Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1088–1094
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of the enantiomer and positional isomer impurities in atomoxetine hydrochloride with liquid chromatography using polysaccharide chiral stationary phases

Jane A. Sellers, Bernard A. Olsen *, Paul K. Owens, Peter F. Gavin

Analytical Sciences Research & Development, Eli Lilly & Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 2 December 2005; received in revised form 25 January 2006; accepted 27 January 2006
Available online 14 March 2006

Abstract

A normal-phase isocratic chiral liquid chromatographic method has been developed and validated for atomoxetine hydrochloride. In addition to the *S*-enantiomer of atomoxetine, the conditions separate both *para* and *meta* positional isomers and the phenyl des-methyl analog. Method development strategies included (a) evaluation of polysaccharide-based chiral stationary phases with nonaqueous mobile phases, (b) the use of an octyl stationary phase with a sulfated-β-cyclodextrin mobile phase additive, and (c) capillary electrophoresis using a single isomer heptakis-6-sulfato-β-cyclodextrin modifier. All three approaches yielded acceptable conditions for the separation of atomoxetine from related molecules with the former fully validated and the latter two held as alternatives if needed. The final method conditions employing a Chiralcel OD-H column and mobile phase of hexane/IPA/DEA/TFA (85/15/0.15/0.2, v/v/v/v) at 1.0 mL/min have been fully validated with acceptable specificity, linearity, accuracy, repeatability, intermediate precision and quantitation limit.

Keywords: Atomoxetine; Liquid chromatography; Chiral separation; Chiralcel; Positional isomer; Chiral mobile phase additive; Sulfated-β-cyclodextrin; Capillary electrophoresis; Heptakis-6-sulfato-β-cyclodextrin

1. Introduction

It is widely recognized that enantiomers have distinct biological interactions and thus potentially different pharmacokinetic, pharmacological and/or toxicological effects [1,2]. To assure patient safety and clinical efficacy, the pharmacological evaluation of stereoisomers is an integral part of new drug development [3,4]. Analytical methods to determine the enantiomeric purity of new investigational drugs are often attained through a series of generic or screening methodologies [5–9]. Although many analytical techniques can be employed to achieve this, the most widely used is liquid chromatography (LC) employing a chiral stationary phase (CSP) [10–15].

In addition to determining enantiomeric purity, methods to identify and quantify other structurally similar impurities must be developed. These impurities can be starting materials, intermediates, reaction by-products or degradation products that are most often determined by reversed-phase LC. When the structure of a drug substance includes substituted aromatic groups, the possibility exists for isomers substituted at different positions on the aromatic ring. Detecting positional isomers in an active pharmaceutical ingredient (API), or gathering evidence to support their absence, poses a particularly difficult problem for the separation scientist. Isomers have chemical and physical characteristics (hydrophobicity) similar to those of the API, which makes their determination difficult from both a separation and a detection perspective. For example, it is difficult to use either diode array UV detection or mass spectrometry coupled with "peak purity" algorithms to determine if positional impurities are present under the main component in a chromatogram.

The separation and quantification of positional isomers can be achieved using many different approaches. Normal or reversed-phase LC separations can be developed by evaluating solvent systems, chromatographic columns, aqueous buffer systems where appropriate, mobile phase pH, and/or temperature. Another option is to examine a more orthogonal chromato-

* Corresponding author. Tel.: +1 317 651 9910; fax: +1 317 433 7477.
E-mail address: olsen_bernard_a@lilly.com (B.A. Olsen).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.01.063

graphic approach [16,17] which may include chiral chromatography either in reversed or normal-phase mode and using either a chiral mobile phase additive (CMPA) [18] or a CSP [19–23]. Furthermore, an orthogonal analytical technique may also be utilized and positional isomer separations have been reported using capillary electrophoresis (CE) [19,24], capillary electrochromatography [25], supercritical fluid chromatography [26] and gas chromatography [27]. Capillary electrophoresis, where the separation mechanism is truly orthogonal to that of reversed-phase chromatography, can also be very effective in separating isomers, both optical or positional, when a chiral medium is employed in the background electrolyte. The inherent high peak efficiencies and resulting resolution advantages that can be obtained using CE have been utilized to great effect [28,29].

In this paper we describe the development and validation of a method for separating atomoxetine, a non-stimulant drug for the treatment of attention deficit/hyperactivity disorder, from its *S*-enantiomer, two positional isomers, and an additional impurity (structures given in Fig. 1). Previous methods for atomoxetine *S*-enantiomer determination employed achiral derivatization followed by chiral HPLC [30] and CE with cyclodextrin (CD) chiral selectors [31]. Positional isomers or other impurities were not considered in previous work. The approaches described here include reversed-phase chromatography with both neutral and charged cyclodextrin CMPAs, CE utilizing both neutral and charged CD additives, and the use of polysaccharide-based CSPs in normal-phase mode. The effect of mobile phase additives, the polar organic component of the nonaqueous mobile phase, and the type of stationary phase are described for the normal-phase LC method.

