Agilent 7100 instrument (Agilent Technologies, Waldbronn, Germany), equipped with a photodiode array detector and the Chemstation software (Openlab CDS Chemstation Edition Rev. C.01.04) for data handling. Untreated fused silica capillaries (50 μm id, 48.5 cm total, 40 cm effective length) were purchased from Agilent. Conditioning of new capillary was conducted by flushing with 1 M NaOH followed with 0.1 M NaOH and water for 30 min each. Prior to all runs the capillary was preconditioned by rinsing with water (1 min), 0.1 M NaOH (2 min), water (2 min), and BGE (background electrolyte, 4 min). The temperature of the capillary was set to 25°C during the preliminary screening experiments

and varied from 15°C to 25°C during method optimization. UV detection was performed at 200 nm and 15–25 kV voltage was applied. Samples were injected hydrodynamically (200–300 mbar·s). The running buffers consisted of 20 mM acetic acid adjusted to pH 4.5 with 1 M Tris in the case of the screening experiments and for method optimization 25–50 mM Tris BGE were adjusted to pH 4.0–5.0 by using acetic acid. The BGE contained CDs at various concentrations (1–10 mM) in the preliminary screening experiments.

In the case of preliminary experiments, stock solutions of each solriamfetol and phenylalaninol enantiomers were prepared separately in methanol (1 mg/mL) and appropriate dilutions with methanol were used to prepare sample solutions.

Method optimization through experimental design was performed using Modde Go 12.01 software from Umetrics/Sartorius (Sartorius AG, Göttingen, Germany).

The developed method was validated for the simultaneous analysis of *R*- and *S*-phenylalaninol and for the enantiomeric purity of the API.

Sunosi® tablets (75 mg) (Jazz Pharmaceuticals Ireland) were obtained from the University Pharmacy, Department of Pharmacy Administration Semmelweis University (Budapest, Hungary). Samples were prepared as follows: 10 tablets were weighted, then ground and mixed in a mortar. In a 5 mL volumetric flask, MeOH was added to an accurately weighed portion of the tablet powder corresponding to about 25 mg *R*-solriamfetol. The suspension was sonicated for 30 min and centrifuged for 5 min applying 3500 rpm (Sartorius 2–16 P benchtop centrifuge, Göttingen, Germany). For the filtration, 0.22 μm pore size Durapore PVDF syringe filter (Millex® GV filter, Millipore, Milford, MA, USA) was used. The final test solution of solriamfetol used for purity testing was about 5000 μg/mL. All impurity level percentages are reported relative to this concentration.

2.3 NMR experiments

^{1}H NMR spectra were recorded at 298 K on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection probe fitted with a gradient module (IDPF probe). Stock solutions were prepared from the *R*- and *S*-solriamfetol sample with D_2O and acidified with CD_3COOD.

Conventional 2D experiments (^{1}H-^{1}H gCOSY, ROESYAD, and ^{1}H–^{13}C gHSQCAD, HMBC) for structural elucidation were acquired on a solution containing 18 mM S-γ-CD (resulting in a 1:7 solriamfetol:S-γ-CD ratio). ROESY experiments were acquired collecting 16 scans on 1258·512 data points, applying various mixing times of 300 and 400 ms.

3 Results and discussion

3.1 Selection of the chiral selector

As phenylalaninol could be treated as both starting material of the API synthesis and decomposition product of solriamfetol (due to carbamate hydrolysis [9]), it was decided to include not only the chiral impurity *S*-solriamfetol but phenylalaninol enantiomers into the screening experiments, to develop a method capable of detecting trace amounts of chiral impurity as well as its major impurity (phenylalaninol) in the *R*-solriamfetol sample.

Due to the primary amine moiety, solriamfetol (pK_a 8.5 [10]) and phenylalaninol (pK_a 9.4 - predicted by the MarvinSketch 16.10.31 software) are positively charged under acidic conditions, thus neutral and charged CDs could also be applied to find the most suitable chiral selector for the enantioseparation of all four components in one system.

