Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 122–129

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Development and validation of a HPLC method for the determination of sertraline and three non-chiral related impurities

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ARTICLE INFO

Article history:
Received 9 November 2009
Received in revised form 20 January 2010
Accepted 20 January 2010
Available online 25 January 2010

Keywords:
HPLC
Method optimization
Pharmaceuticals
Validation

ABSTRACT

In this study, a screening on reversed-phase stationary phases (including C_8, C_{18}, CN, PEG and amide) was carried out in order to obtain an efficient HPLC method for the determination of sertraline and three of its more closely related synthetical and non-chiral impurities, without using ion-pair reagents. The best results in terms of both retention time and resolution of the target analytes were obtained with a Zorbax Bonus-RP column, which contains a polar amide group embedded in a C_{14} alkyl chain.

Once the most suitable stationary phase was chosen, the HPLC method was optimized by using a factorial design, evaluating three quantitative factors (column temperature, buffer pH and buffer concentration) in order to find the best conditions which maximize the resolution between impurities A and B (positional isomers) and minimize the total run time. The final HPLC conditions were set by means of a second experimental design, which allowed optimizing the effects of the buffer pH and the proportion of methanol in the mobile phase.

The optimal conditions for simultaneously determining sertraline and its impurities, being baseline separated in less than 10 min, were finally obtained with Zorbax Bonus-RP column (150 mm × 4.6 mm, 5 μm), under isocratic conditions with phosphate buffer (pH 2.8; 10 mM)–methanol (63:37, v/v) at 50 °C, at the flow-rate of 1.0 mL/min. UV detection was set at 220 nm.

This method was successfully validated following ICH guidelines and it proved to be reliable for the determination of sertraline and related impurities in tablets as pharmaceutical forms.

1. Introduction

Sertraline is a potent selective serotonin reuptake inhibitor (SSRI) in the brain, used in the treatment of all type of depression and effective for acute treatment and long-term management of social anxiety disorder [1]. At clinical doses, sertraline blocks the uptake of serotonin into human platelets. Like most clinically effective antidepressants, sertraline down regulates brain norepinephrine and serotonin receptors in animals [2]. In receptor binding studies, sertraline has no significant affinity for adrenergic, cholinergic, GABA, dopaminergic, histaminergic, serotonergic or benzodiazepine binding sites [2] and this is the main reason for the reduced side effects shown by this active compound. In addition, in placebo-controlled studies in normal volunteers, sertraline did not cause sedation and did not interfere with psychomotor performance [2].

Several analytical methods have been described in the literature for the determination and quantification of sertraline in pharmaceuticals and biological samples. In 2008, Boscha et al. reviewed the analytical methodologies for the determination of this pharmaceutically active compound from 1987 until 2008 [1]. Most of these methods are based on separation techniques, being HPLC the most frequently employed. Compared with other techniques, HPLC offers several advantages, including low cost, simplified sample preparation and easiness of measurement. With relation to the analytical determination of sertraline in pharmaceuticals, the literature indicates also the employment of non-chromatographic techniques, such as UV–vis spectroscopic methods, potentiometric methods and titrations [1]. In particular, spectrophotometric techniques are frequently employed for the determination of sertraline in pharmaceuticals [1].

In the literature different HPLC methods have been described for the analysis of sertraline in biological samples [3–8]. These methods employ different types of detection, including UV–vis, fluorescence and mass spectrometry (MS) detection [1].

Several analytical methods have also been described for the simultaneous identification of different antidepressant drugs, including sertraline, by using HPLC techniques [9–20].

Four methods described in the literature allowed the separation and determination of sertraline diastereoisomers and enantiomers

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doi:10.1016/j.jpba.2010.01.036

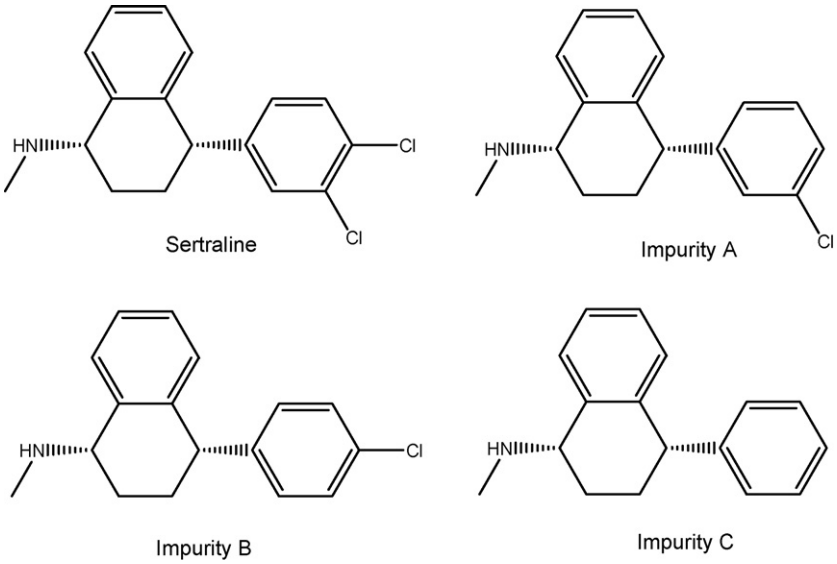

Fig. 1. Chemical structure of sertraline and related impurities A, B and C.

