UV SPECTROPHOTOMETRIC, DERIVATIVE SPECTROPHOTOMETRIC AND RP-HPLC-DAD DETERMINATION OF SIBUTRAMINE

Gokce Kilicarslan¹, Esin Imamoglu¹, Aysel Kucuk^{1,*}, Abdil Ozdemir¹

¹*Department of Chemistry, Faculty of Arts and Sciences,
Sakarya University, Esentepe Campus, 54187, Sakarya, Turkey*

ABSTRACT

Simple and rapid new methods were developed and validated as an alternative to the quantification methods of sibutramine presented in the literature. UV-Vis. and derivative spectrophotometry methods were based on the determination of sibutramine pharmaceutical preparations (capsule dosage forms) in different solvent media (methanol and water). All UV-Vis. absorbance spectra of sibutramine were recorded with maximum absorbance of 224 nm and with spectral bandwidth of 2 nm and all first- and second-order derivative spectra of sibutramine were recorded at 217 and 223 nm with spectral bandwidth of 5 nm in wavelength range of 200-400 nm in methanol and water media. The other method was a new RP-HPLC with DAD detector method developed for the determination of sibutramine by using Donepezil as the internal standard in human plasma samples (in-vitro) and in pharmaceutical preparations (capsule dosage forms). HPLC was performed using mobile phase comprising of methanol-acetonitrile (90:10) on a reversed phase C₁₈ column and the detection was carried out at 224 nm. The recovery of capsule by standard addition method was found to be in the range of 101.10-104.08 % and 91.96-107.68%, respectively. The developed methods were applied directly and easily to the analysis both of pharmaceutical preparations and biological fluids without extraction or evaporation steps prior to drug assays. Also bioanalytical method validation was achieved

*Corresponding Author:
e-mail: ayselk@sakarya.edu.tr

successfully for comparison as statistical of developed methods both in pharmaceutical preparations and plasma levels in vivo outside, and validation results on linearity, specificity, accuracy and precision were effectively performed.

Keywords: Sibutramine, UV-Vis. Spectrophotometry, Derivative Spectrophotometry, High Performance Liquid Chromatography

1. INTRODUCTION

Sibutramine hydrochloride is an orally administrated agent for the treatment of obesity. Its expected activity is weight loss through inhibition of serotonin and norepinephrine reuptake /1/. It is chemically a racemic mixture of the (+) and (-) enantiomers. The chemical name of sibutramine is N-{1-[1-(4-chlorophenyl)-cyclobutyl]-3-methylbutyl}-N,N-dimethylamine hydrochloride monohydrate (Figure 1) and it has an empirical formula of $C_{17}H_{29}Cl_2NO$ /2/.

Fig. 1: Chemical structure of sibutramine

Based on the literature review, a number of analytical methods such as UV-Vis. /3,4/, GC-MS /5,6/, LC-ESI-MS /7,8/, LC-ESI-TMS /9-11/, HPLC-UV /12-15/, Chiral LC for enantiometric separation /16/, HPLC-UV using column-switching in rat serum /17/ etc. have been developed for determination of sibutramine but no method has been developed so far in the literature for determination of sibutramine hydrochloride by using RP-HPLC method with DAD detection in human plasma and no derivative spectrophotometric method in capsule dosage forms for different solvent

media (methanol and water).

The proposed methods are cheaper and simpler than other methods. It might be an alternative to other HPLC techniques by using simple mobile phase for routine analysis in human plasma and there are no extraction processes to eliminate the excipients, which are time consuming and tedious. Another important advantage of these proposed techniques over the other techniques can be applied directly to the analysis of pharmaceutical dosage forms and biological samples without the need for extensive sample preparation, since there was no interference from the excipients and endogenous substances. Quantitation was accomplished by using an internal standard method. This study was conducted in order to develop a new RP-HPLC method of shorter analysis time and a detailed comparison of HPLC method was carried out by UV and derivative spectrophotometric method in capsules.

2. EXPERIMENTAL

2.1. Instrumentation

For spectrophotometric study, Shimadzu UV-2401 PC Model, UV-Vis Recording Spectrophotometer, in a 10-mm quartz cells were connected to (LG) PC Computer and Hewlett-Packard DeskJet printer and Momentum SVC-1000 regulator.

