

Ultraviolet spectrophotometric method for analytical determination of mianserin hydrochloride in coated tablets and comparison with LC

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Ultraviolet spectrophotometric (UV) and Liquid Chromatographic (LC) methods for the determination of mianserin hydrochloride in pharmaceutical formulation were developed and validated. The various parameters, such as specificity, linearity, precision and accuracy were studied according to International Conference on Harmonization (ICH, 2005). For UV method, mianserin hydrochloride was determined at 278 nm using HCl 0.1 M as the solvent. The response was linear in the concentration range of 20.0 - 140.0 µg/mL ($r = 0.9998$). Precision data evaluated by relative standard deviation was lower than 2%. The UV method was simple, rapid and low cost. Chromatographic analyses were performed in an Ace C₁₈ column and the mobile phase was composed of methanol, 50 mM monobasic potassium phosphate buffer and 0.3% triethylamine solution adjusted to pH 7.0 with phosphoric acid 10% (85:15). LC method was specific, linear, precise, exact and robust. The results confirmed that the both methods are valid and useful to the routine quality control of mianserin hydrochloride in coated tablets. Statistical analysis by Student's *t*-test showed no significant difference between the results obtained by UV and LC methods.

Uniterms: Ultraviolet spectrophotometry/qualitative analysis. Liquid chromatographic/qualitative analysis. Mianserin hydrochloride/determination in tablets. Coated tablets/qualitative analysis.

Os métodos por espectrofotometria na região do ultravioleta (UV) e por cromatografia líquida (CL) para determinação do cloridrato de mianserina na forma farmacêutica foram desenvolvidos e validados. Os vários parâmetros, como especificidade, linearidade, precisão e exatidão foram avaliados de acordo com o International Conference on Harmonization (ICH, 2005). Para o método de UV, o cloridrato de mianserina foi determinado utilizando o comprimento de onda de 278 nm e HCl 0,1 M como solvente. A resposta foi linear na faixa de concentração de 20,0 a 140,0 µg/ML ($r = 0,9998$). A precisão foi avaliada pelo valor de desvio padrão relativo (DPR) inferior a 2%. O método por UV é simples, rápido e de baixo custo. As análises cromatográficas foram realizadas em uma coluna Ace C₁₈ e a fase móvel foi composta por metanol, tampão fosfato de potássio monobásico 50 mM com 0,3% de trietilamina com o pH ajustado para 7,0 com ácido fosfórico 10% (85:15). O método de CL foi específico, linear, preciso, exato e robusto. Os resultados confirmam que ambos os métodos são válidos e úteis para o controle de qualidade do cloridrato de mianserina em comprimidos revestidos. A análise estatística por teste *t* de Student não mostrou diferença significativa entre os resultados obtidos para os métodos de UV e CL.

Unitermos: Espectrofotometria na região do ultravioleta/análise quantitativa. Cromatografia líquida/análise quantitativa. Cloridrato de mianserina/determinação em comprimidos. Comprimidos revestidos/análise qualitativa.

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INTRODUCTION

Mianserin hydrochloride (Figure 1), chemically (RS)-2-methyl-1,2,3,4,10,14b-hexahydrodibenzo[*c,f*]pyrazino[1,2-*a*]azepine hydrochloride, is a drug for the treatment of depressive illness and depression associated with anxiety. The antidepressant mianserin hydrochloride exhibits a tetracyclic structure that is different from typical tricyclic antidepressants. Based on its mechanism of action not defined, mianserin is classified as an atypical antidepressant (Pawlowska *et al.*, 2003).

It is metabolized mainly by *N*-demethylation, aromatic hydroxylation, *N*-oxidation and *N*-glucuronidation. The major metabolite of mianserin hydrochloride in plasma, *N*-desmethyalmianserin, contributes substantially to the overall therapeutic effects of mianserin in patients (Chauhan *et al.*, 2005).

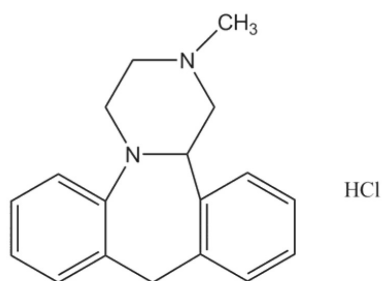


FIGURE 1 - Chemical structure of mianserin hydrochloride.

Although there are studies describing the determination of mianserin hydrochloride in biological fluids by LC-MS (Chauhan *et al.*, 2005), LC-ESI/MS (Xu *et al.*, 2008), LC (Hefnawy, Aboul-Enein, 2004; Brown, Hundt, Swart, 1992; Lukaszewicz *et al.*, 2007) and spectrophotometric methods with detection in the visible region (Khan *et al.*, 2002; Devani, Pandya, Shah, 1990), there are no studies describing quantification methods by UV spectrophotometry for coated tablets. The literature reports few methods for the quantitation of mianserin hydrochloride in pharmaceutical dosage form.