[21–25] and three of them are based on MEKC [23–25]. The fact that sertraline is a chiral compound may explain why these methods are focused on the separation of its isomers and stereoisomers, while the achiral analysis of this biologically active compound and its potential impurities has not been explored.

During the synthesis of setraline, different impurities can remain in the raw material (A, B and C, Fig. 1, corresponding with impurities C, D and B reported in the European Pharmacopoeia). Other two non-chiral intermediates in the synthesis (mandelic acid and (4R)-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1 (2H)-one) have not been included, as they are clearly different and do not pose an analytical challenge. The control of impurities is currently a critical issue in pharmaceutical analysis. Up to date, there is no analytical method covering the determination of sertraline and non-enantiomeric impurities in pharmaceuticals. A possible approach in this context is based on the use of ion-pair reagents as mobile phase additives, since this active compound contains an amino group. It is well-known that ion-pairing chromatography can be applied in the analysis of ionic compounds, which are typically difficult to retain and separate on a reversed-phase (RP) stationary phase. Therefore, the analytical method initially developed for the determination of sertraline and its impurities in our lab was based on RP chromatography at acidic pH, employing 1-octane sulphonate as the ion-paring reagent and acetonitrile as the organic modifier. The drawbacks of ion-pairing chromatography are generally well-known, such as a long equilibration time and, as the ion-pairing compounds interact strongly with the stationary phase, difficult elimination from the column after their use, providing poor retention time reproducibility. The latter is highly undesirable during routine analysis of related compounds.

In the light of all the above, the aim of this work was the evaluation of the chromatographic performance of several HPLC stationary phases to obtain an efficient separation of sertraline and closely related, synthetic, non-chiral impurities, avoiding the employment of ion-pairing reagents. Once the best choice for this purpose was found, the HPLC method was optimized in order to achieve the best compromise between degree of separation and analysis time. Finally, hence this method is intended to be implemented for the quality control of pharmaceutical formulations, a full validation was carried out to demonstrate its reliability in the determination of sertraline and synthetic, non-chiral related substances in sertraline tablets.

2. Experimental

2.1. Chemicals and solvents

Sertraline hydrochloride, (1*S*,4*S*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine) (HPLC purity 99.7%), impurity A (1*S*-cis)-4-(3-chlorophenyl-1,2,3,4-tetrahydro-N-methyl-1-naphthaleneamine) (HPLC purity 97.5%), impurity B (1*S*-cis)-4-(4-chlorophenyl-1,2,3,4-tetrahydro-N-methyl-1-naphthaleneamine) (HPLC purity 87.7%), impurity C (1*S*-cis)-4-(phenyl-1,2,3,4-tetrahydro-N-methyl-1-naphthaleneamine) (HPLC purity 99.6%) as well as sertraline 50–100 mg tablets and excipients (microcrystalline cellulose, monohydrate lactose, povidone, sodium croscarmellose, magnesium stearate, silica colloidal anhydrous, hipromelose, hidroxypropyl cellulose, titanium dioxide (E-171), macrogol 400 (Opadry Y-5-7068)) were kindly provided by CINFA S.A. (Pamplona, Spain).

Methanol (HPLC grade) was purchased from Poch-Sa (Gliwice, Poland) and phosphoric acid (H_3PO_4) (85%) from Carlo Erba Reagenti (Milan, Italy). Water was purified with a Milli-Q plus system from Millipore (Bedford, MA, USA).

2.2. Chromatographic apparatus and conditions

A LaChrom Elite HPLC equipment from VWR, consisting of a quaternary pump, an automatic injector, a single wavelength detector and a column oven, was employed. The optimized method was validated on a Zorbax Bonus-RP column (150 mm × 4.6 mm i.d., 5 μm) (Agilent Technologies, Waldbronn, Germany). The mobile phase consisted of phosphate buffer (pH 2.8; 10 mM)–methanol 63:37 (v/v). The column temperature was set at 50 °C. The flow-rate was 1 mL/min. The injection volume was 7 μL. UV detection was set at 220 nm.

2.3. Standard solutions and sample preparation

The solvent solution (SS) for standards and samples was the mobile phase. The method was validated establishing a concen-

tration level of 0.56 mg/mL of sertraline hydrochloride as a 100% of theoretical concentration. Sertraline hydrochloride standard solution was prepared by weighing approximately 28.0 mg of this substance and dissolving it with SS in a 50 mL volumetric flask. A stock solution containing all the impurities was prepared by weighing approximately 25 mg of each impurity and dissolving in 1000 mL volumetric flask with SS. The concentration of the impurities in this solution was 0.025 mg/mL.

For quantification purpose, 76.5 mg of the pulverized tablets were made up to 50 mL with SS. After bath sonication for 5 min, the samples were filtered with 0.45 μm nylon filters prior to the injection.