In the preliminary screening experiments, the chiral separation of solriamfetol and phenylalaninol enantiomers was investigated using the following cyclodextrins: three native (α-, β- and γ-CD), 10 neutral (RAME- and TRIME-α-, β-, and γ-CD, DIME-β-CD, HP-α-, β-, and γ-CD), and 16 negatively charged derivatives (CM-α-, β-, and γ-CD, CE-β-CD, SBE-α-, β-, and γ-CD, SP- and S-β-, and γ-CD, and the single isomer HS-, HDMS-, HDAS-β-CD, ODMS-γ-CD, and 6-(SB)$_7$-β-CD). All 29 CDs were screened at various selector concentrations in the range of 1–10 mM in 20 mM acetic acid-Tris buffer (pH 4.5). Fifteen of the applied CD derivatives resulted in partial or baseline separation of solriamfetol enantiomers, while 14 selectors showed enantiorecognition toward phenylalaninol. The resolution values are summarized in Table 1. None of the native cyclodextrins displayed enantiorecognition under these conditions. Partial resolution of solriamfetol and/or phenylalaninol enantiomers was observed for one neutral α- and three β-CD derivatives (TRIME-α-CD, RAME-β-CD, DIME-β-CD, and HP-β-CD). While TRIME-α-CD allowed the partial resolution of solriamfetol enantiomers only, and DIME-β-CD exhibited enantioselectivity toward phenylalaninol. The cavity size of beta-derivatives or the extended alpha cavity with permethylation seemed favorable for the enantiorecognition of both compounds, however, permethylation of β-CD (TRIME-β-CD) seemed detrimental for enantiodiscrimination of the studied analytes.

The anionic CDs usually enhance the chiral resolution of cationic racemates, due to the additional ionic interactions supporting the diastereomeric ion-pair formation between negatively charged CDs and positively charged enantiomers. The aromatic ring of solriamfetol (and phenylalaninol) can enter the hydrophobic cavity of the selector, while the positively charged amino group moiety can interact with the negatively charged carboxyalkyl, sulfoalkyl, or sulfate groups at the rim of the selector. The advantage of the countercurrent flow of the negatively charged chiral selector with respect to the EOF, may extend the enantiorecognition abilities compared to neutral CDs [11]. Moreover, several cases highlight the importance of the countercurrent mobility of oppositely charged analyte and selector, which can be more important in enantioseparations than the electrostatic analyte-selector interactions [12,13].

Regarding the neutral CDs, only limited chiral selectivity could be achieved, while most of the studied charged CDs resulted in enantioseparation, instead of CM-α- and γ-CD, and

Table 1. Enantioresolutions and enantiomer migration orders of solriamfetol and phenylalaninol along with the analysis time using 10 mM selector concentration

		1 mM		5 mM		10 mM		EMO		Analysis time (min)
		Solriamfetol	Phenylalaninol	Solriamfetol	Phenylalaninol	Solriamfetol	Phenylalaninol	Solriamfetol	Phenylalaninol	
Neutral	**RAME-β-CD**	–	–	–	–	0.45	0.90	-	*S,R*	2.2
	DIME-β-CD	–	–	–	–	–	0.28	-	*S,R*	2.2
	TRIME-α-CD	–	–	0.6	–	0.78	–	***R,S***	-	2.1
	HP-β-CD	–	–	–	–	0.39	0.19	*S,R*	*S,R*	2.0
Charged	**CM-β-CD**	0.18	–	na	–	0.42	–	*S,R*	-	4.7
	CE-β-CD	0.17	0.32	0.43	na	na	na	*S,R*	*S,R*	3.0*
	SBE-α-CD	0.39	0.34	0.32	0.31	na	na	*S,R*	*S,R*	3.3*
	SBE-β-CD	0.36	1.85	na	na	na	na	*S,R*	*S,R*	2.8**
	SBE-γ-CD	–	–	–	–	–	0.33	-	*S,R*	3.1
	SP-β-CD	0.18	0.27	na	na	0.42	0.87	*S,R*	*S,R*	5.5
	S-β-CD	1.82	0.83	**5.34**	**6.75**	na	na	*S,R*	*S,R*	11.0*
	S-γ-CD	2.40	1.69	**3.95**	**3.58**	4.43	3.52	*S,R*	*S,R*	4.5
	HS-β-CD	–	–	0.67	0.58	0.50	1.40	***R,S***	***R,S***	5.7
	HDMS-β-CD	–	–	0.30	0.35	0.73	1.10	***R,S***	***R,S***	3.4
	HDAS-β-CD	0.27	–	1.99	–	2.87	–	*S,R*	-	5.5
	ODMS-γ-CD	1.05	0.49	2.13	1.21	**2.72**	**1.60**	***R,S***	***R,S***	3.7
	6-$(SB)_7$-β-CD	0.17	–	0.31	0.59	na	na	*S,R*	*S,R*	4.0

na: not analyzed (deteriorated peak shapes).
*at 5 mM selector concentration level.
**at 1 mM selector concentration level.