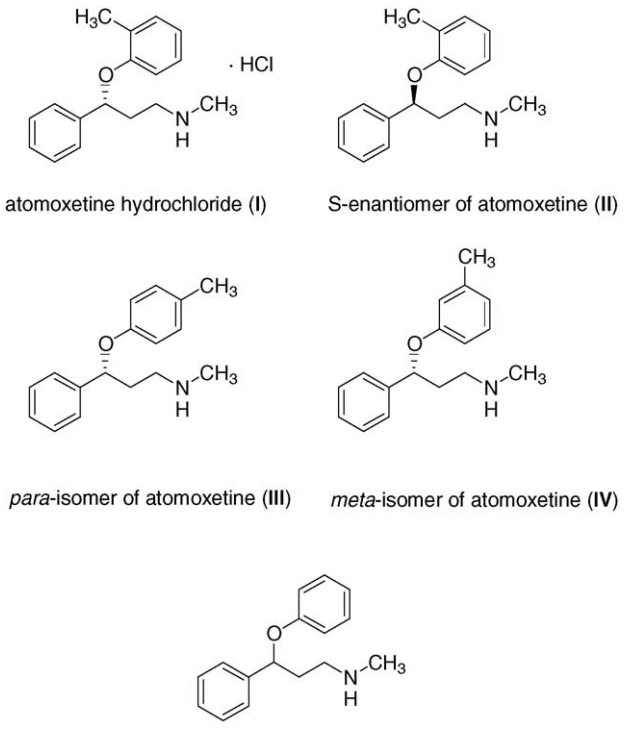

Fig. 1. Atomoxetine HCl and related molecules.

2. Experimental

2.1. Equipment

Chromatography was performed on an Agilent Technologies (Waldbronn, Germany) G1100 system equipped with a vacuum degasser, quaternary pump, autosampler, thermostated oven device and a variable wavelength UV detector. The chromatographic data were acquired and analyzed using Millenium^{32} software, version 3.2 (Waters Corporation, Milford, MA) or an in-house data system based on an HP1000 computer. Capillary electrophoretic separations were performed using the Beckman Coulter P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA).

2.2. Materials

Chiralcel OD-H and Chiralpak AD-H columns (25 cm × 4.6 m i.d.), were purchased from Chiral Technologies (Exton, PA, USA). The HPLC grade solvents (Omnisolv) (hexanes, *n*-propyl alcohol (*n*-PrOH), acetonitrile (MeCN), methanol (MeOH) and isopropanol (IPA)) were obtained from EMD (Darmstadt, Germany). Absolute ethanol (EtOH), A.C.S. reagent grade, trifluoroacetic acid (TFA), 99% and diethylamine (DEA) re-distilled, 99.5%, were purchased and used as received from Aldrich (Milwaukee, WI). Tertiary butyl alcohol (*t*-BuOH), HPLC grade, was purchased from Sigma–Aldrich (St. Louis, MO). Samples of compounds I–V (Fig. 1) were obtained from the Chemical Product Research and Development laboratory at Eli Lilly and Company (Lafayette, IN).

Sodium dihydrogen phosphate monohydrate (Fluka Chemie, Steinheim, Germany), potassium phosphate monobasic (EM Science, Darmstadt, Germany) and *ortho*-phosphoric acid (85%, HPLC grade) were purchased from Fisher Chemicals (Fair Lawn, NJ). Single isomer heptakis-6-sulfato-β-cyclodextrin (HS-β-CD) used in the CE study was purchased from Regis Technologies (Morton Grove, IL) and β-cyclodextrin sulfated sodium salt was purchased from Sigma–Aldrich (Sigma–Aldrich Co, St. Louis, MO). Sodium hydroxide (1.0N) and the Zorbax RX-C8, 5 μm, 250 mm × 4.6 mm LC column were purchased from Agilent Technologies (Waldbronn, Germany) and deionized water (18.2 MΩ) used throughout the study was taken from a Millipore Milli-Q Plus water purification system (Millipore, Billerica, MA). Fused silica capillaries of 59 cm × 50 μm were cut and prepared for use from 100 m rolls (Polymicro Technologies Inc., Phoenix, AZ).

2.3. Preparation of atomoxetine HCl samples for analysis

For normal-phase LC, samples of atomoxetine hydrochloride (3.5 mg/mL) and related impurities were dissolved in EtOH and diluted to volume with hexane to give a final sample solvent composition of hexane/EtOH, (75/25, v/v). For reversed-phase utilizing mobile phase additives, samples of atomoxetine hydrochloride (2.0 mg/mL) and related impurities were prepared in 25 mM potassium phosphate buffer/MeCN, (70/30, v/v). Atomoxetine hydrochloride samples (3.2 mg/mL) for CE analysis

were prepared by dissolving in a 1:1 mixture of methanol/20 mM sodium dihydrogen phosphate (20% of final volume) and diluting to the final volume with deionized water.

3. Results and discussion

3.1. Initial investigation of Chiralcel OD-H conditions

Positional isomer impurities are often very difficult to separate from each other and the main band in conventional reversed-phase impurities methods. During development it is sometimes necessary to consider positional isomers as potential impurities, which may require a separate method. Although the initial chiral method development goal was enantiomer separation, a secondary goal of separating the positional isomer impurities was also identified. Based on success with similar compounds, polysaccharide-based CSPs were investigated for atomoxetine. Separation of atomoxetine and related impurities was achieved using a Chiralcel OD-H column and a mobile phase containing hexane/IPA/DEA (85/15/0.2, v/v/v), however these conditions did not provide robust, reproducible retention and separation as shown in Fig. 2. Three replicate injections of a solution of atomoxetine and impurities showed that not only were there problems with retention time repeatability, but selectivity varied significantly. In each separation, adequate resolution was obtained for atomoxetine and its enantiomer to allow quantitation. Compounds III–V, however, were found to either co-elute or differ significantly in retention from injection to injection. As a consequence, there was a potential risk of overestimating the true level of the undesired enantiomer due to co-elution with other impurities and/or isomers.