2.4. Validation

The validation parameters were tested in two ranges: quantification range and impurity range.

Linearity was tested for the high range by preparing standard solutions at five different concentration levels, from 75 to 125% of the target analyte concentration. In this case, sertraline hydrochloride concentrations were from 0.42 to 0.70 mg/mL. The solutions were prepared in 50 mL volumetric flasks by weighing the exact amount of sertraline hydrochloride (from 21.0 to 35.0 mg) and diluting to volume with SS. Each concentration level was prepared in triplicate and analyzed three times. For the impurity range, a stock solution, containing impurities A, B and C, was prepared at 0.025 mg/mL. Linearity was checked from 0.25 to 5 μg/mL, by adding the corresponding volume of the impurity stock solution (from 0.50 to 10.00 mL) in a 50 mL volumetric flask and diluting to volume with SS.

For accuracy determination, the analytical procedure was applied to synthetic mixtures of the drug components to which known quantities of drug substance and related products to be analyzed were added. It was tested in triplicate at five levels (75, 90, 100, 110 and 125%) and in parallel with the linearity assay. The % recovery and the RSD values were then calculated.

For accuracy in the low range, 48.5 mg of excipients, emulating the pharmaceutical matrix, were weighed in 50 mL volumetric flasks and the corresponding volumes of sertraline impurities stock solution were added to obtain 0.05, 0.1, 0.2, 0.5 and 1.0%. These values ranged from the limit of declaration to a value over the limit of acceptance.

For the intra-assay precision, data were obtained by analyzing standards and samples in one laboratory on one day. For the instrumental precision data were obtained by analyzing ten times the same aliquot of standard. Solutions for the precision assay were prepared in 50 mL volumetric flasks by weighing exactly 28 mg of sertraline hydrochloride and adding 2 mL of impurities stock solution. Ten standards and sample solutions were independently prepared according to the procedure of the method. Data for intermediate precision were obtained by repeating the intra-assay experiments on a different day with newly prepared solutions.

Response factors for impurities A, B and C related to sertraline hydrochloride was obtained by analyzing three aliquots of a mixture of each impurity and sertraline, three times. They were prepared in 50 mL volumetric flasks by adding separately 50 μL of impurities A, B, C and sertraline standards solutions (1 mg/mL). This concentration level corresponds to a 0.5% of the theoretical concentration.

2.5. Software

The HPLC instrument was controlled by Ezchrom software. Method optimization by experimental design was carried out with Statgraphics Plus 5.1 software. The validation calculations were carried out with Microsoft Excel 2003 version Office Package.

3. Results and discussion

3.1. Method development

There are two primary approaches that can be used to increase the RP-HPLC retention of ionic compounds. The first one is based on the addition of an ion-pair reagent to the mobile phase to form non-polar complexes with the analytes. The second strategy is based on the use of RP-HPLC stationary phases that exhibit enhanced polar retention and selectivity.

The initially developed analytical method for the determination of sertraline and its impurities (A, B and C) in our lab was based on the use of ion-pair chromatography on a RP (C_{18}) stationary phase, employing 1-octane sulfonate as the ion-pairing reagent at acidic pH and acetonitrile as the organic modifier (Fig. 2). Although this technique provided a good retention of sertraline and related impurities, the analysis time was not satisfactory. Furthermore, it is well-known that ion-pair chromatography has several drawbacks, including a long equilibration time and, as the ion-pairing compounds interact strongly with the stationary phase, difficult elimination from the chromatographic system, thus causing poor reproducibility and robustness, highly undesirable.

This study was firstly focused on the development of a HPLC method for the separation of sertraline from its impurities, without the employment of ion-paring reagents in the mobile phase, and therefore to avoid all the aforementioned inherent problems related to this methodology. In order to obtain an adequate resolution of the target analytes in isocratic conditions without employing ion-pairing reagents, several stationary phases were tested. In recent years, significant improvements have been made in the quality of bonded silica particles used in HPLC, by increasing the number of RP columns available with different functionalities and selectivities. In this study a screening on RP stationary phases was carried out in order to explore their chromatographic performance for the separation of sertraline and its impurities, including a wide range of polarities such as C_8, C_{18}, CN, PEG and amide. Table 1 shows the list of the all the columns tested in the method development. All the assayed columns are equivalent in dimension.

The columns tested here were checked with a mobile phase consisting of phosphate buffer (pH 3.0; 10 mM)–methanol (60:40, v/v) and all of them were kept at 35 °C during the analysis. The flow-rate was 0.8 mL/min and the injection volume was 20 μL. UV detection was performed at 220 nm. The assays were developed by