For chromatographic study, a Shimadzu mark HPLC system with a SPD-M20A Prominence photodiode array detection system consisting of LC-20AD Prominence Liquid Chromatography, SIL-20A HT Prominence auto sampler, DGU-20A5 Prominence degasser, CTO-10AS VP Column Oven, LC Solution Program, were connected to Samsung Computer and FCM power source.

2.2. Materials and Reagents

Certificated Sibutramine standard was obtained from Fargem A.Ş. (Turkey). Donepezil used as internal standard was donated from a Turkish pharmaceutical industrial firm. The commercial capsules (Reductil, 15 mg) containing the active ingredient sibutramine were kindly supplied by Turkish distributor under license from Abbott Lab. The plasma sample was obtained

from a healthy volunteer in Istanbul-Turkey. All other chemicals were analytical grade and HPLC grade (Merck, Germany).

2.3. Spectroscopic Conditions and Optimisation

All of the UV absorbance spectra of sibutramine were obtained with the spectral bandwidth of 2 nm by scanning wavelength range of 200-400 nm in methanol and water media. In these conditions, a single well-defined maximum peak was monitored and no difference was observed in the maximum wavelengths of all spectra for both solvent media ($n=6$). All of the absorbance measurements and absorbance spectrums of samples contained commercial capsules were taken at a wavelength of 224 nm for methanol and water, respectively (Figure 2a-2b and Figure 3a-3b).

Fig. 2a: UV absorbance spectrums of sibutramine in methanol

Fig. 2b: UV absorbance spectrums of sibutramine in water

Fig. 3a and 3b: UV absorbance spectrums of samples contained commercial sibutramine capsules of $45 \mu\text{g mL}^{-1}$ in methanol and water, respectively.

All of the derivative absorbance spectra of sibutramine were obtained with 5 nm instead of the spectral bandwidth of 2 nm scanning wavelength range of 200–400 nm in methanol and water media. The first-order curve was displayed one maximum at 217 nm and one minimum at 230 and (in Figure 4a and 5a), while the second-order curve was showed one maximum at 223 nm and one minimum at 232 nm in both solvent media (in Figure 4b and 5b). The largest ones of which would be more sensitive were preferred by comparing the regression coefficients and the slopes from calibration curves. So, the absorbance values obtained of wavelengths at 217 and 223 nm were taken. The reason of quantification for sibutramine with derivative spectroscopy was to determinate better of the peak shapes and to eliminate possible impurities. Since water is more a polar solvent (forming the H-bond)

than methanol, the detail structures do not appear. The polarity of methanol is lower than water and its dipole-dipole and dielectric effects are not present. Because of this, the refined structures are seen in more detail.

Fig. 4a: First-order derivative spectra of sibutramine in methanol medium (5, 10, 15, 20, 30, 40, 45 ve 50 $\mu\text{g mL}^{-1}$)

Fig. 4b: Second-order derivative spectra of sibutramine in methanol medium (5, 10, 15, 20, 30, 40, 45 ve 50 $\mu\text{g mL}^{-1}$)

Fig. 5a: First-order derivative spectrums of sibutramine in water medium (5, 10, 15, 20, 30, 40, 45 ve 50 µg mL⁻¹)

Fig. 5b: Second-order derivative spectrums of sibutramine in water medium (5, 10, 15, 20, 30, 40, 45 ve 50 µg mL⁻¹)

2.4. Chromatographic Conditions and Optimisation

It was determined by using isocratic pump system for developed HPLC method and scanning the region between 200-400 nm by using DAD detector that sibutramine showed maximum absorbance at 224 nm. Chromatograms were obtained separately at each wavelength of 212, 224, 229, 254, 256 and 271 nm and then, all chromatograms were recorded as optimal wavelength at

224 nm. A 4.6x150 mm I.D. Inertsil ODS-2 reversed-phase C₁₈ column with a particle size of 5 μ m was employed. The temperature of column oven was operated at 25°C. The flow-rate was set at 1 mL min⁻¹. All standard samples were filtered through a membrane of 0.45-mm pore size before injection into the HPLC system. The injection volume was 15 μ L. Retention time (RT) was determined as 4.52 min for sibutramine and as 2.87 min for the internal standard from chromatograms obtained in these conditions (in Figure 6). The mobile phase was also optimized by using different percentage combinations such as methanol-phosphate buffer, acetonitrile-phosphate buffer, methanol-acetonitrile (50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10 etc.). As a result of the different mobile-phase trials, the most appropriate isocratic mobile phase was determined as a mixture of methanol-acetonitrile mixture in the ratio 90:10. Mobile phase was prepared as fresh daily and all other organic solvents were used filtering through a 0.47- μ m nylon membrane filter after vacuum and degassed ultrasonically before use. So, optimum conditions were chosen for the HPLC system.