Chiral stationary phases of derivatized cellulose or amylose have been utilized and studied extensively for the separation of enantiomers [13,32–39]. It is known that these CSPs can be sensitive to small changes in method parameters such as polar organic modifier concentration or memory effects from the use of a particular additive from a previous method [40,41]. In an effort to understand the large variability shown in Fig. 2 and to develop more robust and reproducible method conditions, various strategies were evaluated. One possibility for the lack of robustness observed is related to the sample solvent. The sample was dissolved as the hydrochloride salt and not neutralized prior to injection. Any variability in mixing after injection and subsequent neutralization of the salt by the DEA in the mobile phase may have contributed to the variability in retention. The addition of DEA to the sample solvent was not explored further, however, because of concerns about potential sample solution instability. Rigorous column washing and more stringent column temperature control were also evaluated but did not improve robustness so efforts were focused toward evaluation of mobile phase additives and various polar organic modifiers.

3.2. Addition of TFA to the mobile phase

A slight molar excess (relative to the DEA) of TFA added to the mobile phase had a positive effect on the observed selectivity of the method (Fig. 3). The resolution between the two enantiomers was enhanced which also provided a larger separation window for the impurities shown in Fig. 1. The impurities were now well resolved from each other and from atomoxetine and its *S*-enantiomer. Also shown in Fig. 3 is the resolution observed for the enantiomers of the phenyl des-methyl impurity (V). Although the first eluting enantiomer of this impurity was not well resolved from the *S*-enantiomer of atomoxetine (II), this is not a significant problem since this impurity enantiomer is not anticipated in atomoxetine HCl. Furthermore, the des-methyl impurity is also controlled in the achiral reversed-phase impurities method for atomoxetine. A preliminary evaluation of the method with TFA in the mobile phase indicated a significantly more robust set of chromatographic conditions (see full validation data below).

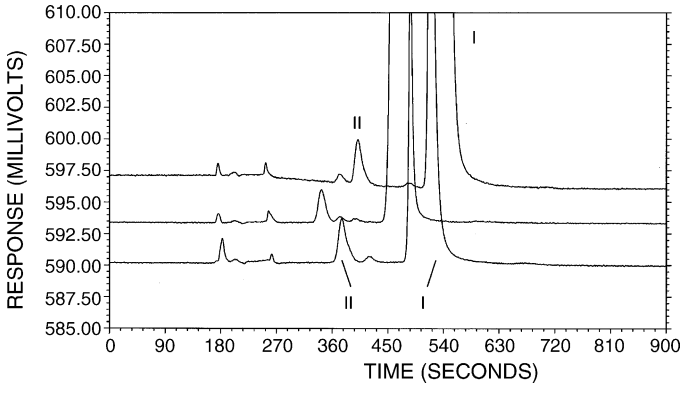

Fig. 2. Three repeat chromatographic injections of an atomoxetine sample containing the impurities shown in Fig. 1. Data indicate poor repeatability for chromatographic peak retention, selectivity and resolution. Atomoxetine enantiomers (I and II) could be identified, however, given the variability of these conditions it was not possible to accurately assign the remaining three impurities. Conditions: Chiralcel OD-H 250 mm × 4.6 mm 5 μm; hexane/IPA/DEA (85/15/0.2, v/v/v); 1.0 mL/min; UV detection at 273 nm; atomoxetine prepared at 3.5 mg/mL; ambient column temperature; 10 μl injection.

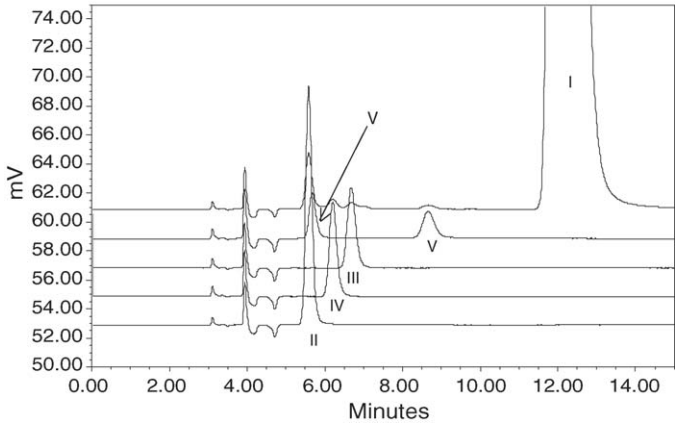

Fig. 3. Final optimized chromatographic conditions for the separation of atomoxetine enantiomers (I and II), atomoxetine positional isomers (III and IV) and the phenyl des-methyl impurity of atomoxetine (V), which was prepared as a racemic mixture. Conditions: Chiralcel OD-H 250 mm × 4.6 mm 5 μm; hexane/IPA/DEA/TFA (85/15/0.15/0.2, v/v/v/v); 1.0 mL/min; UV detection at 273 nm; atomoxetine prepared at 3.5 mg/mL; ambient column temperature; 10 μl injection.