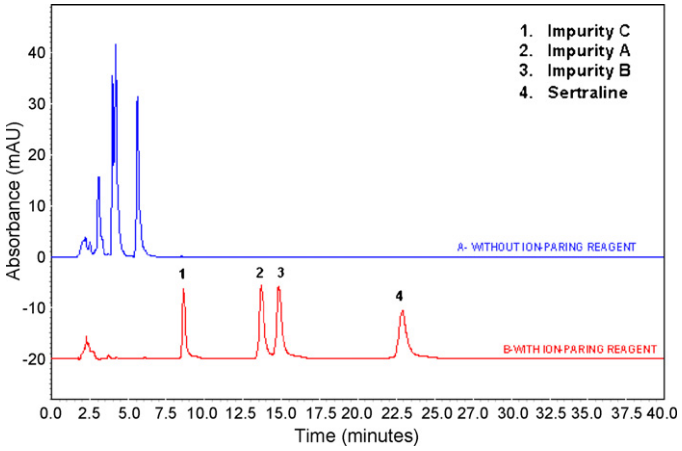

Fig. 2. Chromatogram showing the separation of setraline and impurities A, B, C, Column: Symmetry C18 (2506 mm × 4.6 mm, 5 μm). Mobile phase: phosphate buffer (pH 3.1; 10 mM)–acetonitrile (60:40, v/v). Column temperature: 25 °C. Flow-rate: 1 mL/min. Detection: UV at 220 nm. (A) Analysis without ion-pairing reagent. (B) Analysis with 1-octane sulfonate, as the ion-pairing reagent.

Table 1
HPLC columns tested in the method development.

Manufacturer	Name	Stationary phase	Particle size (μm)	Dimensions (mm)
Supelco	Discovery	CN	5	150 × 4.6
Supelco	Discovery	C18	5	150 × 4.6
Supelco	Ascentis	RP-Amide	5	150 × 4.6
Supelco	Discovery	RP-Amide C16	5	150 × 4.6
Agilent	Zorbax	Bonus-RP	5	150 × 4.6
Supelco	Ascentis	C18	5	150 × 4.6
Supelco	Discovery	HS-PEG	5	150 × 4.6
ACE	ACE5	C18	5	150 × 4.6
Agilent	Zorbax	SB-CN	5	150 × 3.0
Supelco	Discovery	C8	5	150 × 4.6
Agilent	Zorbax Eclipse	XDB-C8	5	150 × 4.6

running sertraline, uracil, impurities A, B and C. Uracil was used to determine void volume of the columns.

Different experimental parameters, including peak asymmetry (As), retention factor (k), sertraline retention time (t_{RSert}) and resolution between impurities A and B (Rs_{A-B}) were evaluated, in order to select the best stationary phase. All the columns tested showed As values from 1.1 to 1.4, with the exception of Zorbax Eclipse XDB-C8 column, that showed an As values over 1.5 and the Ascentis RP-Amide that presented As values for impurities A, B and sertraline over 2.4. Better results on peak asymmetry were achieved with Zorbax Bonus-RP, Zorbax SB-CN, Discovery HS-PEG and Ascentis C18 columns, which presented As values about 1.0 and 1.2, respectively.

Fig. 3 shows the retention factor (k) values for sertraline and its impurities achieved with these stationary phases. Discovery C18 and C8, Ascentis C18, ACE5 C18 and Zorbax Eclipse XDB-C8, presented similar properties, and Ascentis RP-Amide and RP-Amide C16 columns showed very high k values for at least one of the compounds to be separated (over 10). Although these columns presented a good value of Rs_{A-B}, they displayed too high value for t_{RSert} (greater than 50 min), resulting in an analysis time too long for a routine work. Better results on retention factor were obtained with the Zorbax Bonus-RP and the Zorbax SB-CN columns: in fact, the k range for these columns was from 1 to 10.

The value of Rs_{A-B} and t_{RSert} are shown in Fig. 4. Ascentis C18 and ACE5 C18 columns allowed a complete resolution between impurities A and B, but they showed the highest t_{RSert} among all the tested columns. Discovery C18 and Zorbax Eclipse XDB-C showed high t_{RSert} values too. Interestingly, the Ascentis RP-Amide and the Discovery RP-Amide C16 columns, although similar for their stationary phases, showed a very different separation behaviour: in particular, Ascentis RP-Amide provided a high t_{RSert} and a poor separation of all the compounds, with significant peak tailing; on the other hand, Discovery RP-Amide C16 presented a quite high t_{RSert} and gave a good value of Rs_{A-B}. Discovery CN and Zorbax SB-CN showed a low value of t_{RSert} and an insufficient degree of Rs_{A-B}.

Of all the columns tested, Zorbax Bonus-RP and Discovery HS-PEG gave the best results, showing a low t_{RSert} and the best ratio between the t_{RSert} and the Rs_{A-B}. The Discovery HS-PEG column was discarded because it did not provide sufficient Rs_{A-B} (i.e. lower than 1.5). In view of its better chromatographic performance, the Zorbax Bonus-RP column, characterized by a polar amide group embedded in a C_{14} alkyl chain and triple end-capping, was finally selected for the subsequent method optimization.

3.2. Method optimization

Once the Zorbax Bonus-RP column was selected as the best stationary phase for developing a chromatographic method for the separation of sertraline and its impurities, the next step was the optimization of the chromatographic conditions.