Fig. 6: Sibutramine chromatogram of 30 μ g mL⁻¹ (with added Donepezil of 15 μ g mL⁻¹)

Using the parameters optimized, system suitability parameters were calculated: The capacity factors as the retention functions of the peaks for sibutramine and donepezil in flow rate of 1 mL dk⁻¹, k_S' : 1.82, and k_D' : 0.24, the selectivity coefficient, α : 3.97, the total theoretical plate number as an indicator of column efficiency, N : 2663, the resolution as the separation power of column, R_S : 3.07 and the plate height, H : $5.63 \cdot 10^{-3}$ cm. the parameters were compared against those recommend by the Center for Drug Evaluation and Research (CDER) [18].

2.5. Preparation of Stock and Standard Solutions

Stock solutions of sibutramine for UV and derivative determinations were prepared as $100\ \mu\text{g mL}^{-1}$ in methanol and deionized water from pure sibutramine standard and kept stored at 4°C until used. The stock solutions were stable at least 4 weeks when stored at 4°C . No change in the stability of the stock solutions over one month was observed. The standard solutions in $5\text{--}50\ \mu\text{g mL}^{-1}$ concentrations (5, 10, 15, 20, 30, 40, 45 and $50\ \mu\text{g mL}^{-1}$) were prepared by diluting from the stock solutions to a constant volume with methanol and water (Figure 2a and 2b). Calibration curves were prepared for eight different concentrations ($n=6$). The quality control samples were also prepared at 15, 30 and $45\ \mu\text{g mL}^{-1}$ concentrations from stock solutions. Methanol and deionized water was used as reference in all measurements of UV and derivative spectrums.

Stock solution of sibutramine for HPLC determination was prepared as $250\ \mu\text{g mL}^{-1}$ in mobile phase and kept stored at 4°C until used. The stock solution was stable about 4 weeks when stored at 4°C . No change in the stability of the stock solution about one month was observed. The standard solutions in concentrations between $5\text{--}30\ \mu\text{g mL}^{-1}$ (5, 10, 15, 20, 25 and $30\ \mu\text{g mL}^{-1}$) for calibration curve method were prepared by diluting from the stock solutions to a constant volume with mobile phase (Figure 6). Calibration curve was prepared for six different concentrations ($n=6$). Internal standard solution of 1 mL ($15\ \mu\text{g mL}^{-1}$) was added on each of these standards prepared from the main stock.

New standard solutions were prepared in the range of $5\text{--}30\ \mu\text{g mL}^{-1}$ concentrations (5, 7.5, 10, 12.5, 15, 17.5, 20, 25 and $30\ \mu\text{g mL}^{-1}$) from same stock solution for recovery works in HPLC. Internal standard solution of 1 mL was added on each of these new nine different standards prepared and they were completed to 25 mL with mobile phase. A new calibration curve was prepared for nine different concentrations ($n=6$). All standard solutions were kept stored in the refrigerator at 4°C until used. Then, they were brought to room temperature before use and mixed with the vortex about 5–10 minutes.

2.6. Pharmaceutical Sample Preparation

Stock sample solutions of $150\ \mu\text{g mL}^{-1}$ for UV and derivative determinations were prepared in two different solvent media (methanol and

water) from the commercial capsule containing the active ingredient of 15 mg sibutramine hydrochloride. Capsules were mixed for 5-10 minutes mixed with a vortex until thoroughly dissolved. Then capsule standard solutions were prepared by diluting in both solvent media by taking parts of 15, 30 and 45 $\mu\text{g mL}^{-1}$ from these stocks (Figure 3a and 3b). These samples were used in the daily analysis.

Stock sample solution of 150 $\mu\text{g mL}^{-1}$ for HPLC determination was prepared in mobile phase solvent media from the commercial capsule containing the active ingredient of 15 mg sibutramine hydrochloride. Capsules were mixed for 5-10 minutes mixed with a vortex until thoroughly dissolved. Then, six different drug standard solutions were prepared by diluting in mobile phase solvent media by taking parts of 15 $\mu\text{g mL}^{-1}$ from this stock. Internal standard solution of 1 mL was added on each of these six capsule standards prepared and they were completed with mobile phase (Figure 7). These samples were used in the daily analysis.

