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Original Article

DETERMINATION OF MIANSERIN USING TROPAEOLIN-OOO BY ION PAIR FORMATION

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ABSTRACT

Objective: The present study was aimed at the development of a simple visible spectrophotometric method for the assay of mianserin, a drug used for the treatment of depression.

Methods: The method was developed using tropaeolin-ooo (TPooo) as an ion associative complex forming a chromophore. Developed the chromophore by sequential mixing of aqueous solutions of mianserin, hydrochloric acid, and TPooo. Chromophore was extracted into an organic solvent (chloroform) and absorbance values of organic layers were measured. As per the existing guidelines of an international conference on harmonization (ICH), various parameters of the method were tested for validation.

Results: At the optimized reaction conditions, the formed chromophore (λ_{max} 524 nm) was stable and sensitive. Regression analysis (r>0.9999) shows that the plotted calibration curve exhibits good linearity in the studied range of concentration (4–24 µg/ml). Accuracy of the method was evident from the % recovery values (99.50–99.87 range). Satisfactory precision (both intra and inter day) for the proposed method was clear as ranges of percentage of relative standard deviation (%RSD) values were 1.382-1.781 and 1.128-1.765 respectively. Since RSD is less than 2 %, this method was reproducible and accurate.

Conclusion: Due to lack of pre-treatment process for this method, it was simple. All the tested parameters of the method were validated as per ICH guidelines.

Keywords: Mianserin, Tropaeolin-ooo, Ion associative complex, Assay, Method Development, Validation

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INTRODUCTION

Mianserin is an atypical antidepressant. It also works on brain nerve cells. It is useful to relive from depression. In liver, an enzyme cytochrome P450 2D6 metabolizes it via N-oxidation, aromatic hydroxylation, and N-demethylation. $C_{20}H_{20}N_2$ is its molecular formula and is a tetracyclic piperazinoazepine (fig. 1) [1-2]. In spite of its activity in relieving from dyskinesia and PD psychosis, its prospective clinical usage is limited by hindering the action of L-DOPA antiparkinsonian [3]. In stressed animals, mianserin exhibits a protective role on the amounts of cytokine and decreases the levels of IL-6 and TNFa [4]. It exhibits antinociceptive effect along with antidepressant activity and hence it was suggested as a substitute to treat both mood disorders and neuropathic pain associated with diabetes [5]. It is clear from the literature survey that reports were published for its quantitative determination using various analytical methods. Those methods include usage of both UV and visible spectrophotometric [6-9], HPLC [10-13], capillary gas chromatography and electrophoresis [14-17] and Gas chromatography [18-19]. In UV spectrophotometric method proposed by S fair *et al.* [6], linearity was tested in the range of 20.0-140.0 µg/ml and continued study by liquid chromatographic method in which an Ace C18 column was used along with the mobile phase comprising of methanol and KH_2PO_4 buffer (pH 7.0) in the ratio of 85:15 v/v. Farag *et al.* [7] developed an extractive colorimetric method using four dyes where pH has to be maintained carefully using buffer solutions. In the proposed methods for the determination of mianserin concentration in human plasma, lower limits of quantitation were good but poor recovery values were reported [10, 11, 14, 15]. Taking into consideration of the cost of the chromatographic/electrophoresis instruments and difficulty in the maintenance of reaction conditions for the above spectro-photometric methods, in the present study, TPooo was used as a chromogen to develop colour for its determination both in bulk drug and dosage forms.

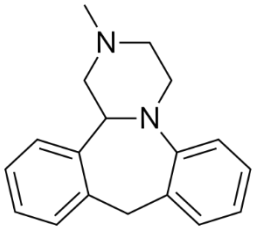


Fig. 1: Chemical structure of mianserin

MATERIALS AND METHODS

TECHOMP (UV 2310) double beam UV-Visible Spectrophotometer with HITACHI software version 2.0 was used to measure the absorbance. Quartz cuvetts (10 mm path length) were used for the analysis. Digital pH meter (Elico LI-120) and balance (Shimadzu AUX-220) were used to weigh the samples and to measure pH respectively. Spectroscopic measurements were conducted at room temperature (30±1 °C). All chemicals used in the present study were AR grade. In the entire process, used water was double distilled.