The method described in this study was validated through the evaluation of the following analytical parameters: specificity, linearity, precision (repeatability and intermediate precision) and accuracy (ICH, 2005). The aim of this study was to develop and validate a simple, fast and low cost method by UV spectrophotometry to quantify mianserin hydrochloride in coated tablets. Besides, the statistical comparison of the results with LC method, previously validated by our research group, was performed (Sfair *et al.*, 2012).

EXPERIMENTAL PROCEDURES

Material

Mianserin hydrochloride reference standard (99.90%) was kindly supplied by Pharmaceutical Industry Organon (São Paulo, Brazil) and the commercial tablets Tolvon® was obtained in the local pharmaceutical market. The tablets were claimed to contain 30 mg of drug and the following excipients: starch, silicon dioxide, magnesium stearate, methylcellulose, calcium dibasic phosphate, hypromellose, macrogol and titanium dioxide. Purified water was obtained by a Millipore® Direct-Q 3UV with pump (Molsheim, France). Hydrochloric acid was purchased from Merck (Darmstadt, Germany).

Instruments and analytical conditions

Spectral and absorbance measurements were performed with an UV-Vis Shimadzu model UV 160A using 10 mm quartz cells and detection at 278 nm.

The LC system (Agilent 1200 series, Santa Clara, USA) consisted of a quaternary pump, vacuum degasser, thermostat column compartment, standard auto sampler and diode array detector set at 278 nm was used. Chromatographic analyses were performed in an Ace RP-18 octadecyl silane column (250 mm×4.6 mm i.d., particle size 5 µm) maintained at ambient temperature (25 °C). The mobile phase was composed of methanol, monobasic potassium phosphate buffer (50 mM) and 0.3% triethylamine solution adjusted to pH 7.0 with phosphoric acid 10% (85:15, v/v) in isocratic mode at a flow rate of 1.0 mL/min and the sample injection volume was 20 µL.

Analytical method development

Different solvents were investigated to develop a suitable UV-spectrophotometric method for the analysis of mianserin hydrochloride in coated tablets. For selection of media the criteria employed were sensitivity of the method, ease of sample preparation, solubility of the drug and cost of solvents. Other parameters were evaluated: wavelength and sample concentration, but the focus was on the solvent used since one of the goals was to use this UV method in the dissolution test.

Method validation

Specificity

A solution was prepared with common excipients present in the dosage form (starch, silicon dioxide,

magnesium stearate, methylcellulose, calcium dibasic phosphate, hypromellose, macrogol and titanium dioxide), the volumetric flask was kept in an ultrasonic bath for 20 minutes and after the solution was filtered. The solution was scanned from 200 to 400 nm.

Linearity

The calibration curve was obtained with seven concentrations of the standard solution (20.0, 40.0, 60.0, 80.0, 100.0, 120.0 and 140.0 $\mu\text{g/mL}$) in triplicate. The linearity was evaluated by linear regression analysis.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days (3 days). Six sample solutions (80.0 $\mu\text{g/mL}$) were prepared and assayed each day. For preparation of sample solutions, were used crushed tablets and the volumetric flask was kept in an ultrasonic bath for 20 minutes and after the solution was filtered.

Accuracy

The accuracy was determined by the recovery of known amounts of mianserin hydrochloride reference standard added to the samples in the beginning of the preparative process. The added levels were 25, 50 and 75% of the 80.0 $\mu\text{g/mL}$ drug concentration.

LC method

The LC method was validated according to ICH. The method was specific and linear over the concentration range of 50.0–110.0 $\mu\text{g/mL}$. Adequate results were obtained for repeatability, intermediate precision, accuracy and robustness.

RESULTS AND DISCUSSION

The development of spectrophotometric methods for drugs determinations has increased considerable, due to their importance, low cost and simplicity. In this study, an UV method was developed and validated for mianserin hydrochloride in coated tablets for routine use. The experimental conditions were chosen after testing different parameters that influence the analysis. The choice of the best solvent for samples was prime accomplished. Considering the solubility of mianserin in methanol, dichloromethane, water and hydrochloric acid, which were tested as diluents, hydrochloric acid

was used, since it is less toxic, can be used in dissolution test later and the drug is stable in it. The development and validation of a UV spectrophotometric method for quantitative determination of mianserin hydrochloride in coated tablets at the wavelength of 278 nm using HCl 0.1 M as the solvent was performed.

The formulation excipients did not interfere, demonstrating the specificity of the method (Figure 2). To assess the linearity, three standard curves for mianserin hydrochloride were constructed by plotting absorbance versus concentrations ($\mu\text{g/mL}$) and showed good linearity on the 20.0–140.0 $\mu\text{g/mL}$ range. The representative linear equation for this drug was $y = 0.0066x + 0.0091$ and the correlation coefficient was $r = 0.9998$. The variance analysis ($P = 0.05$) was performed to verify the good fitting of the method and the results showed that no deviation from linearity was revealed for mianserin ($F_{\text{calculated}} < F_{\text{critical}}$). According to ANOVA there is linear regression and there is no deviation from linearity.