Fig. 7: Commercial sibutramine capsule chromatogram of 15 $\mu\text{g mL}^{-1}$

2.7. Preparation of Standards with Standard Addition Method

In order to detect interactions of excipients (potential interfering compounds), the standard addition method was applied in HPLC. The parts of 2.5, 5, 7.5, 10 and 12.5 $\mu\text{g mL}^{-1}$ from sibutramine stock solution were added into five separate flasks. Then, the parts of 2.5, 5, 7.5, 10 and 12.5 $\mu\text{g mL}^{-1}$ from commercial capsule stock solution were added into the same flasks

and each of the standards was achieved to be total $15 \mu\text{g mL}^{-1}$. Also, internal standard solution of 1 mL was added into the same flasks and then all standards were diluted with methanol.

2.8. Preparation of Plasma Standard Samples

Frozen drug-free human plasma sample was left on the bench to thaw naturally at room temperature and was vortexed prior to its use. Plasma standard solutions for HPLC determination (in vitro) were prepared by spiking into drug-free human plasma with different working standard solutions, which all standards were then further diluted with methanol to give final concentrations of between 5 and $30 \mu\text{g mL}^{-1}$ concentrations of sibutramine (Figure 8). Also, internal standard solution of 1 mL was added into each of human plasma samples. All plasma standards were mixed by vortex for 50-10 min. The parts taken into the centrifuge tubes from these standards were obtained by centrifugation at 5000 rpm for 20-30 min. Thus, all plasma samples were so easily prepared without any extraction procedure for routine analysis.

Fig. 8: Sibutramin chromatogram of $15 \mu\text{g mL}^{-1}$ in plasma

For recovery assays in the plasma, 9 different standards between 5 - $30 \mu\text{g mL}^{-1}$ concentrations from sibutramine stock solution were separately added on 1 mL of human plasma samples. At the same time, internal standard solution of 1 mL was added into each of these plasma standards and was completed to 25 mL with methanol.

2.9. Preparation of the Pharmaceutical Samples in Plasma

Internal standard solution of 1 mL and 1 mL of stock solution prepared from sibutramine containing commercial capsule were added on 1 mL drug-free human plasma sample for HPLC determination. Then, they were diluted with 25 mL of methanol and in this way; total six commercial capsule standards were prepared.

2.10 Preparation of Standards in the Plasma with Standard Addition Method

1 mL plasma sample and 2.5, 5, 7.5, 10 and 12.5 $\mu\text{g mL}^{-1}$ from sibutramine stock solution were added into five separate flasks for HPLC determination. Then, parts of 2.5, 5, 7.5, 10 and 12.5 $\mu\text{g mL}^{-1}$ from commercial capsule stock solution were added into the same flasks and each of the standards reached a total of 15 $\mu\text{g mL}^{-1}$. Also, internal standard solution of 1 mL was added into the same flasks and then all standards were diluted with methanol. All standards prepared were kept stored in the refrigerator at 4°C until used and kept at room temperature before being used. They were ready to be used by mixing for a few minutes by vortex.

3. RESULTS AND DISCUSSION

3.1. Method Validation

The linearity of the methods was determined by analysis of linear regression with standard calibration curves. Linearity of two methods was obtained over a concentration range from 5-50 $\mu\text{g mL}^{-1}$ for UV and derivative and 5-30 $\mu\text{g mL}^{-1}$ for HPLC. The correlation coefficients were obtained and the regression equations were calculated as shown in Table 1 and Table 2 for UV and derivative and in Table 5 for HPLC. The precision of the method, expressed as the relative standard deviation ($\text{RSD}=100 \times \text{SD}/\text{Mean}$) was evaluated. Accuracy was expressed as the mean percentage of analyte recovered in the assay. Both statistical parameters were calculated in each concentration level for the sensitivity of the method. The all RSD values were found as lower than 10 % for all developed methods. LOQ and LOD were calculated using the following equations: $\text{LOQ}=10.S/m$, $\text{LOD}=3.S/m$ (S is the standard deviation; m is the slope of the calibration curve) as shown in Table 1 and Table 2 for UV and derivative and in Table 5 for HPLC /18/.