Preparation of reagents

Tropaeolin-ooo solution (0.2% w/v): 200 mg of tropaeolin-ooo (TPooo) was dissolved in 100 ml of distill water.

Preparation of standard drug solution: The standard mianserin (25 mg) was weighed accurately and transferred to 25 ml volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain the final concentration of 1000 µg/ml (stock solution). 2.0 ml from the stock solution was further diluted to 10.0 ml to get a standard stock solution having 200 µg/ml of mianserin.

RESULTS AND DISCUSSION

Among the various existing techniques for quantitative estimation of pharmaceutical drugs, the prevalent approach is an establishment of the coloured complex involving ion-association. This method can be extended to those drugs consisting of nitrogen, which accepts a proton in an acid medium (i.e., undergoes protonation) to form a cation. Most of the anion dyes develop a complex with the above-formed cation. Visible spectrophotometry is used to measure the absorbances of those complexes after extraction into organic solvents [20]. An added advantage of ion-association extract method is its application to the determination of the exact compound in spite of its presence among different constituents of formulations. Impelled by these advantages, present study describes the establishment of a procedure centered on creation of an ion-association complex with the help of chromogenic dye like tropaeolin-ooo (TPooo). An absorption maximum was observed at 524 nm for the developed chormophore using TPooo in the determination of mianserin by visible spectrophotometry (fig. 2).

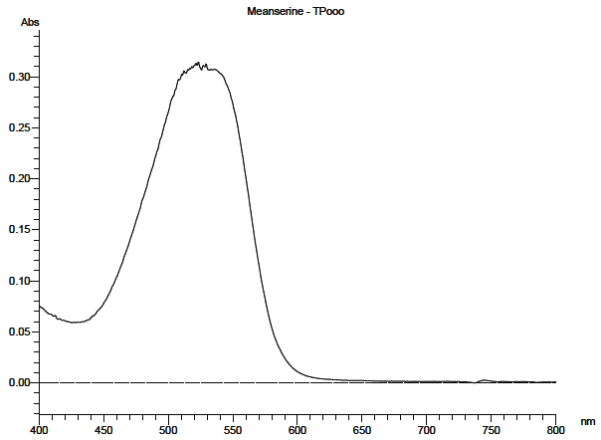
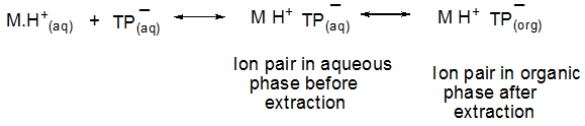


Fig. 2: Visible spectrum of mianserin-TPooo complex

Optimization of reactions conditions

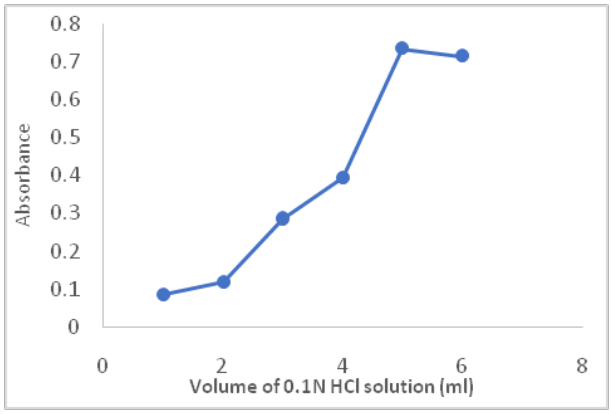
At ambient temperature (30±1 °C), performed the optimization of reaction conditions. Coloured solution exhibited maximum absorbance using HCl (0.1 N; 5 ml) (fig. 3a). Optimized conditions for TPooo regarding volume and concentration were 2.0 ml and 0.2% (w/v) respectively (fig. 3b). Instant formation of colour was noticed by mixing of reactants and observed the maintenance of stable colour intensity for two hours. Out of the tested solvents (C_6H_6, $C_6H_5NH_2$, $C_6H_5NO_2$, CH_2Cl_2 and $CHCl_3$), chloroform was identified as the best solvent for extraction (fig. 4). 10 ml of organic solvent ($CHCl_3$) addition to the aqueous layer (15 ml) resulted in highest and constant absorbance. Therefore, two minutes of contact time was fixed for the organic and aqueous phase (2:3 v/v). Effective sequential addition was mianserin, HCl, and TPooo. As per the Job's continuation method (Job, 1928), confirmed the formation of 1:1 ion-association complex between protonated mianserin and TPooo anion. Scheme-1 shows the formation of coloured ion association pair between TPooo anion (TP–) and mianserin-cation (MH+).