Table 2. Summary of data obtained during method validation

Parameter	Concentration (μg/mL)	*S*-phenylalaninol	*R*-phenylalaninol	*S*-solriamfetol
Range (μg/mL)		5–60	5–60	5–60
Range (%)		0.1–1.2	0.1–1.2	0.1–1.2
Equation		$y = 1.112x+0.3275$	$y = 1.032x+0.6099$	$y = 1.136x+0.128$
r^2		0.9986	0.9980	0.9972
Accuracy (%)*	5	95.1 ± 2.8	102.5 ± 5.0	96.3 ± 3.1
	20	98.0 ± 1.5	97.2 ± 1.1	100.2 ± 2.0
	60	100.4 ± 1.0	98.6 ± 2.2	103.9 ± 4.4
Intraday precision (RSD%)*	5	2.95	5.11	3.01
	20	0.40	0.30	0.69
	60	1.45	3.05	0.77
Intermediate precision (interday) (RSD%)*	5	5.79	4.89	4.12
	20	0.78	0.47	0.89
	60	1.54	2.72	1.65

*Peak area.

SP-γ-CD, which failed to separate the enantiomers of solriamfetol and its impurity. SBE-γ-CD exhibited enantiorecognition only in the case of phenylalaninol enantiomers at 10 mM selector concentration, while SBE-α- and β-CD allowed partial separations. Regarding the carboxyalkylated and sulfopropylated CDs, only the beta analogues were suitable selectors for solriamfetol and phenylalaninol and resulted in partial enantioseparation.

Outstanding enantioresolutions could be observed in the case of negatively charged randomly sulfated-CDs: 5 mM S-β-CD resulted in $R_S = 5.34$ and $R_S = 6.75$ for solriamfetol and phenylalaninol enantiomers, respectively in 11 min analysis time, while using 10 mM S-γ-CD resolution values of $R_S = 4.43$ and $R_S = 3.52$ were achieved for solriamfetol and phenylalaninol enantiomers in 4.5 min analysis time. Highly sulfated CDs are among the most widely used chiral selectors in CE [14–23]. To gain a deeper insight into the inclusion behavior, a well-defined structure of the selector would be essential [24]. Thus, single isomer sulfated analogues were also subjected to the screening. Among the single isomer CD derivatives, the best resolution was achieved with the HDAS-β-CD ($R_S = 2.87$ for solriamfetol) and the ODMS-γ-CD ($R_S = 2.72$ and $R_S = 1.60$, for solriamfetol and for phenylalaninol), however, HDAS-β-CD failed to discriminate the phenylalaninol enantiomers. Comparing these results with those obtained with the randomly sulfated CDs, the single isomer analogues were found to be less effective; and a high degree of sulfation seems to be necessary for the remarkably high resolution values. Solriamfetol and phenylalaninol enantiomers could be also partially resolved by using the single isomer 6-$(SB)_7$-β-CD, with similar results to the randomly substituted analogue, SBE-β-CD.

The influence of the selector concentration on the enantioseparations was also evaluated, and usually the increase in selector concentration resulted in higher resolution values. The temporary diastereomeric complexes migrate as cations at low cyclodextrin concentration, however, higher (e.g., carboxyalkylated and sulfoalkylated) CD concentrations led to the formation of apparently uncharged complexes with similar migration behavior as that of the EOF. The proximity of the EOF resulted in deteriorated peak shapes and thereby resolution values could not be deduced in these cases. Further increase in selector concentration (e.g., for randomly sulfated-CDs) resulted in negatively charged complexes [25].

Not only resolution values, but the enantiomer migration order (EMO) holds a great significance in chiral analysis since the main component can disturb the quantification of the minor component(s) via system overloading or due to heading/tailing peaks. In the case of solriamfetol, the ideal migration order is as follows: *S*-solriamfetol, followed by the *R*-isomer. This criterion is fulfilled by most of the randomly substituted CDs and the single isomer HDAS-β-CD and 6-$(SB)_7$-β-CD, while the single isomer sulfated CDs (HS-β-CD, HDMS-β-CD, and ODMS-γ-CD) and the permethylated TRIME-α-CD showed opposite migration order (see Table 1).