3.2. UV and Derivative methods

The spectra are more detailed and sharper with derivatization and thus the bands from the sample matrix and showing the disruptive effects are eliminated. Derivative UV spectrophotometry was preferred for the analysis of sibutramine since amplitude of the signal of derivative spectra was greater the peak shape was well defined and the separation of the peak was better in this method. Based on this, the work involved in the quantification of sibutramine by using derivative spectrophotometry is the first derivative method performed in the literature.

From results obtained for all UV and derivative spectrums, the RSD values in water are smaller than RSD values found in methanol as shown in Table 1 and 2. It can be said that the precision values in water are higher than the values in methanol. So, the repeatability and the precision are higher in water medium, and RSD, LOQ and LOD values of the second-order derivative are smaller than those of the first-order derivative for both solvent media as shown in the same Tables. The second-order derivative UV spectrum analysis of sibutramine might be more appropriate when compared with the first-order derivative spectrum of sibutramine in both solvent media according to the results in these Tables. As given in Table 3, the recoveries in water are higher than these of methanol and the recoveries of first-order derivative are higher when compared with these of the second-order derivative in both solvent media. According to the results of *t*-test, there is a difference between both solvent media from all spectra results as shown in Table 4. Comparison of the original and first-, second-order derivative spectra of sibutramine in all standards and drug formulation in both solvent media show that the wavelength of maximum absorbance did not change. The impurities did not interfere with the quantitation of sibutramine in capsule.

3.3. HPLC Method

For HPLC determination, it has been seen that the features of the calibration curves of sibutramine standard in Table 5 ($n=6$). These data indicated that have good linearity in the concentration range of $5\text{--}30\text{ }\mu\text{g mL}^{-1}$ and the developed method have a good repeatability both in mobile phase solvent medium and in plasma. Good recoveries were obtained from sibutramine standards and analysis of the commercial capsules in Table 6 and 7.

Table 1
Features of the calibration curves of sibutramine in methanol for UV and derivative spectrophotometric determination

FEATURES	Zero-order derivative	First-order derivative	Second-order derivative
Regression Equation	$y=0.0324x+0.3358$	$y=0.0012x-0.0045$	$y=0.0006x+0.0059$
RSD* %	0.08-1.45	1.24-2.80	0.90-3.00
Correlation Coefficient (r)	0.9995	0.9997	0.9999
Linear Range ($\mu\text{g mL}^{-1}$)	5-50	5-50	5-50
LOQ ($\mu\text{g mL}^{-1}$)	4.00	3.16	1.82
LOD ($\mu\text{g mL}^{-1}$)	1.20	0.95	0.54

*RSD= Standard deviation / Mean value x 100

Table 2
Features of the calibration curves of sibutramine in water for UV and derivative spectrophotometric determination

FEATURES	Zero-order derivative	First-order derivative	Second-order derivative
Regression Equation	$y=0.0343x+0.1347$	$y=0.0018x-0.0001$	$y=0.0007x+0.0031$
RSD * %	0.04-0.66	0.55-2.29	0.36-1.98
Correlation Coefficient (r)	0.9996	0.9998	0.9998
Linear Range ($\mu\text{g mL}^{-1}$)	5-50	5-50	5-50
LOQ ($\mu\text{g mL}^{-1}$)	3.60	2.69	2.58
LOD ($\mu\text{g mL}^{-1}$)	1.10	0.81	0.77

*RSD= Standart deviation / Mean value x 100

Table 3
The data from UV and derivative spectrophotometric method for commercial capsule standard solutions in methanol and water media.

	Methanol			Water		
	Added Concentration ($\mu\text{g mL}^{-1}$)	Found Concentration ($\mu\text{g mL}^{-1}$)	Accuracy* (Relative error, %)	Recovery** (%)	Found Concentration ($\mu\text{g mL}^{-1}$)	Accuracy* (Relative error, %)
Zero-order derivative (224 nm)	15	14.34	-4.39	95.60	14.33	-4.46
	30	29.66	-1.13	98.86	28.43	-5.22
	45	44.40	-1.32	98.67	44.07	-2.04
First-order derivative (217 nm)	15	13.06	-12.95	87.05	13.93	-7.27
	30	26.17	-12.66	87.24	27.67	-7.79
	45	43.99	-2.24	97.76	40.93	-8.99
Second-order derivative (223 nm)	15	12.95	-13.66	86.34	13.67	-8.70
	30	24.68	-17.73	82.27	25.19	-16.02
	45	38.51	-14.62	85.57	40.01	-10.94

