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Validation of an Analytical Method for Determination of Sibutramine Hydrochloride Monohydrate in Capsules by Uv-Vis Spectrophotometry

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SUMMARY. The pharmacopeias have not provided an official method for the quantification of sibutramine hydrochloride monohydrate (SHM). Therefore a sensitive, robust and selective ultraviolet spectrophotometric method was developed and validated for the SHM quantitative determination in capsules. This study was carried out for all validation parameters established by the international guidelines. The measurements were conducted in triplicate and the mean and standard deviations were reported. Validation results on linearity, specificity, accuracy and precision were effectively performed. In the aqueous solution, the linearity was obtained with a correlation coefficient of 0.9997 for the analytical range from 5.0 to 30.0 μg mL^{-1}. Accuracy was 101.4 ± 1.2 %, 99.1 ± 0.9 % and 102.2 ± 1.9 %, respectively for the concentrations of 10.0, 20.0 and 30.0 μg mL^{-1}. The reproducibility presented a relative standard deviation (RSD) of 1.4359 and the repeatability showed RSD of 1.9234. The proposed method is a simple, low cost and easy handling approach for the SHM quantitative determination in capsules.

RESUMEN. "Validación de un Método Analítico para la Determinación de Sibutramina Clorhidrato Monohidrato en Cápsulas por Espectrofotometría UV-VIS". Las farmacopeas todavía no tienen un método oficial para la cuantificación de la sibutramina clorhidrato monohidrato (SHM). Por consiguiente un método espectrofotométrico de ultravioleta sensible, robusto y selectivo fue desarrollado y validado para la cuantificación de SHM en cápsulas. Este estudio se realizó para todos los parámetros de validación establecidos por los criterios internacionales: linealidad, precisión, exactitud y especificidad. Las medidas se hicieron por triplicado y las medias y desviaciones estándares fueron informadas. En la solución acuosa, la linealidad se obtuvo con un coeficiente de correlación de 0,9997 para el rango de concentración de 5,0 a 30,0 μg mL^{-1}. La exactitud mostró los valores de 101,4 ± 1,2 %, 99,1 ± 0,94 % y 102,2 ± 1,9 %, respectivamente para las concentraciones de 10,0, 20,0 y 30,0 μg mL^{-1}. La reproducibilidad presentó un coeficiente de variación (RSD) de 1,4359 y la repetitibilidad reveló un RSD de 1,9234. El método propuesto es simple, de bajo costo y fácil manejo para la cuantificación de SHM en cápsulas.

INTRODUCTION

Sibutramine hydrochloride monohydrate (SHM), chemically identified as a racemic mixture of enantiomers (+) and (-) of {*N*-[1-(4-chloro-phenyl)-cyclobutyl]-3-methyl-butyl}-*N*-*N*-dimethyl-amine (Fig. 1), is an effective serotonin (5-HT) and noradrenaline (NA) re-uptake inhibitor which acts increasing both satiety and metabolism [1-4]. Its satietogenic effect occurs by enhancing central 5-HT and NA functions. The metabolic effects are based on stimulation of thermogenesis due to the activation of the β_3-adrenoceptores in the adipose tissue [2-4]. Therefore, SHM shows a considerable effect on weight loss [5].

SHM is a white and crystalline powder, molecular weight 334.3 g mol^{-1}, melting point 191.0-192.0 °C, soluble in methanol and water (2.9 mg L^{-1} at pH 5.2) [6-7]. It is commercially available as pharmaceutical dosage forms in capsules. The United States Pharmacopeia has not yet incorporated a monograph for SHM [8], but some methods have been described for the substance determination. The high performance

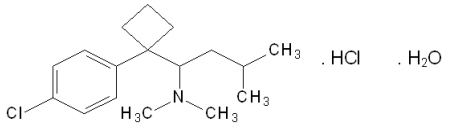

Figure 1. Chemical structure of SHM.