3.3. Investigation of mobile phase polar organic modifier

It is also very important to consider the role of polar organic mobile phase modifier. There have been several reports on the influence and effects of various mobile phase modifiers on the structure and chiral selectivity of substituted polysaccharide CSPs [42–50]. More recently, Wang and Wenslow [43] studied the effects of the modifier concentration together with modifier structure on a polysaccharide CSP. It was concluded that using modifiers of different concentrations, size and shape may cause different degrees of twisting of the glucose units on the CSP helix. In effect, different modifiers generate different structural environments for both achiral and chiral interaction. As a result of this, there is a combination of structural factors that may influence the chiral cavities, which control the degree of fit and interaction between the solute and the CSP. Linear and branched alcoholic modifiers, including EtOH, *n*-PrOH, IPA and *t*-BuOH (combined with hexane, DEA, and TFA in the mobile phase), were studied to observe their effect on selectivity for atomoxetine and related molecules using a Chiralcel OD-H CSP. Ethanol and *n*-PrOH (separately and together) in the mobile phase did not provide the needed selectivity to resolve the two enantiomers and the three related molecules. Shown in Fig. 4A is the best chromatographic separation obtained using *n*-propanol as modifier. While the two enantiomers were well resolved from all other peaks of interest, the two positional isomers (III and IV) were not resolved from one another. Similar to Wang and Wenslow [43], we also observed that the branched alcohol modifiers interacted with the CSP through a sufficiently different mechanism to provide better selectivity. This is shown for the separation of all five analytes in Fig. 3 and Fig. 4B for IPA and *t*-BuOH modifiers, respectively. We have also noted this phenomenon in an additional development project where enantiomeric resolution could only be obtained with branched alcohol *t*-BuOH as modifier. This example illustrates the utility and importance of evaluating a number of different linear and branched alcohol modifiers when developing chromatographic conditions on polysaccharide CSPs. Comparing the results for IPA and *t*-BuOH, shown in Fig. 3 and Fig. 4B, respectively, the former was chosen for validation since it (a) afforded a shorter analysis time and (b) had more favorable handling properties compared with *t*-BuOH (melting point 25 °C) which can often be a solid at ambient temperatures.

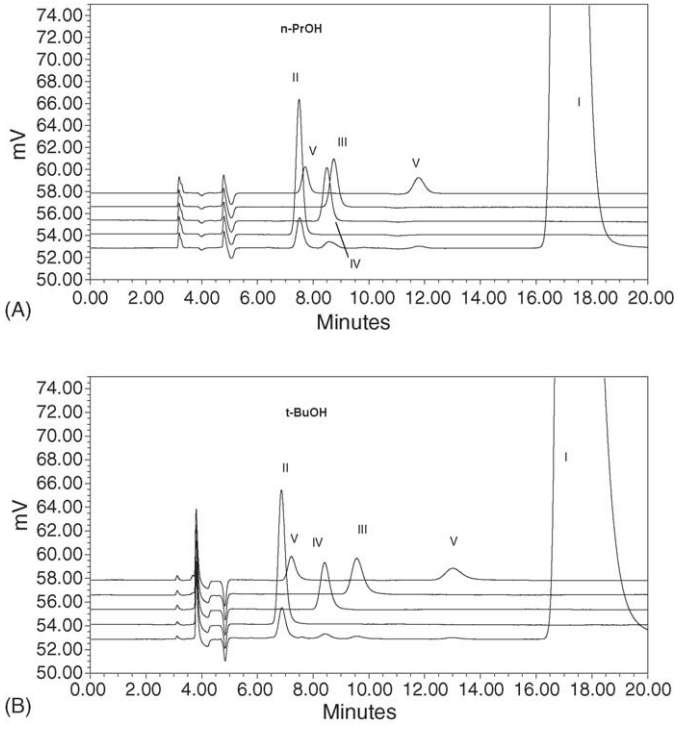

Fig. 4. Chromatographic separations comparing the use of linear and branched alcohol modifiers in chiral chromatography. Separation of atomoxetine HCl from related molecules using (A) *n*-propanol and (B) *tert*-butyl alcohol. Conditions: Chiralcel OD-H 250 mm × 4.6 mm 5 μm; 1.0 mL/min; UV detection at 273 nm; 10 μl injection of atomoxetine prepared at 3.5 mg/mL; using (A) hexane/*n*-propanol/DEA/TFA (93/7/0.15/0.2, v/v/v/v) at 25 °C column temperature; (B) hexane/*t*-BuOH/DEA/TFA (80/20/0.15/0.2, v/v/v/v) at 35 °C column temperature.