*Accuracy= [(found-added)/added] x 100, **Recovery = Found concentration / Added concentration x 100

Table 4
Statistical values from UV and derivative spectrophotometric method of sibutramine in methanol and water media
($\alpha=0.05$ and 95 % confidence level)

Statistical Values	Zero-order derivative			First-order derivative			Second-order derivative		
	Methanol	Water	t-test	Methanol	Water	t-test	Methanol	Water	t-test
Number of determination (n)	6	6	t_T t_C	6	6	t_T t_C	6	6	t_T t_C
Mean Value ($\bar{X}$)	0.7856	0.6385		0.0122	0.0253		0.0115	0.0129	
Standard Deviation (SD, %)	1.05	0.76		0.03	0.03		0.03	0.02	
Relative Standard Deviation (RSD, %)	1.34	1.19	2.23 3.14	2.35	1.05	2.23 3.16	3.01	1.80	2.23 2.96
Standard Error* (%)	0.43	0.31		0.010	0.010		0.010	0.009	

*Standard error = Standard deviation / $\sqrt{n}$, t_T : tabulated t-value, t_C : calculated t-value

Table 5
Features of the calibration curves of sibutramine standard for HPLC determination (n=6)

FEATURES	In mobile phase solvent medium		In plasma	
	The calibration curve values	Recovery values	The calibration curve values	Recovery values
Regression Equation	$y=0.0455x-0.0120$	$y=0.0440x+0.0405$	$y=0.0405x+0.176$	$y=0.0385x+0.0565$
RSD %	2.02	1.95	1.70	1.29
Correlation Coefficient (r)	0.9985	0.9973	0.9992	0.9978
Linear Range ($\mu\text{g mL}^{-1}$)	5-30	5-30	5-30	5-30
LOQ ($\mu\text{g mL}^{-1}$)	4.45	4.43	4.21	3.29
LOD ($\mu\text{g mL}^{-1}$)	1.33	1.33	1.26	0.99

Table 6
The data of recovery for sibutramine standard for HPLC determination

Added Concentration ($\mu\text{g mL}^{-1}$)	In mobile phase solvent medium				In plasma	
	Found Concentration ($\mu\text{g mL}^{-1}$)	Accuracy (Relative error, %)	Recovery (%)	Found Concentration ($\mu\text{g mL}^{-1}$)	Accuracy (Relative error, %)	Recovery (%)
5	5.65	13.00	113.04	5.74	14.80	114.81
7.5	8.61	14.80	114.84	8.36	11.47	111.53
10	10.66	6.60	106.61	10.56	5.60	105.58
12.5	13.06	4.48	104.46	13.07	4.56	104.57
15	14.85	-1.00	99.03	13.70	-8.67	91.31
17.5	17.59	0.51	100.50	16.26	-7.09	92.94
20	20.04	0.20	100.18	20.01	0.05	100.03
25	24.47	-2.12	97.88	24.02	-3.92	96.08
30	30.74	2.47	102.46	28.13	-6.23	93.75

Table 7
The data from commercial capsule solutions for sibutramine for HPLC determination (n=6)

Sibutramine/Donepezil Rates (for 15 $\mu\text{g mL}^{-1}$)		
In mobile phase solvent medium	In plasma	
0.705874	0.6593	
0.840733	0.6523	
0.731905	0.6676	
0.712523	0.6537	
0.696156	0.6439	
0.682371	0.6531	
Mean Rate	0.7282	
Standard Deviation (SD)	0.058	
Relative Standard Deviation (% RSD)	7.90	
Found Mean Concentration ($\mu\text{g mL}^{-1}$)	15.43	
Recovery (%)	102.87	
	14.32	
	95.48	

Table 8
The data from the standard addition method for sibutramine for HPLC determination (n=6)

Added Sibutramine Standard Concentration ($\mu\text{g mL}^{-1}$)	Added Capsule Concentration ($\mu\text{g mL}^{-1}$)	Found Total Concentration ($\mu\text{g mL}^{-1}$)	Recovery (%)
2.5	12.5	15.38	102.54
5	10	15.17	101.10
7.5	7.5	15.23	101.54
10	5	15.39	102.60
12.5	2.5	15.61	104.08
Mean Recovery (%)			
102.37			
Found Mean Concentration ($\mu\text{g mL}^{-1}$)			
15.36			
Standard Deviation (SD)			
0.049			
Relative Standard Deviation (% RSD)			
6.98			

