Analytical Methods

Cite this: *Anal. Methods*, 2011, **3**, 985

www.rsc.org/methods

PAPER

Development and validation of an analytical method by RP-HPLC for quantification of sibutramine hydrochloride in pharmaceutical capsules

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Received 6th October 2010, Accepted 18th February 2011
DOI: 10.1039/c0ay00598c

Sibutramine is a monoamine re-uptake inhibitor used for the treatment of obesity. In the present study a rapid, sensitive and economical HPLC method was developed and fully validated for analysis of sibutramine HCl in pharmaceutical capsules. HPLC analyses were carried out by using an *isocratic* elution mode with a mobile phase constituted by sodium phosphate buffer (pH 2.5) and methanol (30 : 70, v/v), flow rate at 1.0 mL min^{-1}, column temperature at 40 °C, UV detection wavelength at 225 nm and 20 μL of injection volume. The validation parameters were in accordance with FDA and ICH specifications, showing accuracy, precision, selectivity, robustness and linearity from 4.5 to 19.5 mg L^{-1} of sibutramine HCl. The limits of detection and quantification were 0.666 and 2.018 mg L^{-1}, respectively. The validated method is suitable for quality control applications and its advantages over the already existing methods are simplicity and reduced analysis time.

1. Introduction

Sibutramine belongs to the first class of compounds used for the treatment of obesity.[1] It was initially developed as an antidepressant medication and subsequent studies showed a significant effect of the drug on weight loss due to its satietogenic and calorigenic effects.[2] Sibutramine *is* a centrally acting drug and its *mechanism* of *action is a* selective serotonin and noradrenaline reuptake inhibition.[3] It is usually available as sibutramine hydrochloride and the drug is a racemic mixture of the (+) and (−) enantiomers of cyclobutanemethanamine, 1-(4-chlorophenyl)-*N*,*N*-dimethyl-A-(2-methylpropyl), hydrochloride.[4] Sibutramine HCl is a white to cream crystalline powder, with an empirical formula of $C_{17}H_{26}ClN$, a pK_a value of 9.6, a molecular weight of 279.86 and a solubility of 2.9 mg mL^{-1} in water at pH 5.2. Its octanol : water partition coefficient is 30.9 at pH 5.0.[5]

On Thursday, 21st January 2010, the Agency's Committee for Medicinal Products for Human Use (CHMP) recommended the suspension of the marketing authorization for medicines containing sibutramine throughout Europe because its benefits as a weight-loss aid did not outweigh its cardiovascular risks.[6] However, sibutramine is still extensively commercialized in different countries and many methods have been reported for the analysis of this drug.

Sibutramine and its metabolites in human plasma and biological samples have been determined by using high performance liquid chromatography (HPLC) with UV spectrophotometric detection,[7] liquid chromatography-mass spectrometry (LC-MS),[8–16] gas chromatography-mass spectrometry (GC-MS)[17,18] and ultra-high-pressure liquid chromatography coupled to quadrupole-time-of-flight (UHPLC-QTOF) mass spectrometry.[19] For applications in pharmaceutical products, there are methods that make use of spectrophotometry[20–22] and HPLC.[21,23–27]

Singh and coworkers[21] proposed an enantiomeric separation and a quantitative HPLC method for analysis of sibutramine HCl. The enantiomeric separation was obtained on an α-1-acid glycoprotein (chiral-AGP) column in less than 5 min. The quantitative HPLC method used a C18 (Synergi Hydro-RP) column as stationary phase and the separation was achieved in about 4 min. Radhakrishna and coworkers[23] developed two analytical HPLC methods for the determination of purity and assay of sibutramine HCl using as stationary phases a Hypersil C18 BDS (method A) and a Partisphere C18 (method B). These methods have running times of about 10 min. A chiral chromatography method was also described and the separation was obtained on a cellulose-based L40 column (Chiralcel OD). However, the elution time was longer (approximately 15 min). The method described by Segall and coworkers[24] for the determination of sibutramine HCl in the presence of its oxidatively induced degradation products employed a reversed-phase C18 column (Microsorb-MV) at 7 min running time. Chandorkar and coworkers[25] achieved a separation of sibutramine HCl and its impurity in bulk as well as formulation using a stainless steel Lichrosphere C-18 in about 5 min. Diefenbach and coworkers[26] described a method for the determination of sibutramine HCl in capsules that uses a Luna Phenomenex (Torrance, CA) RP-18 column as stationary phase. The authors achieved the separation in about 5 min. Martins, Froehlich and Bergold[27] developed

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a method for the determination of sibutramine hydrochloride in the presence of its degradation products in bulk and capsules using a Macherey-Nagel Nucleosil C8 column with a running time of about 8 min.