3.4. Evaluation of Chiralpak AD-H CSP

As with the evaluation of different alcohol modifiers when using polysaccharide CSPs, the nature of the phase itself offers the potential for a change in selectivity including reversal of enantiomer elution order [11,51–53]. In most chromatographic analyses, it is desirable to detect a minor component eluting before a major component rather than on the tail of the major component both from a detectability and a robustness perspective. Although this was the case with the atomoxetine separation using the Chiralcel OD-H CSP, investigation of an amylose-based CSP (Chiralpak AD-H) was conducted to check for selectivity differences. The separation shown in Fig. 5 obtained using the Chiralpak AD-H column can be compared with data shown in Fig. 3. In contrast to the separation on the Chiracel OD-H column, all impurity peaks eluted after the main peak using a Chiralpak AD-H column. Although the impurities were separated from atomoxetine, these conditions were not favored over those shown in Fig. 3. Similar peak reversals have been observed

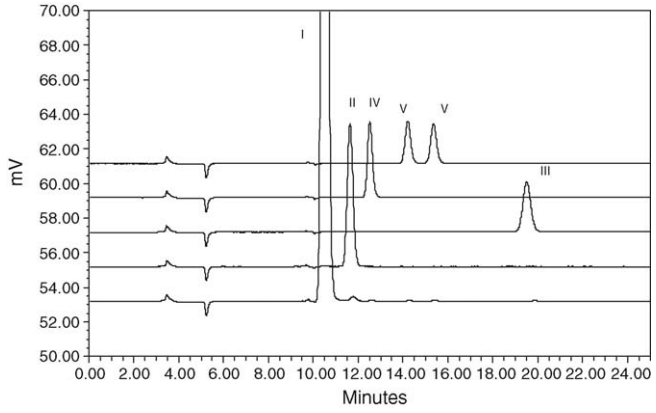

Fig. 5. Different selectivity for separation of atomoxetine HCl and related molecules using a Chiralpak AD-H column (compare to Fig. 3 using a Chiralcel OD-H). Conditions: Chiralpak AD-H 250 mm × 4.6 mm 5 μm; hexane/*n*-propanol/DEA (98/2/0.2, v/v/v); 10 μl injection of a 3.5 mg/mL sample; 1.0 mL/min; UV detection at 273 nm; 25 °C column temperature.

in our laboratory for other compounds, further illustrating the utility of investigating both the Chiracel OD-H and Chiralpak AD-H CSPs.

3.5. Alternative separation modes for atomoxetine isomers and impurities

We investigated other separation modes for the determination of chiral and positional isomers in atomoxetine hydrochloride to check for potential advantages compared to the polysaccharide CSPs. Work on alternative methods focused on using CD mobile phase and electrolyte additives in reversed-phase LC and CE, respectively. Native and modified neutral CDs were initially tested as mobile phase additives in conjunction with a C_8 stationary phase, however, incomplete resolution was obtained and peak shapes were poor. All components in Fig. 1 were separated with good resolution and peak shape when sulfated-β-cyclodextrin was utilized as an additive in an acetonitrile/phosphate buffer mobile phase (Fig. 6). Although the separation appeared to be sufficiently robust for validation and implementation as a long-term control method, concerns about availability and reproducibility of a derivatized CD additive with potential for varying degrees of substitution were noted. Variability among derivatized CD vendors was also a concern. These conditions were therefore not pursued further but could be used in the future should problems be experienced with the Chiralcel OD-H method.

Successful separations were also obtained using CE as shown in Fig. 7. The single isomer sulfated CDs were evaluated using heptakis-6-sulfato-β-cyclodextrin (HS-β-CD) in a low pH phosphate background electrolyte. The system was found to work particularly well for atomoxetine isomers and impurity. Shown in Fig. 7A is the resolution between atomoxetine and its *S*-enantiomer together with selectivity obtained for both the *meta* and *para*-positional isomers and the racemic phenyl des-methyl impurity. These conditions, although not fully optimized, were

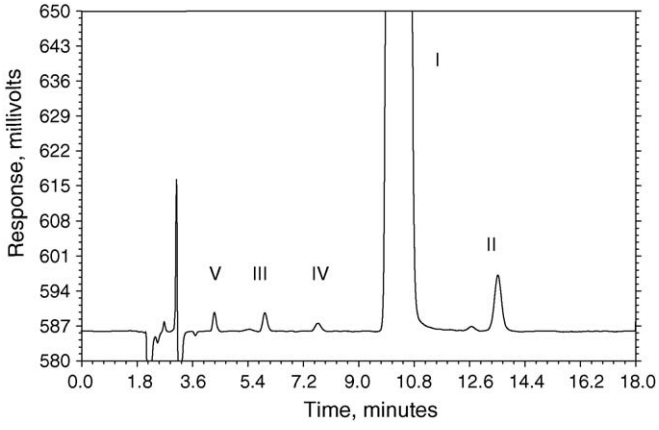

Fig. 6. Separation of atomoxetine from its enantiomer and positional isomers including impurities using a sulfated-β-cyclodextrin. A fluorinated analog is eluted between atomoxetine and its enantiomer. Conditions: Zorbax RX-C8 5 μm 250 mm × 4.6 mm column and using a mobile phase of MeCN/25 mM potassium phosphate at pH 2.5 containing 20 mg/mL sulfated-β-cyclodextrin (75/25, v/v); 1.0 mL/min; 215 nm.