KEY WORDS: Analytical validation, Sibutramine hydrochloride monohydrate, UV-VIS spectrophotometry.
PALABRAS CLAVE: Espectrofotometría UV-VIS, Sibutramina clorhidrato monohidrato, Validación analítica.

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liquid chromatography (HPLC) has been used for the enantiomers separation and for SHM quantification in capsules. The preparation of the pure enantiomer and its determination by single crystal X-ray analysis have been also established. In addition, the quantification of sibutramine and its active metabolites in biological fluids by liquid chromatography-electrospray ionization tandem mass spectrophotometry have been reported [1,9-11].

The goal of this paper is to propose an alternative, low cost, easy handling method to determinate SHM in capsules by UV-VIS spectrophotometry. Also, the complete analytical validation was performed according to the literature [12].

MATERIAL AND METHODS

Sibutramine hydrochloride monohydrate (SHM) (99.9 % purity) was purchased from Pharma Nostra (Rio de Janeiro, Brazil). The SHM capsules (10.0 mg per unit) were obtained from Medley Indústria Farmacêutica (Campinas, Brazil) as a multisource (generic) pharmaceutical product. Other chemicals were analytical grade. Purified Milli-Q® water (Millipore, Bedford, USA) was used throughout the study.

Melting Point

The melting point of SHM was measured with a Büchi SMP 20 instrument (Büchi, Flawil, Switzerland) and the result was compared with literature data [7].

Preparation of standard and sample solutions

For the standard solution of SHM 125.0 µg mL^{-1}, 12.5 mg of SHM was accurately weighed and transferred to a volumetric flask of 100.0 mL. The volume was made up with Milli-Q® water.

The sample solution was obtained from the SHM capsules. Twenty capsules were weighed and the contents was removed, as completely as possible, and mixed. An accurately weighed portion of the combined contents, equivalent to about 10.0 mg of SHM, was dispersed in Milli-Q® water using a 100.0 mL volumetric flask. The dispersion was kept into the ultrasonic bath (Elma, Transonic Digital, Singen, Germany) for 30 min at 25.0 °C. The volume was completed with Milli-Q® water to 100.0 mL and filtered through a quantitative cellulose filter (Framex, 11 cm diameter, black band, 0.00007 ashes, Blumenau, Brazil). After, 5.0 mL of the solution were diluted to 25.0 mL Milli-Q® water resulting in a theoretical concentration of 20.0 µg mL^{-1}.

Spectrophotometric measurements

Sample solutions (n = 3) were analyzed at 223.0 nm in a UV-VIS spectrophotometer (Shimadzu Corp., UV-1601PC, Kyoto, Japan). The absorbance measurements were obtained in a quartz curvette (1 cm optical path length), from 200 to 800 nm of spectral range, with a scan speed of 2400 nm min^{-1} and a 1.0 nm of data interval.

Analytical validation

Analytical validation was performed according to the International and Brazilian guidelines [12,13]. The linearity was established from three analytical curves prepared from three different standard solutions of SHM 125.0 mg L^{-1}. Ten different concentration solutions (5.0 µg mL^{-1}, 7.5 µg mL^{-1}, 10.0 µg mL^{-1}, 12.5 µg mL^{-1}, 15.0 µg mL^{-1}, 17.5 µg mL^{-1}, 20.0 µg mL^{-1}, 22.5 µg mL^{-1}, 25.0 µg mL^{-1} and 30.0 µg mL^{-1}) were exactly obtained and UV absorption measured at 223.0 nm. Absorbance values were plotted and the correlation coefficient was determined for each analytical curve. Also an average analytical curve was carried out to calculate the detection and quantification limits using the equation [**1**] and [**2**], respectively.

$$DL = \frac{3.3x\sigma}{S} \qquad [\mathbf{1}]$$

$$QL = \frac{10x\sigma}{S} \qquad [\mathbf{2}]$$

where DL is the detection limit, QL is the quantification limit, σ is the standard deviation of responses and S is the slope of the calibration curve.