Considering that most of these methods are time consuming and complex and some are very expensive, in the present work we have developed and validated a rapid, sensitive and economical new procedure for analysis of sibutramine HCl in pharmaceutical capsules by RP-HPLC, as an alternative to existing methods.

2. Experimental

2.1. Chemical and reagents

All reagents were of analytical grade. Sodium dihydrogenphosphate (NaH_2PO_4) was purchased from Merck® (Darmstadt, Germany) and orthophosphoric acid was from QM® (São Paulo, Brazil). Methanol was of HPLC grade and acquired from J. T. Baker® (Phillipsburg, USA). The sibutramine HCl reference standard (assigned purity 94.5%) was supplied by Dr Reddy's® (Hyderabad, India). Pharmaceutical capsules claimed to contain 15 mg of sibutramine HCl were manufactured in our laboratory. HPLC grade water was prepared by Milli-Q reverse osmosis (Millipore®, Bedford, USA) and meets USA Pharmacopoeia requirements. Cellulose ester membranes with a pore size of 0.45 μm were used to filter the mobile phase (Millipore®, Bedford, USA) and Millex® syringe filters PVDF membrane (13 mm, 0.45 μm pore size) from Millipore® (Bedford, USA) were used to filter the samples.

2.2. Equipments and chromatographic conditions

The following equipments were used: Metrohm® digital pH meter model 744 (Herisau, Switzerland); Branson® ultrasonic bath model 8510 (Danbury, USA); Mettler Toledo® analytical balance model AG 285 (Greifensee, Switzerland); Agilent® UV–visible spectrophotometer model 8453 (Wilmington, USA) equipped with a quartz cuvette; Waters Alliance® 2690 liquid chromatograph (Milford, USA) equipped with a Waters® high pressure pump model 600, Waters® 2487 UV–vis detector and a 717 autosampler. The chromatograms were analyzed using EmpowerT 2.0 software (Empower Pro, Waters Corporation®, Milford, USA) and the separation was performed on a Waters Symmetry® C-18 column (4.6 mm × 250 mm, 5.0 μm, Waters®, Milford, USA). Data were analyzed by using the Microcal® Origin 6.0 software (Northampton, USA).

2.3. HPLC analytical procedure

HPLC analyses were carried out using an *isocratic* elution mode with a mobile phase constituted by sodium phosphate buffer (pH 2.5) and methanol (30 : 70, v/v), a flow rate of 1.0 mL min^{-1}, a column temperature of 40 °C, a UV detection wavelength at 225 nm and 20 μL of injection volume. All solutions were filtered through a 0.45 μm Millex-LCR filter before injection into the column and the mobile phase was filtered under vacuum through a 0.45 μm membrane and degassed ultrasonically for 30 min prior to use. Peak areas were taken as the analytical signal.

2.4. Preparation of solutions

2.4.1 Stock and reference standard solutions of sibutramine HCl. Stock standard solution of 150 mg L^{-1} sibutramine HCl was prepared by accurately weighing 15 mg of sibutramine HCl, then transferring it into a 100 mL calibrated flask and adding 80 mL of methanol. The flask was sonicated for 1 min and filled to volume with methanol. The reference standard solution was prepared immediately before use by diluting 1 mL of stock standard solution to 10 mL methanol to obtain a solution having a known concentration of 15 mg L^{-1} sibutramine HCl.

2.4.2 Sample capsules solution. For sample analyses, 20 capsules were weighed and the content of the capsules was removed. The emptied shells were weighed, and the average weight of the contents was obtained by subtracting the weight of the shell from the weight of the 20 full capsules, as recommended by the European Pharmacopeia.[28] The weight equivalent to the average dosage of one unit was transferred into a 100 mL volumetric flask, 80 mL of methanol was added and the flask was sonicated for 30 min. The flask was filled to volume with methanol and 1 mL was transferred into a 10 mL calibrated flask and diluted to the mark with methanol in order to obtain a solution having a concentration of 15 mg L^{-1} sibutramine HCl.