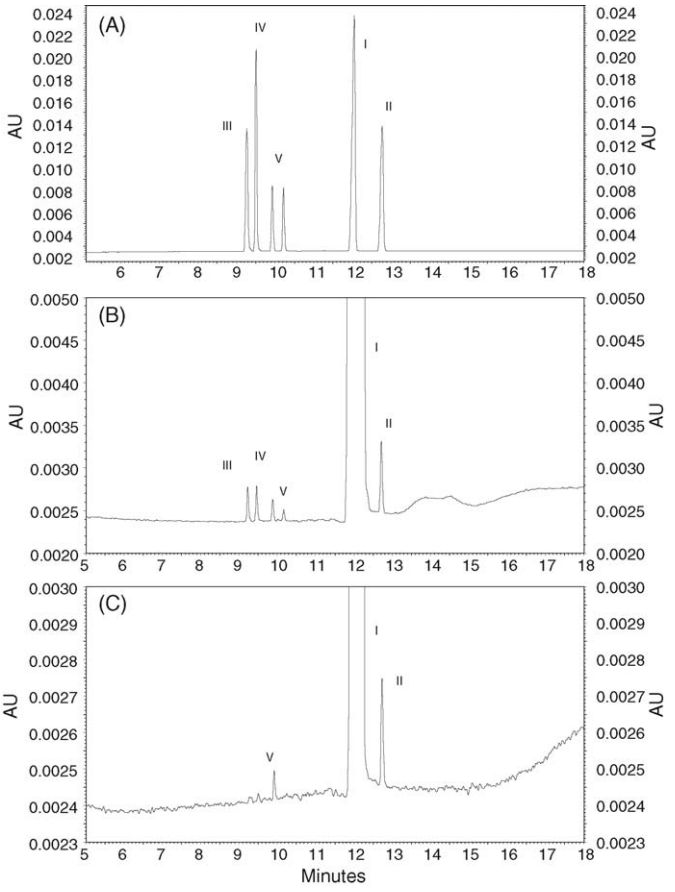

Fig. 7. Separation by CE using a single isomer HS-β-CD of: (A) a selectivity mixture of atomoxetine, its enantiomer, two positional isomers and the phenyl des-methyl impurity; (B) a typical atomoxetine hydrochloride batch spiked with each impurity at 0.1% w/w; (C) a typical atomoxetine HCl batch indicating absence of any discernable amounts of *meta* or *para*-isomer impurities. Conditions: uncoated fused silica 50 μm i.d. × 48 cm (total length 59 cm); 200 ± 10 nm; collection rate 2 Hz; capillary cassette temperature 20 °C and sample temperature 20 °C; background electrolyte of 20 mM sodium phosphate buffer (pH 2.3) containing 20 mM HS-β-CD; 0.5 psi injection for 5 s; reversed polarity (anode at detector end) 339 V/cm; ramped over 0.17 min (approximately 65 μA) initial capillary flush for 30 min with 1.0N sodium hydroxide; 30 min water and 5 min with electrolyte prior to each separation.

capable of detecting each impurity in atomoxetine when spiked at a 0.1% level (Fig. 7B). The data in Fig. 7C on a typical batch of atomoxetine HCl material can be directly compared to the chromatogram shown in Fig. 8, which was collected using the final LC conditions. As noted above with LC, concern around the long-term availability of a specialized chiral reagent was taken into consideration and the CE method was not pursued further. It also has the potential as a backup to the LC method, if necessary.

3.6. Validation of atomoxetine HCl LC method

The CSP LC method conditions described above in Fig. 3 for the determination of the atomoxetine enantiomer and two positional isomer impurities, have been fully validated according to ICH guidelines with respect to specificity, linearity, precision (repeatability and intermediate precision), accuracy, limit

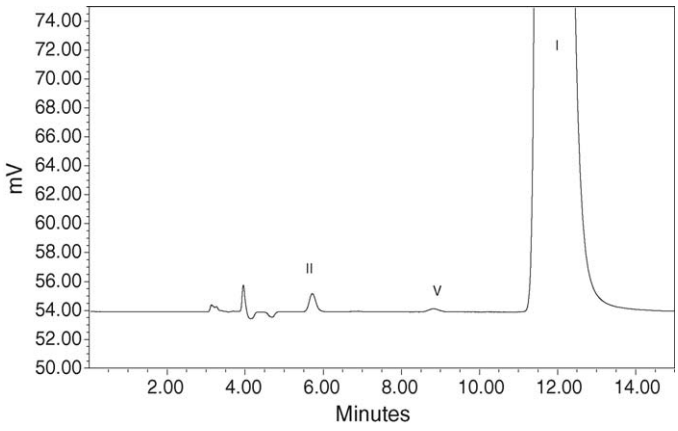

Fig. 8. Chromatographic evaluation of a typical atomoxetine HCl batch indicating separation of enantiomer and des-methyl impurity. The chromatogram also indicates absence of any discernable amounts of *meta* or *para*-isomer impurities. Conditions as shown above in Fig. 3.

of quantitation, and range. A summary of the data can be found in Table 1. Specificity has been demonstrated and shown in Fig. 3. Complete separation was not attained between the *S*-enantiomer of atomoxetine and *S*-phenyl des-methyl atomoxetine, however, the latter was not anticipated to be present in atomoxetine HCl. This was confirmed as shown in Figs. 7 and 8. As noted above, the des-methyl impurity (V) is controlled separately in the impurities method and was thus not included in this validation work. Acceptable linearity was obtained for the main component at a nominal sample concentration of 3.5 mg/mL and for the enantiomer and the two positional isomers, from the reporting limits to well above the permitted specifications. Repeatability and accuracy were evaluated for each impurity at three concentrations across the range with three replicates at each concentration and were found to be acceptable (Table 1). Intermediate precision data were also collected using three analysts, four instruments, three columns and two laboratories, totaling 57 experimental evaluations. Data in Table 1 indicate acceptable intermediate precision. Quantitation limits (QL) for each