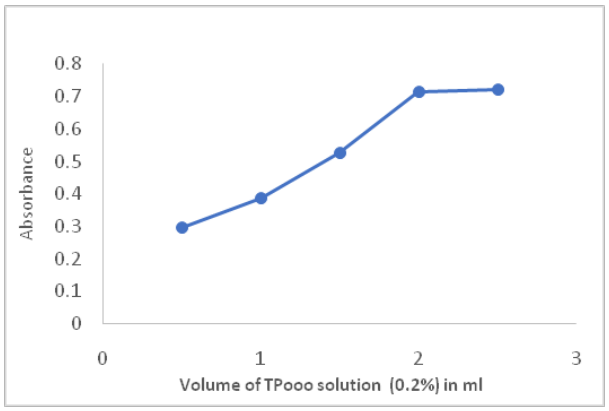
Scheme-1: Formation of coloured ion association pair

Optimized method procedure

Suitable aliquots of mianserin standard solution (200 µg/ml) were taken into a sequence of separating funnels (125 ml volume). It was followed by successive addition of 5.0 ml of hydrochloric acid (0.1 N) and 2.0 ml of TPooo solution (0.2%). Addition of distilled water made the total volume of the aqueous layer to 15 ml. Then shaken the contents for two minutes after the addition of chloroform (10 ml). Absorbance values of organic layers were measured after their separation from aqueous layers.



a



b

Fig. 3: Effect of volumes of (a) acid and (b) TPooo solution

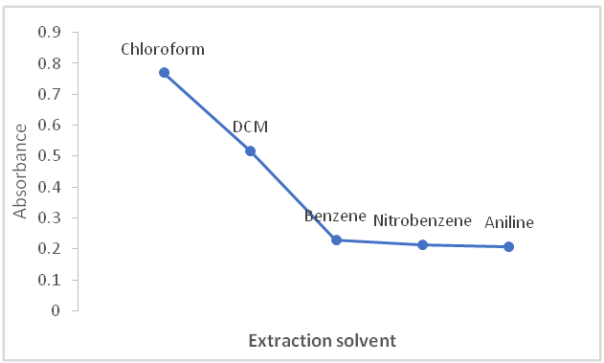


Fig. 4: Effect of extraction solvent

Chromophore formation and chemistry

In acidic medium, sodium sulphonate of TPooo undergoes hydrolysis to give TPooo anion. A stoichiometry of 1:1 for ion-association complex indicates that out of the two nitrogen atoms present on mianserin, only one was protonated. Probably, the lone pair of electrons present on the other nitrogen (of azepine) were involved in resonance with the adjacent benzene ring. Hence, a lone

pair of electrons on this nitrogen was least available for protonation. Therefore, the second nitrogen gets protonated. Fig. 5 shows the chemical reactions involved in the formation of coloured ion pair complex.