In the present study, the optimization of the HPLC method for the determination of sertraline and its impurities on the Zorbax Bonus-RP column was carried out by using a factorial design, evaluating three quantitative factors (column temperature, buffer pH and buffer concentration). The chosen levels for the first assay were 35, 45 and 55 °C for column temperature; 2.0, 2.5 and 3.0 for buffer pH; 10, 17.5 and 25 mM for buffer concentration. The remaining chromatographic parameters were set as follows: phosphate buffer as the buffer type, methanol as the organic modifier and ratio buffer/methanol in the mobile phase equal to 65:35 (v/v).

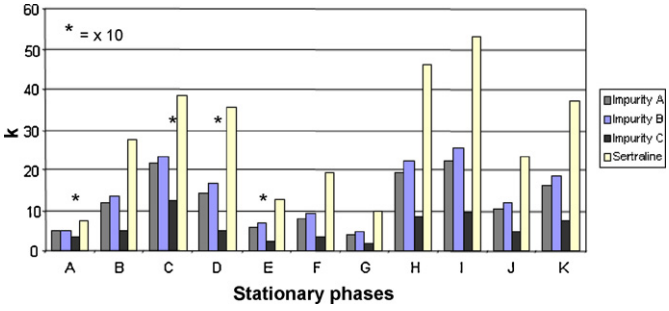

Fig. 3. Comparative behaviour of sertraline and impurities A, B, C on different stationary phases. Column A: Discovery CN, B: Discovery C18, C: Zorbax SB-CN, D: Zorbax Bonus-RP, E: Discovery HS-PEG, F: Ascentis RP-Amide, G: Discovery RP-Amide C16, H: Ascentis C18, I: ACE5 C18, J: Discovery C8, and K: Zorbax Eclipse XDB-C8. Mobile phase: phosphate buffer (pH 3.0; 10 mM)–methanol (60:40, v/v). Column temperature: 35 °C. Flow-rate: 0.8 mL/min. Detection: UV at 220 nm.

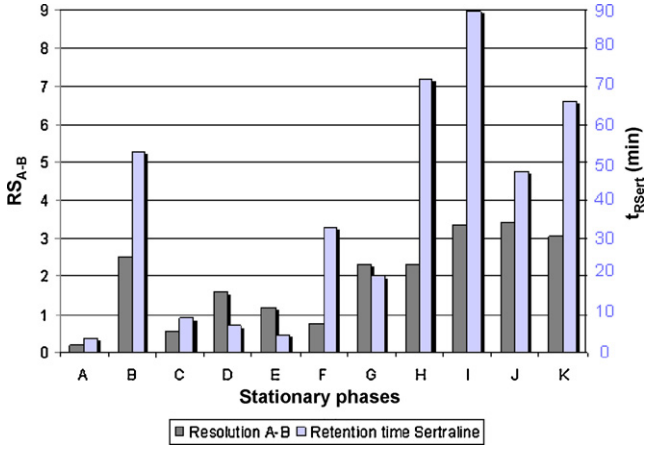

Fig. 4. Resolution between impurities A and B (Rs_{A-B}) and sertraline retention time (t_{RSert}) on different stationary phases. Column A: Discovery CN, B: Discovery C18, C: Zorbax SB-CN, D: Zorbax Bonus-RP, E: Discovery HS-PEG, F: Ascentis RP-Amide, G: Discovery RP-Amide C16, H: Ascentis C18, I: ACE5 C18, J: Discovery C8, and K: Zorbax Eclipse XDB-C8. Mobile phase: phosphate buffer (pH 3.0; 10 mM)–methanol (60:40, v/v). Column temperature: 35 °C. Flow-rate: 0.8 mL/min. Detection: UV at 220 nm.

Table 2
Experimental design no. 1.

Exp. no.	Column temperature (°C)	Buffer pH	Buffer concentration (mM)
1	35	2.0	10.0
2	45	2.0	10.0
3	55	2.0	10.0
4	35	2.5	10.0
5	45	2.5	10.0
6	55	2.5	10.0
7	35	3.0	10.0
8	45	3.0	10.0
9	55	3.0	10.0
10	35	2.0	17.5
11	45	2.0	17.5
12	55	2.0	17.5
13	35	2.5	17.5
14	45	2.5	17.5
15	55	2.5	17.5
16	35	3.0	17.5
17	45	3.0	17.5
18	55	3.0	17.5
19	35	2.0	25.0
20	45	2.0	25.0
21	55	2.0	25.0
22	35	2.5	25.0
23	45	2.5	25.0
24	55	2.5	25.0
25	35	3.0	25.0
26	45	3.0	25.0
27	55	3.0	25.0

Methanol was selected in view of its actual lower price and higher availability in comparison with acetonitrile. The factors, their levels and the complete 3^3 matrix corresponding to the factorial design are presented in Table 2.