The low values for relative standard deviation (RSD) 0.75%; 0.77%; 1.04% (intra-day precision) and 1.08% (inter-day precision) showed the good precision of the method (Table I).

The accuracy expresses the agreement between the accepted value and the value found. The mean recovery was found to be 99.65% (Table II).

Comparison between LC and UV methods

The proposed analytical methods were compared using statistical analysis. The Student's *t*-test was applied and did not reveal significant difference between the experimental values obtained in the sample analysis by the two methods, demonstrating equivalence. The calculated *t*-value ($t_{\text{calc}} = 1.13$) was found to be less than the critical *t*-value ($t_{\text{crit}} = 2.03$) at 0.05 significance level (Table III).

The main difference between techniques is the selectivity. LC method is more selective for being a separation technique while in UV method more than one molecule is able to absorb in the same wavelength. When these techniques are applied to coated tablets, UV method has limitations since it may suffer interference of the formulation excipients. In addition, UV method cannot be used in stability studies since degradation products can absorb in the same wavelength of the drug, making it impossible to quantify both and compromises the accuracy of the analytical method.

CONCLUSION

The proposed method was simple, rapid, accurate, precise and inexpensive and can be used for routine

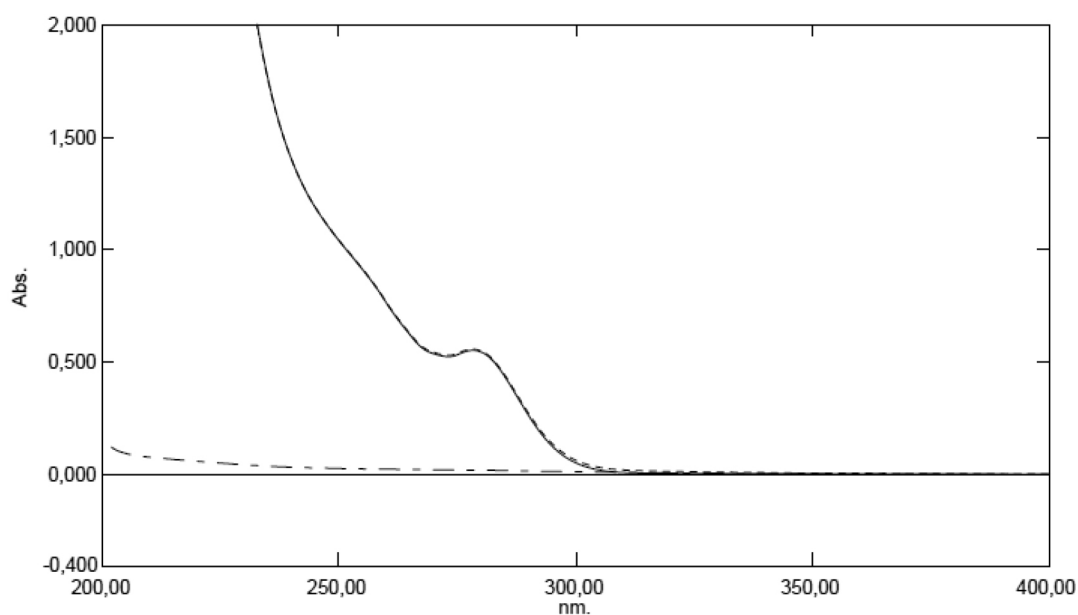


FIGURE 2 - UV spectrum of formulation excipients (---), mianserin hydrochloride reference standard (---) and sample (continuous line) in HCl 0.1 M – 80.0 µg/mL.

TABLE I - Precision of UV method for determination of mianserin hydrochloride

	Intra-day precision (%)	RSD (%)*
Day 1	100.31	0.75
Day 2	101.34	0.77
Day 3	99.64	1.04
	Inter-day precision (%)	RSD (%)
	100.43	1.08

* n=6

TABLE II - Accuracy of UV method for determination of mianserin hydrochloride

Added concentration (µg/mL)	Mean concentration found (µg/mL)	Mean recovery (%) ± RSD*
20.0	19.94	99.70 ± 1.06
40.0	39.73	99.32 ± 0.72
60.0	59.96	99.93 ± 0.54

* Relative standard deviation (n=3)

TABLE III - Results of the determination of mianserin hydrochloride in coated tablets

	LC method Experimental amount (mg/tablet)	R.S.D. (%)	UV method Experimental amount (mg/tablet)	R.S.D. (%)
Day 1*	29.77	0.39	30.09	0.75
Day 2*	29.98	0.44	30.40	0.77
Day 3*	30.31	0.23	29.89	1.04

* Mean of six determinations.

analysis of mianserin hydrochloride in coated tablets. Moreover, the method can be employed to quantify the drug in dissolution test in performance quality control of the formulation.

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