2.4.3 Excipient solutions. Excipient solutions were prepared by transferring a homogeneous mixture of the placebo mixture with the same quantitative composition as the pharmaceutical formulations into a 100 mL volumetric flask and by adding 80 mL of methanol. The solutions were then treated *as section* sample capsules solution.

2.5 Method development

The chromatographic method was developed to yield an adequate analytical performance in a short running time. The optimal conditions were determined by investigating the effects of mobile phase pH, mobile phase composition, column type, and temperature on the separation.

2.6 Method validation

Method validation was performed following ICH and FDA specifications[29,30] for system suitability, stability, selectivity, linearity, precision, accuracy, detection limit, quantitation limit and robustness.

2.6.1 System suitability. System suitability testing was carried out by injecting six times a reference standard solution of sibutramine HCl at 15 mg L^{-1} (100% level of the test concentration). According to the USA Pharmacopoeia,[31] the *K* prime must be higher than 0.5, USP Tailing factor less than 2.5 and theoretical plate number higher than 500. These parameters were calculated by using Empower® 2.0 software.

2.6.2 Solution stability. The stability of the sibutramine HCl standard solution at a concentration of 15 mg L^{-1} in methanol was investigated 7 hours after the solution was prepared. The percentage of relative standard deviation (RSD) of the Sibutramine HCl peak areas at the moment of solution preparation

(injected in triplicate) and 7 hours after solution preparation (injected in triplicate) was calculated.

2.6.3 Selectivity. The selectivity of the method was evaluated by comparing the results obtained from a reference standard solution of sibutramine HCl at 15 mg L^{-1} added to the placebo mixture with the results obtained from a reference standard solution of sibutramine HCl at 15 mg L^{-1} without addition of the placebo mixture. Each solution was injected into the HPLC system in triplicate and the method is considered selective if an RSD $\leq$ 2.0% between peak areas is obtained.

2.6.4 Linearity. The linearity was evaluated by the analysis of reference standard solutions of sibutramine HCl prepared in triplicate at concentrations of 4.5, 7.5, 10.5, 15.0 and 19.5 mg L^{-1} (ranging from 30 to 130% level of the test concentration). One curve was generated by plotting the average of the peak area at each level concentration ($n = 3$) *versus* drug concentration. From the obtained results, the calibration equation ($y = ax + b$) as well as the relative standard deviations for each point ($n = 3$) were calculated.

2.6.5 Precision. Precision of the assay was determined by system precision, repeatability (intra-day) and intermediate precision (inter-day). The system precision was verified by using a reference standard solution of sibutramine HCl at 15.0 mg L^{-1}, which was analyzed on the same day for six times. The RSD of peak areas ($n = 6$) was evaluated.

The repeatability was demonstrated by the assay of six samples, at the same concentration (15.0 mg L^{-1}), on the same day. The RSD of peak areas ($n = 6$) was evaluated. The intermediate precision of the method was determined by assay of six samples, at the same concentration (15.0 mg L^{-1}), prepared and analyzed on each of two successive days. The RSD of peak areas ($n = 12$) was evaluated.

2.6.6 Accuracy. The accuracy of the method was evaluated by addition of three different amounts of sibutramine HCl to placebo mixtures. The amounts added were equivalent to 70, 100 and 130% of the method concentration (15 mg L^{-1}). At each level, samples were prepared in triplicate and the recovery percentage was determined.

2.6.7 Detection and quantitation limit. The detection limit (DL) and quantitation limit (QL) of the methods were obtained from eqn (1) and (2):

$$DL = 3(SD/a) \quad (1)$$

$$QL = 10(SD/a) \quad (2)$$

where SD is the standard deviation of the *y*-intercepts and *a* is the slope of the calibration curves obtained in the linearity study.