The robustness was analyzed through the effects of pH, temperature and measurement time. For the evaluation of pH variations, eight solutions were prepared by the dilution of the sample solution of SHM until a final concentration of 20.0 µg mL^{-1}. Using HCl-KCl buffer and NaOH-phosphate buffer, resultant solutions were adjusted for pH values 1.3, 2.3, 4.5, 5.5, 6.0, 7.0, 8.0 and 9.0 in a digital potentiometer (Hanna instruments, HI 8519N, Woonsocket, USA) and spectrophotometrically measured from 200.0 to 800.0 nm. The influence of the temperature was investigated in triplicate by the proposed UV method at 4.0, 25.0 and 30.0 °C from a 20.0 µg mL^{-1} aqueous sample solution of SMH. Finally one of the analytical curves described was also evaluated at different time intervals, e.g. 0, 1, 2, 3, 4, 8 and 24 h after the

preparation. The UV measurements at 0 and 24 h were statistically compared by the analysis of variance (ANOVA).

Precision was performed by the repeatability and the intermediate precision parameters. Under the same conditions, *e.g.* day and analyst, nine different sample solutions divided into three groups with 10.0 μg mL^{-1}, 17.5 μg mL^{-1} and 25.0 μg mL^{-1} were assayed by UV method at 223.0 nm. For the intermediate precision, nine sample solutions containing 17.5 μg mL^{-1} were prepared and separated into 3 groups. In three non-consecutive days, each group was spectrophotometrically determined by different analysts, instruments and laboratories. During the intermediate precision, the UV-VIS diode array spectrophotometer (Hewlett Packard, 8452A, Boeblingen, Germany) was also used. The repeatability and intermediate precision results were evaluated from the obtained relative standard deviations (RSD). Concerning the influence of these different equipments, the ANOVA was carried out.

For accuracy, nine sample solutions divided into three groups with 10.0 μg mL^{-1}, 17.5 μg mL^{-1} and 25.0 μg mL^{-1} were prepared. A known quantity of SMH equivalent to 2.0 μg mL^{-1} was added to each solution, resulting in three groups with 12.0 μg mL^{-1}, 19.5 μg mL^{-1} and 27.0 μg mL^{-1}. The absorbance values were obtained by UV measurement at 223 nm and the percentage recovery was calculated.

In order to study the influence of the excipients, standard solution of SHM was dissolved to achieve final concentrations of 10.0 μg mL^{-1}, 20.0 μg mL^{-1} and 30.0 μg mL^{-1}. After that, 0.2 g of an excipient mixture (containing 0.5 % magnesium stearate, 1.0 % colloidal silicon dioxide, 40.0 % cellulose microcrystalline and 58.5 % lactose) was added to each solution, sonicated, filtered and measured as described above. As a complementary analysis, the same amount of the excipient dispersed in Milli-Q® water as a negative assay was used to evaluate the selectivity.

RESULTS AND DISCUSSION

The SHM used as a chemical standard showed a melting point of 191.0 °C. According to Jeffery *et al.* [7] who reported the synthesis of SHM, this satietogenic drug presented a narrow melting range between 191.0 and 192.0 °C. Thus, the purity of the available standard was previously confirmed.

From the measured sample solutions (n = 3), the SHM capsules presented drug content of 10.2 ± 0.1 mg per unit, which corresponds to 101.9 % of labeled value. These results are in accordance to the usual pharmacopeial data [8, 14-16], where a range from 90.0 to 110.0 % of the nominal value is acceptable.

Regarding to validation parameters, the linearity (Fig. 2) results on the y = 0.0368999 . x –0.0008524 as a regression equation for the SHM determination in capsules. The Pearson correlation coefficient *(r)* of 0.9997 was obtained as a mean value for the three plotted analytical curves. Ribani *et al.* [17] proposed that an $r > 0.999$ indicates an ideal data adjustment as observed in the present investigation. The calculated values of 0.3 and 1.0 μg mL^{-1} were verified for the detection and quantification limits, respectively.