The response factor was determined as the ratio of the following two limiting factors: resolution between impurities A and B and retention time of the last eluting compound in the chromatogram (sertraline), indicated as Rs_{A-B}/t_{RSert}. Fig. 5 shows a graphical description (Pareto plot) of the effect of each of the investigated parameter on the response parameter (Rs_{A-B}/t_{RSert}). As shown in Fig. 5, buffer pH, buffer concentration and the interaction between both these variables showed a significant negative effect on the response parameter, meanwhile temperature presented a positive effect. On the other hand, the interactions between pH and temperature and between buffer concentration and temperature did not show a significant effect. The main effect plot, shown in Fig. 6, indicated that the response parameter decreased with pH and buffer concentration without getting the maximum, while the increase of the response parameter with temperature reached the top values at 55 °C. The outcome of the first experimental design allowed to set up the column temperature and the buffer concentration at 55 °C and 10 mM, respectively, hence, with these values the response parameter to be optimized got maximum at this point.

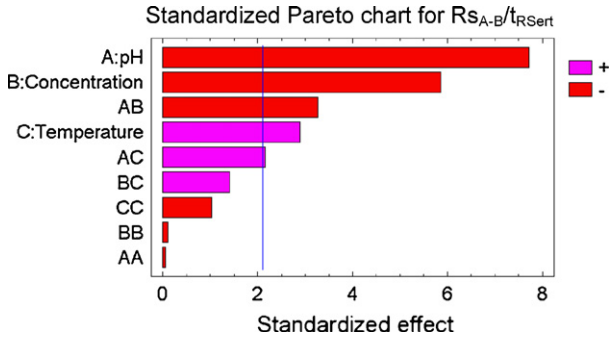

Fig. 5. Standardized Pareto plot for response parameter Rs_{A-B}/t_{RSert}. Variables: (A) buffer pH, (B) buffer concentration, and (C) column temperature.

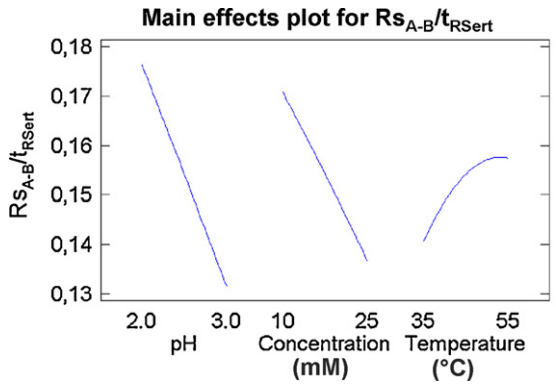

Fig. 6. Main effects plot (buffer pH, buffer concentration, column temperature) for the response parameter Rs_{A-B}/t_{RSert}.

Table 3
Experimental design no. 2.

Exp. no.	Buffer pH	% MeOH (v/v)
1	2.6	40.0
2	2.8	40.0
3	3.0	40.0
4	2.6	37.5
5	2.8	37.5
6	3.0	37.5
7	2.6	35.0
8	2.8	35.0
9	3.0	35.0
10	2.6	32.5
11	2.8	32.5
12	3.0	32.5
13	2.6	30.0
14	2.8	30.0
15	3.0	30.0

The column temperature was finally set at 50 °C, which represents a better choice for the chromatographic column on routine work.

To deeper investigate the effect of buffer pH and methanol content in the mobile phase, a second factorial design was assayed with these two factors of variation, in order to find the best conditions which maximized the resolution between impurities A and B and minimized the total run time. Three levels for the first variable (buffer pH) and five levels for the second variable (% of methanol) were assayed. The experiment organization is summarized in Table 3.

The results for the second experimental design are shown in Fig. 7. The response parameter (Rs_{A-B}/t_{RSert}) increased with increas-

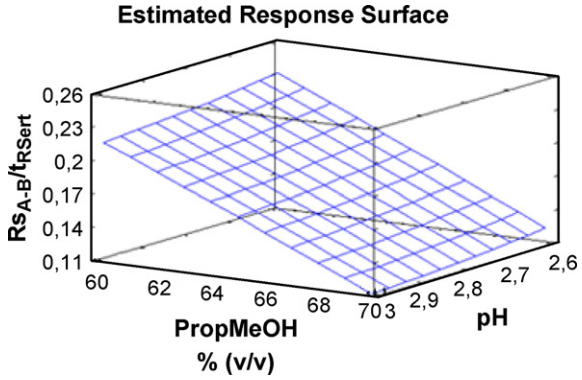

Fig. 7. Estimated response surface for the response parameter Rs_{A-B}/t_{RSert} depending on the proportion of methanol and buffer pH. Column: Zorbax Bonus-RP (150 mm × 4.6 mm, 5 μm). Mobile phase: phosphate buffer (10 mM)–methanol (v/v). Column temperature: 50 °C. Flow-rate: 1 mL/min. Detection: UV at 220 nm.

Table 4
Linearity validation parameters for sertraline, impurities A, B and C.