2.6.8 Robustness. The robustness of the method was evaluated by analyzing data after varying the ±2% mobile phase flow rate (0.98 mL min^{-1} and 1.02 mL min^{-1}), ±2% column temperature (39.2 °C and 40.8 °C), ±2% mobile phase pH (2.4 and 2.6), mobile phase proportion (sodium phosphate buffer at pH 2.5-methanol (29.4 : 70.6, v/v) and sodium phosphate buffer at pH 2.5-methanol (30.6 : 69.4, v/v)) and by using a different lot of chromatographic columns. The sibutramine HCl reference standard at a concentration of 15 mg L^{-1} (100% level) was used in these experiments. Each assay was performed in triplicate. Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

2.7 Analysis of the commercial products

The validated method was applied for determination of sibutramine HCl in the capsule dosage form. The sample was treated as in the section sample capsules solution. The result was obtained by comparison of the sample peak areas ($n = 3$) with those obtained from Sibutramine HCl standard solutions ($n = 6$) at the same concentration level and was expressed as percentage drug related to label claim.

3 Results and discussion

3.4 Method development

Because sibutramine HCl exhibits basic characteristics and, consequently, an acid pH could improve the ionization efficiency of this drug, a mobile phase *with a* pH close to 2.5 was selected, which provided an adequate separation of the chromatographic peaks. Regarding mobile phase composition, several concentrations of sodium phosphate buffer (pH 2.5) and methanol were evaluated, and a good separation in a short running time (approximately 3.7 min) was obtained by using an elution system of sodium phosphate buffer (pH 2.5)—methanol (30 : 70 (v/v)) as the mobile phase. Regarding the stationary phase, it is reported in the literature that tertiary amines with $pK_a > 9.0$ are much more affected by free silanol groups than primary or secondary amine compounds with $pK_a < 9.0$.[32] For this reason, a C18 endcapping column was used in order to produce a decrease of the number of surface silanols. The column temperature was set at 40 °C because it was experimentally observed that the resolution between the chromatographic peaks increases with increase of the column temperature. Column temperature values above 40 °C were not tested because the use of higher temperatures can decrease the lifetime of the column. Sibutramine HCl was found to exhibit one UV absorption maxima at 225 nm (results not shown). Then, this wavelength was used for the HPLC quantitation throughout the study.

3.5 Method validation

3.5.1 System suitability testing. A representative chromatogram of system suitability testing is shown in Fig. 1. The following results were found: *K* prime of 0.78, USP tailing of 1.17 and USP plate count of 1975. Thus, all parameters are in agreement with the USA Pharmacopeia recommendations.[31]

3.5.2 Solution stability. Sibutramine HCl standard solution at concentration of 15 mg L^{-1} in methanol remained stable at

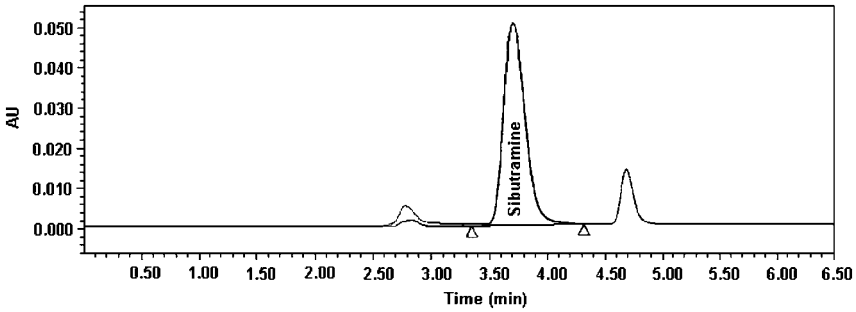

Fig. 1 Chromatogram of sibutramine HCl reference substance. Conditions: sodium phosphate buffer (pH 2.5) and methanol (30 : 70, v/v) as mobile phase, flow rate at 1.0 mL min^{-1}, column temperature of 40 °C, Waters Symmetry® C18 analytical column, UV detection wavelength at 225 nm and 20 μL of injection volume.

room temperature for 7 h after the solution preparation. The overall RSD of the sibutramine HCl peak areas was 0.28% ($n = 6$).

3.5.3 Selectivity. The results of the method selectivity are shown in Table 1. The overall RSD did not exceed 2.0%, demonstrating suitable selectivity of the method.

3.5.4 Linearity. The results of linearity are shown in Table 2. The calibration equation obtained from the HPLC method was $y = 42\,917x - 44\,282$. The least squares regression showed an excellent correlation coefficient of 0.9969. The relative standard deviation of each point ($n = 5$) was smaller than 2%.

3.5.5 Precision. The observed RSD values for the precision study were <2%, confirming that the method is sufficiently precise (Table 3).