Table 1
Validation data obtained for chiral LC conditions for atomoxetine hydrochloride including data for *S*-enantiomer of atomoxetine, *para* and *meta*-positional isomers

Validation criterion	Range or experiment	Result
Specificity	Impurities of interest spiked into atomoxetine HCl and run under the final chiral LC conditions	All analytes were adequately resolved as shown in Fig. 3
Linearity		
Atomoxetine HCl (I)	80–120% of nominal sample concentration (3.5 mg/mL)	$R^2 = 0.997$; $Y = 7402X + 936$
S-enantiomer of atomoxetine HCl (II)	0.05–0.76% in parent	$R^2 = 0.992$; $Y = 8740X + 37$
Para-isomer (IV)	0.03–0.81% in parent	$R^2 = 1.000$; $Y = 9006X + 21$
Meta-isomer (III)	0.03–0.81% in parent	$R^2 = 1.000$; $Y = 9186X + 22$

Accuracy and repeatability	Percentage vs. atomoxetine	% Recovery, $n = 3$	% RSD, $n = 3$
S-enantiomer of atomoxetine (II)	0.05	106	12.3
	0.25	103	0.2
	0.50	102	3.3
Para-isomer (IV)	0.025	91	7.3
	0.05	105	6.3
	0.10	104	4.5
	0.20	106	4.0
	0.39	106	3.7
Meta-isomer (III)	0.025	110	5.1
	0.05	114	6.4
	0.10	113	3.9
	0.20	111	3.6
	0.39	110	4.0

Impurity, level in sample	% RSD, $n = 57$, 3 analysts, 4 instruments, 3 columns, 2 laboratories
Intermediate precision	
S-enantiomer of atomoxetine (II), 0.47%	1.7
Para-isomer (IV), 0.09%	8.0
Meta-isomer (III), 0.08%	8.5
Quantitation limit (QL): calculated as outlined in ICH Q2B from the standard deviation of the response and the slope using data from accuracy studies	
S-enantiomer of atomoxetine (II)	0.07%
Para-isomer (IV)	0.02%
Meta-isomer (III)	0.02%

peak of interest were calculated as outlined in ICH Q2B from the standard deviation of the response and the slope using the data obtained during the accuracy studies. The range for each impurity was therefore reported from the QL to the highest concentration used in the precision and accuracy studies. Finally, shown in Fig. 8, is a chromatogram of a typical atomoxetine sample demonstrating the separation of the *S*-enantiomer of atomoxetine and the des-methyl impurity from atomoxetine as well as the absence of any amounts of either the *meta* or *para*-substituted isomer impurities.

4. Conclusions

A polysaccharide-derived CSP (Chiralcel OD-H) was found to be successful in separating atomoxetine hydrochloride from the undesired *S*-enantiomer, *meta* and *para*-positional isomers and des-methyl related impurity. Preliminary conditions based on the cellulose CSP were found to be insufficiently robust for long-term control use and were optimized further. It was noted that in addition to a DEA additive in IPA and hexane to improve peak shape and efficiency, the addition of TFA to the mobile phase was necessary to facilitate method robustness. Furthermore, a wider set of parameters were also investigated and optimized including the stationary phase itself and mobile phase solvent composition. It was concluded for atomoxetine, as others have previously noted for other compounds, that selectivity, chromatographic performance and enantiomeric peak elution could be influenced and controlled by switching from an amylose to a cellulose based polysaccharide phase or through the use of either a linear or branched alcohol mobile phase modifier. During method development, additional chromatographic and electrophoretic separation conditions were also considered. It was shown that selectivity for atomoxetine and related molecules could also be attained using either a chiral mobile phase additive with a C_8 column or with CE when a derivatized cyclodextrin was added to the background electrolyte. The final LC conditions were fully validated according to the ICH guidelines with acceptable results obtained for specificity, linearity, accuracy, repeatability, intermediate precision and quantitation limit.

Acknowledgement

The authors gratefully acknowledge the contributions of Frank Akey and John Stafford to this work.