Table 9
The data from the standard addition method for sibutramine in plasma for HPLC determination (n=6)

Added Sibutramine ^a Standard Concentration ($\mu\text{g mL}^{-1}$)	Added Capsule Concentration ($\mu\text{g mL}^{-1}$)	Found Total Concentration ($\mu\text{g mL}^{-1}$)	Recovery (%)
2.5	12.5	13.79	91.96
5	10	15.04	100.25
7.5	7.5	14.50	96.65
10	5	16.15	107.68
12.5	2.5	14.96	99.76
Mean Recovery (%)			
Found Mean Concentration			
($\mu\text{g mL}^{-1}$)			
Standard Deviation (SD)			
Relative Standard Deviation (% RSD)			
			99.26
			14.89
			0.0382
			5.61

Table 10

Statistical values from in mobile phase solvent medium and in plasma for commercial capsule standard solutions by HPLC method ($\alpha=0.05$ and 95 % confidence level) ($15 \mu\text{g mL}^{-1}$)

Statistical Values	In mobile phase solvent medium	In plasma	<i>t</i> -test	
Number of determination (n)	6	6	t_T	t_C
Mean Value ($\bar{X}$)	0.7283	0.6566	2.23	2.18
Standard Deviation (SD, %)	5.25	0.77		
Relative Standard Deviation (RSD, %)	7.21	1.18		
Standard Error (%)	2.14	0.32		

Table 11

Statistical comparison of UV and HPLC method for determination of sibutramine in commercial capsule standard solutions ($\alpha=0.05$ and 95 % confidence level) ($15 \mu\text{g mL}^{-1}$)

Statistical Values	UV	HPLC	<i>t</i> -test	
Number of determination (n)	6	6	t_T	t_C
Mean Value ($\bar{X}$)	0.7856	0.7283	2.23	1.91
Standard Deviation (SD, %)	1.05	5.25		
Relative Standard Deviation (RSD, %)	1.34	7.20		
Standard Error (%)	0.43	2.14		

The recovery of drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding to analyze commercial sibutramine capsules as shown Table 8 and 9. Also, high analytical recoveries from plasma were obtained such as mean 99.26 % with the standard addition method (in Table 9).

To evaluate by comparing statistically plasma levels of developed method was applied all the validation parameters such as the specificity, sensitivity, precision, accuracy, linearity and repeatability and bioanalytical method validation were made. Also the results were evaluated in terms of biostatistics by using statistical Student's *t*-test at a 95% confidence level. According to the results of *t*-test in Table 10, it's showed that there is no difference between both media when compared with statistical values obtained from in mobile phase solvent medium and in plasma for commercial capsule standard solutions of $15 \mu\text{g mL}^{-1}$ by HPLC method ($\alpha=0.05$ and 95 % confidence level). As shown in Table 11, since t_T value is higher than that of t_C , there is no a significant difference between both methods from all chromatogram results with regard to accuracy and precision. This table summarizes the statistical comparison of the results obtained by applying the proposed UV and HPLC methods. The numerical values of all statistical parameters calculated in Table 11 are in acceptable determination limits in application of two methods to the commercial capsules.

4. CONCLUSION

In this study, new UV and derivative spectrophotometric methods were developed and validated for determination of sibutramine in capsule dosage forms in two different solvent media (methanol and water), but also for comparison in two different solvent media. As well as, a new reverse-phase HPLC method with DAD detector was developed and validated to determine the amounts of sibutramine both in commercial capsule preparations and in human plasma (as in vitro). These methods are more simple, rapid and cheaper than some other methods in literature that might be an alternative to some classical methods of existed time-consuming in terms of easily of usage in practice for a routine laboratory. It was obtained high recovery values in plasma without any extraction process thanks to this new alternative method.

Also, this new alternative reverse-phase HPLC method has some advantages such as a short run time, addition of internal standard and simplicity in plasma studies. Satisfactory results were obtained for drugs and were in good agreement with the label claims. The obtained results were statistically compared with each other as well as those obtained by HPLC method and they showed good agreement.

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