Moving from highly sulfated randomly substituted CDs to single isomer HS-β-CD, HDMS-β-CD, or ODMS-γ-CD, a change in the EMO occurred. Among the single isomer sulfated CD derivatives, substituent dependent EMO reversal could be also observed in case of HS-β-CD or HDMS-β-CD (*R*-, *S*-solriamfetol) and HDAS-β-CD (*S*-, *R*-solriamfetol). These chiral selectors carry the sulfate groups at the 6-*O* position (primary side) and they differ in the substitution (unmodified, methylated, or acetylated) at 2-*O* and 3-*O* positions, thus the enantioselectivity resides in the substitution type- and pattern of the secondary side of the hosts.

In order to develop a fast method capable of detecting trace amount of decomposition products in in *R*-solriamfetol, the migration order of phenylalaninol should also be examined. In the majority of the cases, the migration order was ideal, the peaks of phenylalaninol enantiomers were followed by the peaks of solriamfetol enantiomers, apart from the application of HS-β-CD (where the first migrating component was the API), and randomly S-β-CD, (where the high enantioresolution of solriamfetol allowed the insertion of the

phenylalaninol enantiomers between the two peaks of solriamfetol enantiomers). Phenylalaninol enantiomers showed the same migration order as that of solriamfetol in all cases (see Table 1).

To determine the impurities at 0.1% level in the presence of *R*-solriamfetol, the preferred systems are those where the eutomer migrates last. This criterion has been fulfilled in the case of sulfated-γ-CD with remarkable resolution values in a short analysis time, thus this chiral selector was chosen for further optimization.

3.2 CE method development

In an attempt to increase resolution and/or decrease analysis time, a multivariate method optimization approach using Modde Go 12.01 software was undertaken. A fast, linear screening experimental design was applied to observe the impact of selected experimental variables upon method performance parameters. During the optimization phase, the following factors were considered: pH of the BGE (pH 4.0/4.5/5.0), buffer concentration of BGE (25 mM/37.5 mM/50 mM), CD concentration (3 mM/5 mM/7 mM), capillary temperature (15°C/20°C/25°C) and applied voltage (15 kV/20 kV/25 kV). The monitored responses were the migration time of the last peak (t_{migr4}), R_S between phenylalaninol enantiomers (R_{s1}), R_S between *R*-phenylalaninol and *S*-solriamfetol (R_{s2}), and R_S between the solriamfetol enantiomers (R_{s3}).

A fractional factorial design of resolution III was chosen with a total of 11 runs, including 3 center, replicate runs. The worksheet of the experimental design is summarized in Supporting Information Table 1. The obtained experimental data were fitted with a partial least squares (PLS) method.

Upon analysis of the obtained regression models, significant results were obtained in all cases, except for t_{migr4} (Supporting Information Fig. 1). In the latter case, low model validity was observed, which was due to the high reproducibility of replicate runs and thus, low pure error inside the models. Similar observations were described during several chromatographic method optimizations [26–28].

The analysis of the coefficient plots (Supporting Information Fig. 2) revealed that as expected t_{migr4} displays a significant inverse correlation with capillary temperature and the voltage applied, both leading to a decrease in analysis time. In the case of the monitored R_S values, temperature had a negative effect on enantioresolution in all cases, i.e., lower values being obtained at elevated temperatures. CD concentration also significantly influenced all R_S values monitored: an increase in CD concentration led to decreased R_{s1}, but increased R_{s2} and R_{s3} values.

Based on the obtained results, after model fitting and refinement, an optimization run was carried out based on the suggested parameter settings provided by the DoE software. The predicted and experimentally obtained values showed good correlation (Supporting Information Table 2). The optimized, final method parameters were as follows: BGE composition: 45 mM Tris-acetate buffer, pH 4.5, 4 mM S-γ-CD; capillary temperature maintained at 21°C and 19.5 kV voltage applied. The application of these settings resulted in baseline separation of all analytes, with R_S values greater than 4, in 7 min.