Variable	Quantification range		Impurity range			
	Specification	Sertraline	Specification	Impurity A	Impurity B	Impurity C
Range (μg/mL)	–	425–700	–	0.24–4.70	0.23–4.70	0.22–4.70
Correlation coefficient (r)	0.999	0.99964	0.99	0.99998	0.99998	0.999994
Linearity test						
Response factor RSD (%)	≤5%	0.63%	≤5%	1.70%	1.81%	1.52%
Slope	–	90341793.57	–	92063.68	96896.77	67618.78
Confidence interval	0 value not included	88879567.92–9180419.22	0 value not included	91798.01–92329.35	96604.67–97188.88	67490.13–67747.44
Experimental "*t*"	"*t*"exp > "*t*"tab	133.48 > 2.16	"*t*"exp > "*t*"tab	748.64 > 2.16	716.64 > 2.16	1135.45 > 2.16
Proportionality test						
Intercept	–	928199.86	–	(−)376.97	215.49	(−)238.98
Confidence interval	0 value included	92117.0–1764282.70	0 value included	(−1014.14)–260.19	(−446.33)–877.32	(−559.36)–81.39
Experimental "*t*"	"*t*"exp < "*t*"tab	2.40 > 2.16	"*t*"exp < "*t*"tab	1.23 < 2.16	0.70 < 2.16	1.61 < 2.16

ing the proportion of methanol in mobile phase and with decreasing pH.

In Fig. 8 the chromatograms of the separation of sertraline and its impurities at different pH values (2.6, 2.8 and 3.0) with phosphate buffer (10 mM)–methanol (62.5:37.5, v/v) at 50 °C are shown. The pH corresponding to 2.8 was finally selected, being a good compromise between the separation of impurities A and B and the analysis time.

Finally, the best chromatographic conditions were identified as follows: column Zorbax Bonus-RP (150 mm × 4.6 mm, 5 μm) kept a 50 °C, with phosphate buffer (pH 2.8; 10 mM)–methanol (63:37, v/v) as the mobile phase at the flow-rate of 1.0 mL/min and UV detection at 220 nm. Under these chromatographic conditions all the impurities and sertraline were separated in less then 10 min. The achieved chromatographic resolution was greater than 1.5. A representative chromatogram of the separation of the target compounds at the optimized working conditions is shown in Fig. 9.

3.3. Method validation

The validation of the optimized method was performed in agreement with ICH guidelines [26], with both standards and sertraline tablets. The selectivity was tested by running solutions containing the excipients of the formulation in the same quantities and conditions as the samples, to show that there was no peak at the retention time corresponding to the analytes of interest.

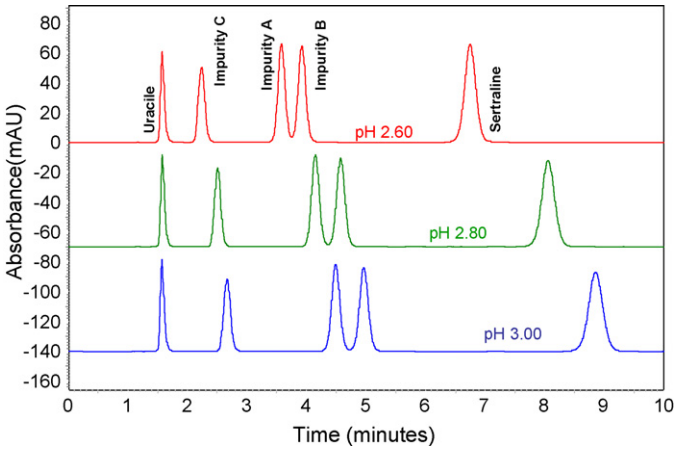

Fig. 8. Chromatogram showing the separation of uracil, impurities A, B, C and setraline at different buffer pH (2.6, 2.8, 3.0). Column: Zorbax Bonus-RP (150 mm × 4.6 mm, 5 μm). Mobile phase: phosphate buffer (pH 2.60, 2.8 and 3.0; 10 mM)–methanol (62.5:37.5, v/v). Column temperature: 50 °C. Flow-rate: 1 mL/min. Detection: UV at 220 nm.

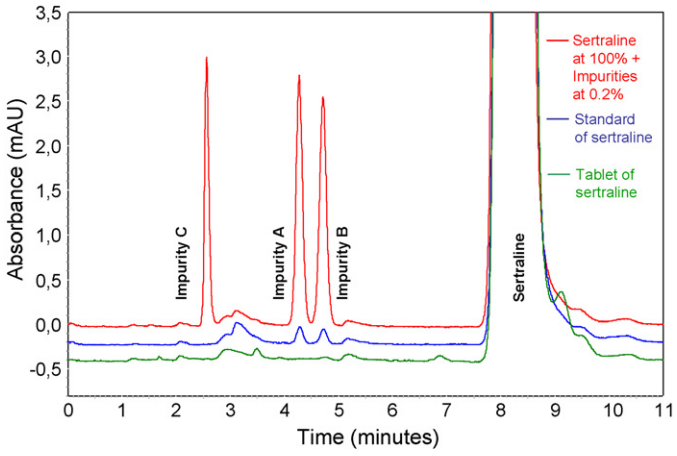

Fig. 9. Chromatogram showing the analysis of: setraline (at 100%) and impurities A, B, C (at 0.2%); sertraline standard (at 100%); sertraline tablet. Column: Zorbax Bonus-RP (150 mm × 4.6 mm, 5 μm). Mobile phase: phosphate buffer (pH 2.8; 10 mM)–methanol (63:37, v/v). Column temperature: 50 °C. Flow-rate: 1 mL/min. Detection: UV at 220 nm.