References

[1] E.J. Ariens, E.W. Wuis, J. Roy. Coll. Phys. Lond. 28 (1994) 395–398.
[2] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3–33.
[3] W.H. De Camp, Chirality 1 (1989) 2–6.
[4] W.H. De Camp, J. Pharm. Biomed. Anal. 11 (1993) 1167–1172.
[5] N. Matthijs, C. Perrin, M. Maftouh, D.L. Massart, Y.Y. Heyden, J. Chromatogr. A 1041 (2004) 119–133.
[6] N. Matthijs, C. Perrin, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Pharm. Biomed. Anal. 27 (2002) 515–529.
[7] C. Perrin, N. Matthijs, D. Mangelings, C. Granier-Loyaux, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 966 (2002) 119–134.
[8] C. Perrin, V.A. Vu, N. Matthijs, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 947 (2002) 69–83.
[9] M.E. Anderson, D. Aslan, A. Clarke, J. Roeraade, G. Hagman, J. Chromatogr. A 1005 (2003) 83–101.
[10] C. Roussel, A. Del Rio, J. Pierrot-Sanders, P. Piras, N. Vanthuyne, J. Chromatogr. A 1037 (2004) 311–328.
[11] M. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 401–407.
[12] F. Gasparrini, D. Misiti, C. Villani, J. Chromatogr. A 906 (2001) 35–50.
[13] P. Franco, A. Senso, L. Oliveros, C. Minguillon, J. Chromatogr. A 906 (2001) 155–170.
[14] G. Felix, J. Chromatogr. A 906 (2001) 171–184.
[15] T.J. Ward, D.M. Hamburg, Anal. Chem. 76 (2004) 4635.
[16] J. Grainger, P.C. McClure, Z. Liu, B. Botero, S. Sirimanne, D.G. Patterson Jr., M. Sewer, C. Gillyard, K. Kimata, K. Hosoya, T. Araki, N. Tanaka, S. Terabe, Chemosphere 32 (1996) 13–23.
[17] P.J. Slonecker, X.D. Li, T.H. Ridgway, J.G. Dorsey, Anal. Chem. 68 (1996) 682–689.
[18] P.K. Owens, A.F. Fell, M.W. Coleman, J.C. Berridge, Chirality 8 (1996) 466–476.
[19] P.D. Ferguson, D.M. Goodall, J.S. Loran, J. Chromatogr. A 768 (1997) 29–38.
[20] P.K. Owens, L. Karlsson, E.S.M. Lutz, L.I. Andersson, TRAC-Trend. Anal. Chem. 18 (1999) 146–154.
[21] M.C. Ringo, C.E. Evans, J. Microcol. Sep. 10 (1998) 647–652.
[22] D.S. Rao, M.K. Srinivasu, C.L. Narayana, G.O. Reddy, J. Pharm. Biomed. Anal. 25 (2001) 21–30.
[23] S. Piperaki, M. Parissipoulou, M. Koupparis, J Liq. Chromatogr. 16 (1993) 3487–3508.
[24] J.H.T. Luong, A.L. Nguyen, J. Chromatogr. A 792 (1997) 431–444.
[25] D. Hindocha, N.W. Smith, J. Chromatogr. A 904 (2000) 99–106.
[26] L. Zhou, Y. Wu, B.D. Johnson, R. Thompson, J.M. Wyvratt, J. Chromatogr. A 866 (2000) 281–292.
[27] Z.R. Zeng, M. Liu, Chromatographia 48 (1998) 817–822.
[28] T.K. Natishan, J. Liq. Chromatogr. R. T. 28 (2005) 1115–1160.
[29] G. Gubitz, M.G. Schmid, Electrophoresis 25 (2004) 3981–3996.
[30] R.J. Bopp, J.H. Kennedy, LC–GC 6 (1988) 514, 516, 518, 520, 522.
[31] L. Li, M.A. Nussbaum, J. Pharm. Biomed. Anal. 19 (1999) 679–694.
[32] B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 1042 (2004) 55–60.
[33] T. Kubota, C. Yamamoto, Y. Okamoto, J. Polym Sci., Part A: Polym. Chem. 42 (2004) 4704–4710.
[34] Y. Okamoto, E. Yashima, C. Yamamoto, Top. Stereochem. 24 (2003) 157–208.
[35] C. Yamamoto, Y. Okamoto, Bull. Chem. Soc. Jpn. 77 (2004) 227–257.
[36] C. Yamamoto, Y. Okamoto, Methods Molecular Biology, 243, Totowa, NJ, United States, 2004, pp. 173–181.
[37] H.Y. Aboul-Enein, I. Ali, Farmaco 57 (2002) 513–529.
[38] E. Yashima, J. Chromatogr. A 906 (2001) 105–125.
[39] B.A. Olsen, D.D. Wirth, J.S. Larew, J. Pharm. Biomed. Anal. 17 (1998) 623–630.
[40] R.W. Stringham, K.G. Lynam, B.S. Lord, Chirality 16 (2004) 493–498.
[41] Y.K. Ye, B. Lord, R.W. Stringham, J. Chromatogr. A 945 (2002) 139–146.
[42] S.C. Lin, C. Engelsma, J. Liq. Chromatogr. R. T. 20 (1997) 2181–2192.
[43] T. Wang, R.M. Wenslow, J. Chromatogr. A 1015 (2003) 99–110.
[44] R.M. Wenslow, T. Wang, Anal. Chem. 73 (2001) 4190–4195.
[45] H.Y. Aboul-Enein, I. Ali, J. Biochem. Bioph. Meth. 48 (2001) 175–188.
[46] G.S. Yang, W. Du, S. Jin, L. Zhou, P.P. Vazquez, A.G. Frenich, J.L.M. Vidal, H.Y. Aboul-Enein, Anal. Lett. 36 (2003) 1423–1435.
[47] K.M. Kirkland, J. Chromatogr. A 718 (1995) 9–26.
[48] Y. Tang, Chirality 8 (1996) 136–142.
[49] Y. Tang, J.C. Reepmeyer, L.K. Revelle, J.A. Wilson, J. Chromatogr. A 752 (1996) 93–99.
[50] A. Kunath, F. Theil, K. Jahnisch, J. Chromatogr. A 728 (1996) 249–257.
[51] K. Balmer, B.A. Persson, P.O. Lagerström, J. Chromatogr. A 660 (1994) 269–273.
[52] B.A. Persson, S. Andersson, J. Chromatogr. A 906 (2001) 195–203.
[53] F. Wang, T. O'Brien, T. Dowling, G. Bicker, J. Wyvratt, J. Chromatogr. A 958 (2002) 69–77.