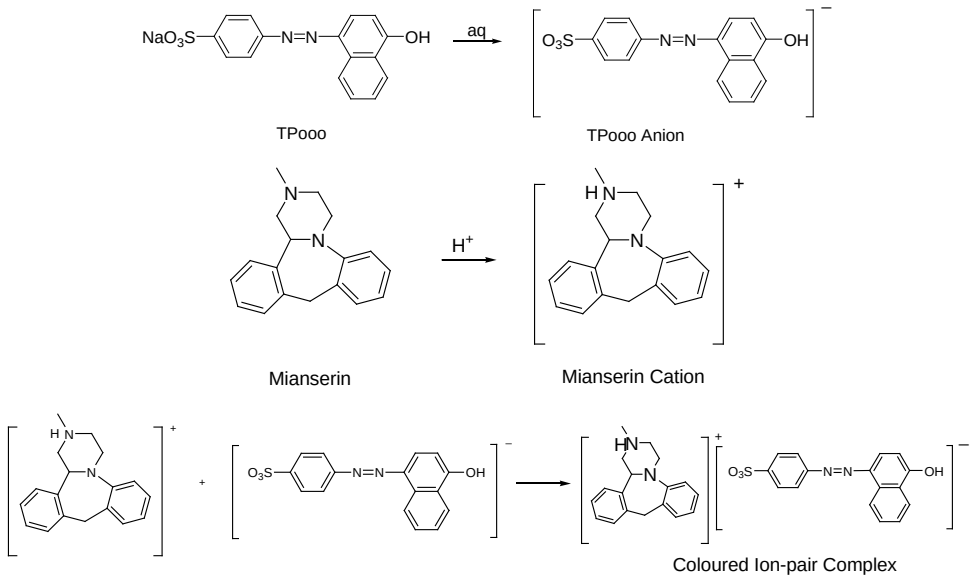


Fig. 5: Reaction of mianserin with TPooo

Validation of method

Linearity and range

By following the above-developed method, the colour was developed by taking different concentrations of mianserin in the range 4–24 µg/ml. For each concentration of mianserin, mean of three measurements for absorbance was taken (table 1). A linear calibration curve was obtained by plotting mean absorbance against mianserin concentration (fig. 6). y = 0.0493x-0.0103 was the equation obtained by linear regression of the data. The correlation coefficient was found to be greater than 0.9999. It indicates the successful testing of linearity of the suggested analytical method.

Table 2 represents key parameters of method development and validation.

Table 1: Calibration curve values

Concentration (µg/ml)	Absorbance* (mean±SD)
4	0.192±0.003
8	0.381±0.002
12	0.578±0.003
16	0.773±0.001
20	0.976±0.002
24	1.175±0.001

*Average of three determinations

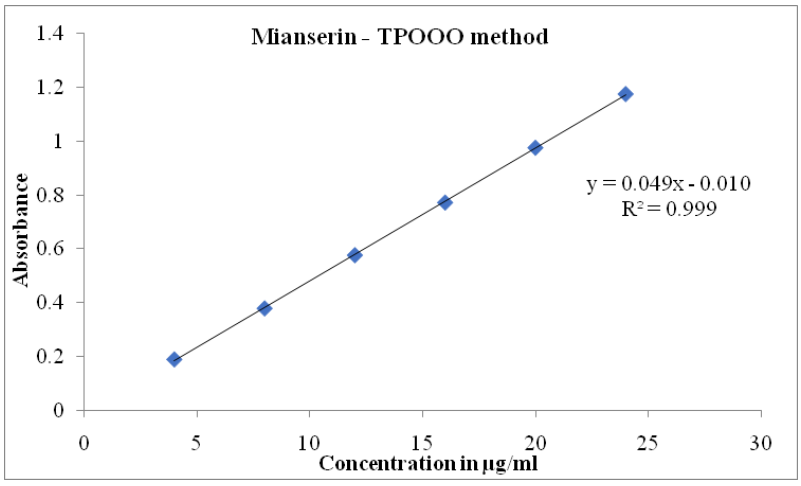


Fig. 6: Calibration graph of mianserin

Table 2: Key parameters of method development and validation

S. No.	Parameter	Observation
Optical characteristics		
1.	Apparent molar absorptivity	1.27×10^4 l/mol/cm
2.	Sandell's sensitivity	0.0207 µg/cm^2/A
Regression analysis		
1.	Slope	0.0493
2.	Intercept	-0.0103
3.	Regression coefficient (*r*)	0.9999
Validation parameters		
1.	λ_{max}	524 nm
2.	Linearity (Beer's Law Limit)	4–24 µg/ml
3.	Limit of detection	0.30 µg/ml
4.	Limit of quantitation	1.00 µg/ml
5	Stability period	18 h

Accuracy

In order to test the accuracy of the method, % recovery studies were carried out and values were given in table 3. In this regard, different amounts of the drug sample in the range of 50 to 150% were added to fixed amounts of mianserin so that total concentration lies within the range of linearity study. The observed percent recoveries of were in between 99.50 to 99.87. As both SD and % RSD values were<1%, the suggested method was accurate.