3.3 Validation and method application

Validation of the optimized method was performed according to International Council for Harmonization guideline Q2 (R1) for all investigated impurity (*R*-and *S*-phenylalaninol as well as *S*-solriamfetol) with respect to sensitivity, linearity, accuracy, and precision [29]. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on signal-to-noise ratios of 3:1 and 10:1 for the LOD and LOQ, respectively. Determination of impurities was validated in the range of 5–60 μg/mL (0.1–1.2%) uniformly for all impurities. Based on the results, the linearity of the method was evaluated at six concentration levels for all impurities and calibration plots were represented by plotting peak areas against corresponding concentrations (expressed in μg/mL). The correlation coefficient was determined by linear least squares regression analysis and it was found to be higher than 0.997 in all cases.

The accuracy and precision were analyzed by performing intra- (repeatability) and inter-day evaluation (two consecutive days) of three concentration levels (5, 20, and 60 μg/mL) for all impurities, covering the linearity range, each solution being injected five times. The accuracy (expressed in mean recovery%) ranged from 95.1% to 103.9%. The repeatability of peak area (expressed as RSD%) determined by five parallel injections of the solutions on the same day was between 0.4% and 5.1%. Intermediate precision of the method (expressed in RSD%) regarding the peak area was investigated on two consecutive days and was lower than 5.8%. The obtained values are summarized in Table 2. The precision of migration time was also investigated. In the intraday repeatability study, the RSD% of migration time was lower than 1.2%. In the interday study the RSD% of migration time was lower than 2.3%.

According to the obtained results, the optimized method proved to be reliable, linear, precise, and accurate for the determination of 0.1% *R*- and *S*-phenylalanol as well as *S*-solriamfetol in *R*-solriamfetol sample. Thereafter, the optimized and validated method was applied to the analysis of real samples, Sunosi® tablets containing 75 mg solriamfetol. The representative electropherograms recorded for the sample solution and the sample solution spiked with impurities (representing 0.1%) are shown in Fig. 2A and B, respectively. No impurities were detected in the tablet.

3.4 NMR experiments

Since CE does not provide any molecular level information on the interaction between solriamfetol and the selector, NMR

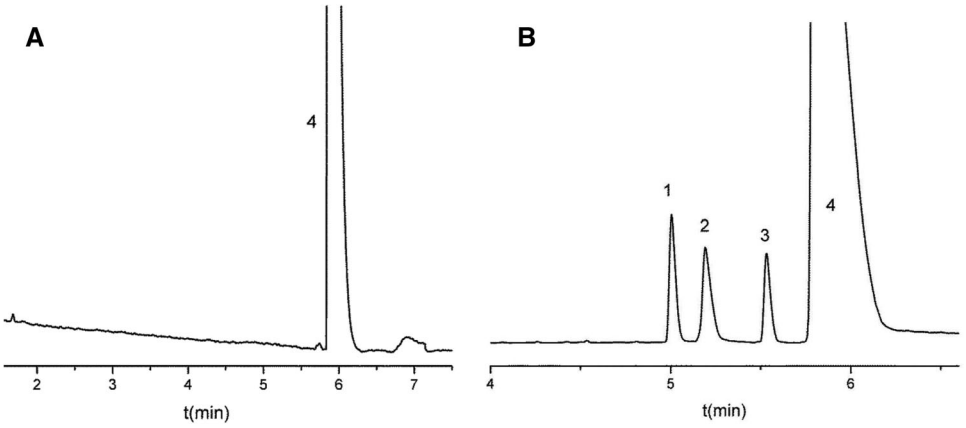

Figure 2. Representative electropherograms recorded for the Sunosi® sample solution (A) and the sample solution spiked with impurities (B) (CE conditions: 45 mM Tris-acetate buffer, pH 4.5, 4 mM S-γ-CD; 200 nm, 21°C, 19.5 kV, 1: *S*-phenylalaninol, 2: *R*-phenylalaninol, 3: *S*-solriamfetol, 4: *R*-solriamfetol, 0.1% impurities in 5000 μg/mL *R*-solriamfetol sample solution).