3.5.6 Accuracy. The values of the method accuracy are summarized in Table 4. The mean percentage recoveries at 70, 100 and 130% of the method concentrations were 100.5, 102.6 and 100.8, respectively, indicating the suitability of the developed method in quantifying the concentration of sibutramine HCl in pharmaceutical capsules.

3.5.7 Detection limit and quantitation limit. The detection limit and quantitation limit of the HPLC method were found to be 0.666 and 2.018 mg L^{-1}, respectively. The precision experiments at the QL level yielded a RSD of 0.15% ($n = 6$). These results have demonstrated that the analyses were being performed in a region above the quantitation limit value.

3.5.8 Robustness. %RSD between the analyte peaks at each variable condition confirmed the robustness of the HPLC assay, since the obtained values were within the acceptance limits (%RSD of the analyte peak less than 5.0%) in all cases.

3.6 Analysis of the commercial products

The validated method was applied for determination of sibutramine HCl in the capsule dosage form. A representative chromatogram is shown in Fig. 2. It was obtained a mean result of 100.54%, showing a good conformity with the label claim.

Table 1 Results for sibutramine HCl capsules method selectivity

	Sibutramine HCl added of the placebo mixture	Sibutramine HCl without addition of the placebo mixture
Areas	592 209	609 920
	593 999	611 005
Mean	599 335	613 327
	595 181	611 417.3
RSD (%)	1.54 ($n = 6$)	

Table 2 Results for method linearity

Concentration/mg L^{-1}	Area (mean)	RSD (%)
4.5	131 973	0.06
7.5	258 700	0.08
10.5	420 353	0.03
15.0	611 417	0.28
19.5	782 415	0.34

Table 3 Results for sibutramine HCl capsules method precision

Level	Concentration level/mg L^{-1}	RSD (%)
System precision	15	0.45 ($n = 6$)
Repeatability	15	1.67 ($n = 6$)
Intermediated precision	15	1.93 ($n = 12$)

Table 4 Results for sibutramine HCl capsules method accuracy

	Concentration/mg L^{-1}		
Level	Theoretical	Found	Mean recovery (%)
70%	10.53	10.67	100.5
	10.53	10.60	
	10.53	10.47	
100%	15.04	15.51	102.6
	15.04	15.47	
	15.04	15.31	
130%	19.55	19.93	100.8
	19.55	19.57	
	19.55	19.64	

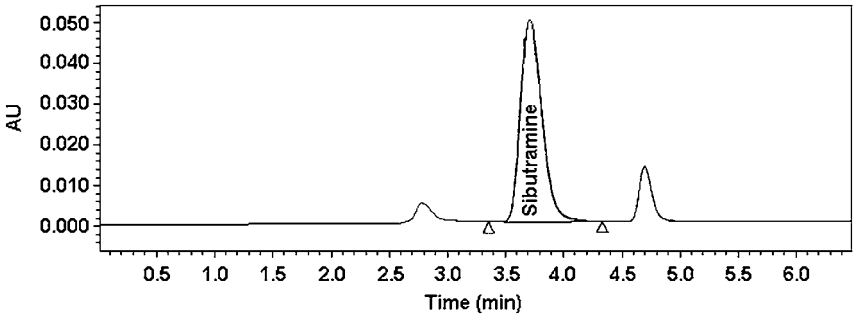

Fig. 2 Chromatogram of sibutramine sample solution (capsules). Conditions: sodium phosphate buffer (pH 2.5) and methanol (30 : 70, v/v) as mobile phase, a flow rate of 1.0 mL min^{-1}, a column temperature of 40 °C, Waters Symmetry® C18 analytical column, a UV detection wavelength at 225 nm and 20 μL of injection volume.

4. Conclusion

In this study, an analytical method by RP-HPLC for quantification of sibutramine HCl in pharmaceutical capsules was developed and fully validated. All parameters meet the acceptance criteria for method validation according to the FDA and ICH specifications and the method shows accuracy, precision, selectivity, robustness and linearity. The validated method is rapid, sensitive and economical and may be used to quantify sibutramine HCl in capsules in a short analysis time (about 3.7 min), which shows an advantage of the proposed method over those described in the literature.

Acknowledgements

The authors are grateful to FAPESP (São Paulo, Brazil), CNPq (Brasília, Brazil) and PADC/FCF/UNESP (Araraquara, Brazil) for research fellowships.

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