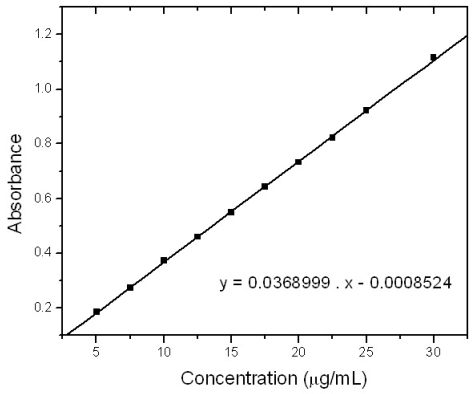

Figure 2. Analytical curve for SHM quantitative determination in capsules.

Concerning to the robustness, when the spectrophotometrical measurements were performed between 200.0 and 800.0 nm, a bathochromic shift was detected for the alkaline pH values of 8.0 and 9.0 structurally related to the effect of the substitution series. In acid and neutral conditions no change was seen in the SHM spectra with a maximum wavelength (λ = 223.0 nm). For the analytical conditions, the temperatures (4.0, 25.0 and 30.0 °C) showed no influence in the absorbance and wavelength data. The results obtained at different time intervals were remarkably similar. One-way ANOVA revealed no significant differences in the UV measurements performed at 0 and 24 h [F(8.28) = 0.000362, P = 0.9850]. Therefore acid and neutral aqueous media, temperatures from 4.0 to 30.0 °C and the measurement performed over the entire 24 h period are suitable conditions where the analytical method can be safely used.

The results of repeatability and intermediate precision for the Shimadzu UV-1601PC spectrophotometer as the RSD from the triplicate are summarized in the Table 1. For both parame-

concentration (μg mL^{-1})	RSD day 1 (%)	RSD day 2 (%)	RSD day 3 (%)	Mean (%)
10.0	2.7			
17.5	1.4	1.4	1.9	1.6
25.0	1.2			
mean (%)	1.8			

Table 1. Repeatability and intermediate precision obtained for validated UV spectrophotometric method at 223.0 nm. * Shimadzu UV-1601PC spectrophotometer.

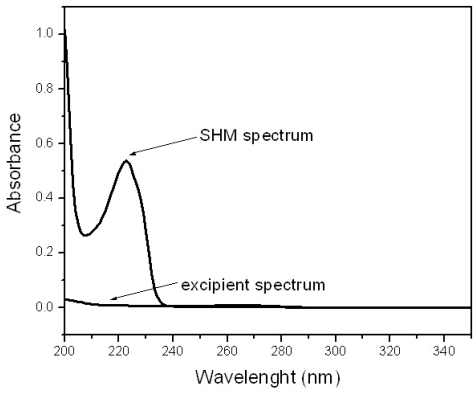

Figure 3. UV spectra of SHM and excipients.

ters, the values obtained for the RSD were less than 5.0 % in accordance to the literature [13]. Thus an adequate precision with a high degree of agreement [8,12] was obtained for the current analytical assay. Furthermore the one-way ANOVA showed no significant differences in the UV measurements comparing Shimadzu UV-1601PC and Hewlett Packard 8452A equipments [F(8.86) = 0.00018, P = 0.9895].

Accuracy was 101.4 ± 1.2 %, 99.1 ± 0.9 % and 102.2 ± 1.9 %, respectively, for the concentrations of 10.0, 20.0 and 30.0 μg mL^{-1}. The closeness showed by accuracy results showed that the proposed method represents an appropriate strategy according to the pharmacopeial guidelines [8] for SHM determination in capsules. However the described method can not be used in stability studies due to the influence of degradation products on the particular SHM absorption region.

Regarding to selectivity, no influence of the excipients was established and a suitable ability to measure SHM accurately in the presence of these interferences was demonstrated. In addition the negative assay (Figure 3) showed no absorption at 223.0 nm. Hence the analytical method was perfectly selective for the SHM.

CONCLUSION

The reported UV-VIS spectrophotometric method was successfully validated as a suitable approach for SHM determination in capsules. Its advantages include simplicity, fastness, low cost and non-polluting conditions.

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