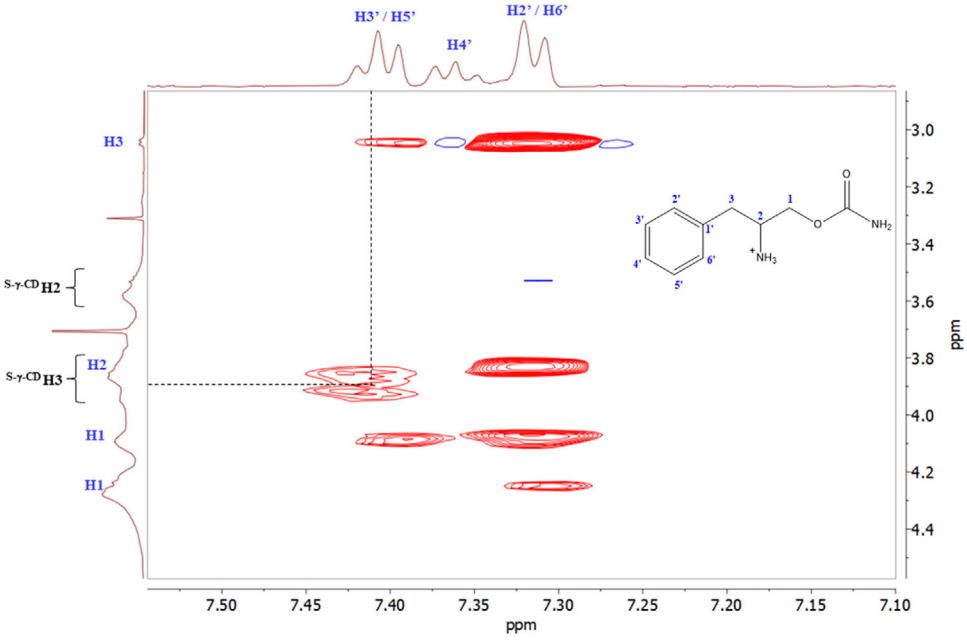

Figure 3. Partial 2D ROESY NMR spectra of solriamfetol and S-γ-CD, showing cross-peaks between the inner H3 proton of S-γ-CD and the aromatic H4, H5 protons of solriamfetol.

measurements were carried out to complement the CE experiments [30,31]. Before the investigation of the intermolecular interactions, ^{1}H NMR resonances of solriamfetol were assigned (Supporting Information Fig. 3–5.) based on 1D ^{1}H and ^{13}C, 2D COSY, HSQC, and HMBC experiments. The substitution of the gamma cyclodextrin resulted in a statistical sulfation pattern at 2OH, 3OH, and 6OH positions therefore the ^{1}H resonances could not be assigned unambiguously to any particular resonances of the macrocycle. Chen et al. studied the ^{13}C chemical shift changes induced by sulfation in the case of various sulfated CDs [32]. Using extensive homonuclear 2D correlation experiments however the H3 resonances of the S-γ-CD were successfully identified, thus possible inclusion could be studied.

To explore the structure of the inclusion complex, 2D ROESY NMR spectrum was recorded on a 1:7 solriamfetol:S-γ-CD solution. The partial ROESY spectra is shown in Fig. 3. In addition to the trivial intermolecular H3, H2, H1, and H2', H6' cross-peaks, further indicative cross-peaks between the aromatic H3' and H5' of solriamfetol and H3 of the S-γ-CD suggested the formation of an insertion complex. Complexation-induced chemical shift changes could also be used to confirm the protons mainly involved in the interaction. The most pronounced changes were observed at H2', H6', and H3 resonances in the presence of S-γ-CD.

4 Concluding remarks

The enantiorecognition study of solriamfetol and its major impurity phenylalaninol was performed by CE and NMR techniques providing an optimized, validated cyclodextrin-based chiral CE method for the separation of solriamfetol enantiomers and its impurities for the first time. In the preliminary screening experiments three native, 10 neutral, and 16 negatively charged cyclodextrin derivatives were investigated for the chiral separation of solriamfetol and phenylalaninol, the latter being degradation product/starting

material of the API. The charged cyclodextrin derivatives outperformed the neutral ones in terms of enantioselectivity. Outstanding enantioresolutions could be observed in the case of negatively charged sulfated-CDs, among which the S-γ-CD was chosen for further method optimization, due to the optimal migration order of enantiomers. After the optimization of the critical parameters by a fractional factorial experimental design the chiral CE method was validated according to ICH guidelines. The developed method was proven to detect 0.1% chiral and related impurities. It has also been applied to real samples, Sunosi® tablets containing 75 mg solriamfetol, where no impurities could be detected. This method could be implemented in routine drug analysis laboratories or in Pharmacopoeial monograph.

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The authors have declared no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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