Precision

Three different concentrations were selected in the linearity range (4–24µg/ml) to check the precision of the proposed method. A series of 6 independent analyses were done for each concentration on concurrent days (of 6 numbers) (table 4). The proposed method satisfied precision studies as % RSD values for intraday and interday were in between 1.382-1.781 and 1.128-1.765 respectively.

Table 3: Recovery of mianserin

Level of recovery (%)	Amount of drug recovered (µg/ml) (practical)	Statistical evaluation		% Recovery = practical x 100/theoretical
50	11.98	Mean	11.96	99.83
	11.94	SD	0.017	99.50
	11.97	%RSD	0.142	99.75
100	15.96	Mean	15.97	99.75
	15.98	SD	0.008	99.87
	15.97	%RSD	0.051	99.81
150	19.94	Mean	19.95	99.70
	19.97	SD	0.012	99.85
	19.95	%RSD	0.062	99.75

Nominal concentration used (a): 8 µg/ml, Amount of drug added (b): 4, 8 and 12 µg/ml respectively for 50%, 100% and 150% recovery levels, Theoretical amount: Total amount of drug (a+b) = 12, 16, 20 µg/ml respectively for 50%, 100% and 150% recovery levels

Table 4: Intraday and inter-day precision readings

Concentration of mianserin (µg/ml)	Concentration*			
	Intraday (mean±SD) (µg/ml)	% RSD	Inter-day (mean±SD) (µg/ml)	% RSD
4	4.042±0.072	1.781	4.042±0.071	1.765
16	15.767±0.229	1.453	15.767±0.299	1.453
24	23.921±0.331	1.382	23.921±0.269	1.128

*Average of six determinations

Table 5: Ruggedness data of mianserin

Test concentration of mianserin (µg/ml)	Concentration*	
	Analyst change	
	mean±SD (µg/ml)	% RSD
4	4.144±0.061	1.472
16	15.767±0.229	1.454
24	23.921±0.270	1.128

*Average of six determinations

Ruggedness

Assay of different amounts of mianserin (4, 16 and 24 µg/ml) was carried out by two different analysts on different days under the above-given method optimized conditions in order to appraise the ruggedness of the current developed method. Lack of significant difference in the values produced by different analysts indicates the evidence for reproducible results (table 5). Hence, ruggedness of this method was confirmed.

Limits of detection and quantification (LOD and LOQ)

As per the ICH guidelines (2005), LOD and LOQ were calculated to determine the sensitivity of the proposed method using formula (3.3

× σ/S) and (10 × σ /S) respectively taking into consideration of ratio between signal and noise [21-22], where S (calibration curve slope) and σ (SD of the response). The corresponding calculated values for mianserin determination were given below.

LOD = 0.30 µg ml^{-1} and

LOQ =1.00 µg ml^{-1}

Analysis of pharmaceutical formulations

Mianserin tablet (Depnon®) extracts were treated with chromogen to develop the chromophore and measured the absorbances in order to determine the API amount in the formulation (tablet) taking into consideration of average weight as a basis (table 6). To determine the amount of mianserin present in the tablet formulations, the above-suggested method can be used because the recovery values of the API was good. It indicates the non-interference to the above method from common excipients. In developing countries, the most opted analytical technique is spectrophotometry to carry out the routine analysis in QC laboratories of industries [23-28].

Hence, the above method which comprises mianserin as a complexing agent can be applied to determine the quantity of Depnon present in pure and tablet formulations.

Table 6: Estimation of mianserin from its formulation

Formulation	Labeled amount (mg)	Amount found* (mg)	% Drug recovered	%RSD
Depnon®	30	29.946±0.0638	99.82	0.213

*Average of three determinations

CONCLUSION

The method suggested using TPooo as an ion-pair forming agent was simple as there is no need to maintain complicated conditions (like an elaborate procedure for sample treatment, maintenance of critical optimum pH etc). Hence, no need of sophisticated or costly instruments. These benefits encourage the usage of the current method in quality control wings for mianserin routine analysis, both in the tablet dosage form and bulk drug.

AUTHOR CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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