The validation parameters for linearity are shown in Table 4. The linearity parameters were found to be highly satisfactory for both sertraline and its impurities in the tested ranges, with correlation coefficients over 0.999.

The ranges established to consider acceptable accuracy were between 98 and 102% for sertraline in the quantification range and between 95 and 105% for impurities A, B and C in the impurity range. As can be seen in Table 5, recoveries do not statistically differ from 100% (*t*-test, $p < 0.05$) in all cases and fulfill the established specifications.

Table 6 shows the required specifications for the concentration assayed assuming a single injection per standard/sample and the results achieved for the instrumental and method precision. The RSD values were low enough to consider the method precise as well as for standards and for sertraline samples in all cases.

The quantification limit of an analytical method (LOQ) is defined as the lowest level of analyte that can be accurately and precisely

Table 5
Accuracy validation parameters for sertraline, impurities A, B and C.

	Sertraline	Impurity A	Impurity B	Impurity C
Specification	98–102%	95–105%	95–105%	95–105%
Standard recovery (%)	100.00	99.83	99.63	99.89
Standard RSD (%)	0.49	1.42	1.73	1.30
Sample recovery (%)	99.50	101.94	101.06	101.34
Sample RSD (%)	1.10	2.50	2.40	2.61

Table 6
Precision validation parameters for sertraline, impurities A, B and C.

Test	Variable	Specification	Sertraline	Impurity A	Impurity B	Impurity C
Instrumental precision						
Intra-assay	RSD	<1.37%	0.23%	0.66%	0.63%	0.26%
Intermediate	RSD	<2.74%	0.66%	1.05%	0.96%	0.39%
Standard method precision						
Intra-assay	RSD	<1.94%	0.78%	0.66%	0.88%	0.64%
Intermediate	RSD	<3.88%	1.02%	1.32%	1.61%	0.68%
Sample method precision						
Intra-assay	RSD	<1.94%	0.52%	0.91%	1.21%	0.55%
Intermediate	RSD	<3.88%	0.88%	0.96%	1.3%	0.66%

Table 7
Limits of detection and quantification for impurities A, B and C.

Assay	Compound	Concentration ((g/mL)	Concentration (%)
LOD	Impurity A	0.0098	0.0020
	Impurity B	0.0152	0.0030
	Impurity C	0.0153	0.0031
LOQ	Impurity A	0.0326	0.0065
	Impurity B	0.0508	0.0102
	Impurity C	0.0510	0.0102

Table 8
Response factors for impurities relative to sertraline.

Analyte	Response factor
Impurity A	0.87
Impurity B	0.89
Impurity C	0.64

measured. The LOQ for impurities A, B and C was determined by applying the EURACHEM method, where LOQ is calculated when RSD value is equal to 10%. The values achieved for impurities A, B and C are shown in Table 7.

The detection limit of an analytical method (LOD) is defined as the lowest analyte concentration that produces a response detectable above the noise level of the system. The LOD was calculated by means of the equation LOD = (3/10) × LOQ and checked experimentally. The LOQ and LOD values obtained through the EURACHEM method are summarized in Table 7.

Nevertheless, the real limit of quantification of the method is considered to be the lowest concentration value where it was validated and, therefore, it is 0.05% of the theoretical concentration of active substance in real samples for all the compounds. Limits of detection were lower than 0.004%, which are below the necessary concentration required for the method.

The objective of validating the method at impurity level is to use a dilution of the standard compound to quantify all the impurities by using the corresponding response factors, because the impurities are usually not commonly available. Response factors for impurities A, B and C, relative to sertraline, were calculated by running three mixture containing all the impurities plus sertraline at 0.2%, three times. Response factors for the different impurities related to sertraline are shown in Table 8.

4. Conclusion

In this study, the Zorbax Bonus-RP column provided the adequate chromatographic properties for the effective separation of sertraline and three of its non-chiral impurities, those more chemically closely related, under reversed-phase conditions. This column allowed a baseline separation of the target analytes under isocratic elution, avoiding the employment of ion-pairing reagents, in less than 10 min of total run time. Furthermore, the replacement of acetonitrile with methanol implies a reduction in the analysis cost, when applying this method for quality control analysis.

The method was validated and it demonstrated to be reliable for the determination of sertraline and its impurities, being linear, accurate and precise both in the upper and lower concentration range. Therefore, this method can be considered suitable for the quantification of sertraline and its impurities in the quality control of pharmaceutical products.

Acknowledgments

This work was carried out thanks to a research grant awarded to Dr. Alessia Ferrarini for her master thesis in the lab of Prof. Coral Barbas from the University of Modena and Reggio Emilia (Italy), in the ambit of the Project LLP/Erasmus (Faculty of Pharmacy, University of Modena and Reggio Emilia, area code 12.5 Pharmacy, Coordinator Prof. Federica Pellati).

The authors are also grateful to Cinfa Laboratories for providing the test materials and to Agilent Technologies for providing the HPLC column.

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