ORIGINAL ARTICLE

Ion-pair Formation for the Determination of Mianserin Using Fast Sulphon Black F

Giri Prasad Gorumutchu[1], Venkata Nadh Ratnakaram[2], Sireesha Malladi[3]

[1]Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India,[2]Department of Chemistry, GITAM University, Bengaluru, Karnataka, India,[3]Department of Science and Humanities, Vignan's Foundation for Science, Technology and Research, Vadlamudi, Andhra Pradesh, India

Abstract

Aim: The objective of the current study is to develop a colorimetric method for the determination of mianserin, an antidepressant drug. **Materials and Methods:** Fast Sulphon Black F, an acidic dye was used to develop a soluble colored ion-pair complex. The complex was extracted into an organic solvent and absorbance was measured. **Results:** Reaction conditions were optimized to obtain a sensitive and stable chromophore (λ_{max} 554 nm) in dichloromethane. Good linearity was observed for the calibration curve plotted in the studied concentration range (4–14 µg/mL) with regression analysis ($r > 0.9997$). High percentage recovery values (98.25–101.40) show that the method is accurate. Reproducibility of the method is evident from lower relative standard deviation (<2%) for both intra- and inter-day precision studies. **Conclusions:** The proposed method is validated as per the existing ICH guidelines. This method is simple as it does not require any pre-treatment process.

Key words: Assay, Fast Sulphon Black F, ion-pair complex, method development, mianserin, validation

INTRODUCTION

Mianserin is used to get relief from depression by working on nerve cells of brain. It is metabolized in liver by enzyme cytochrome P450 2D6 through a sequence of reactions such as N-oxidation, aromatic hydroxylation, and N-demethylation. It is a tetracyclic piperazinoazepine with molecular formula $C_{20}H_{20}N_2$ [Figure.1].[1,2] Hindering the role of L-DOPA antiparkinsonian limits its prospective clinical usage, though it is active in relieving from PD psychosis as well dyskinesia.[3] Mianserin modulates (a) the decrease in levels of interleukin-6 and tumor necrosis factor-alpha and (b) regulation of cytokine amounts in stressed animals.[4] A thorough literature survey shows that spectrophotometric,[5-9] high-performance liquid chromatography,[10-13] capillary gas chromatography and electrophoresis,[14-16] and gas chromatography[17,18] based analytical methods were published for quantitative determination of it. In view of high cost of the above stated instruments, Fast Sulphon Black F (FSBF) was used as a chromogen for color development to determine mianserin spectrophotometrically in bulk drug as well as tablet dosage forms.

MATERIALS AND METHODS

TECHOMP (UV 2310) double-beam UV-visible spectrophotometer with HITACHI software version 2.0 was used to measure the absorbance. Quartz cuvettes (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 (25 ± 5°C). All chemicals used in the present study were AR grade. In the entire process, used water was double distilled.

Preparation of reagents

FSBF solution

About 300 mg of FSBF is dissolved in 100 mL of distilled water. Then, the solution was washed with chloroform to remove soluble impurities in the organic solvent.

Address for correspondence:
Dr. Venkata Nadh Ratnakaram, Department of Chemistry, GITAM University, Bengaluru Campus, Nagadenahalli, Doddaballapur Taluk, Bengaluru, Karnataka, India.
Phone: +91-9902632733. E-mail: doctornadh@yahoo.co.in

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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 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

Out of the available different techniques, ion association involved colored complex formation is a widespread approach in the quantitative determination of pharmaceutical drugs. This method can be adopted to all those drugs consisting a heteroatom (for example, nitrogen) which bears lone pair of electrons. Hence, they undergo protonation by accepting proton(s) and form a cation. Dyes capable of attaining anionic form can cultivate an ion-pair complex with the above formed cation from a drug. Organic solvent is used to extract this complex and its absorbance is measured by visible spectrophotometry.[19] Applicability to determine the precise compound even in the presence of different constituents of formulations is the additional benefit of this method. Prompted by the above gains, the current study explains the establishment of a process which is based on the development of a soluble ion-pair complex in the presence of an acidic chromogenic dye like FSBF. The developed chromophore has shown an absorption maximum at 554 nm [Figure 2].

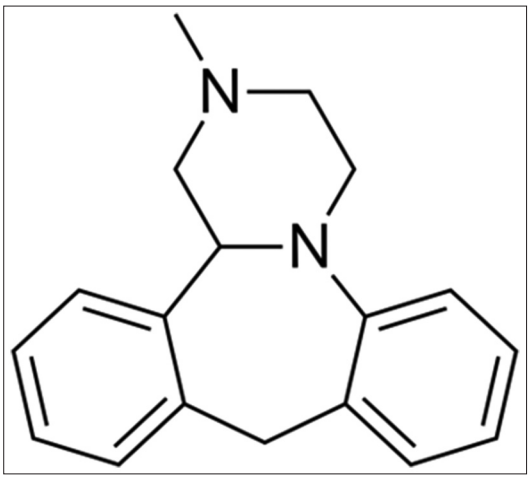


Figure 1: Chemical structure of mianserin

Optimization of reactions conditions

Reaction conditions were optimized at 30 ± 1°C (ambient temperature). At initial volumes of 0.1 N HCl, absorption increased with an increase of acid volume up to 4 mL. Further, increase in acid volume resulted in a decrease of the absorbance due to reversal of FSBF hydrolysis which results in lowering the availability of the number of FSBF anions [Figure 3a]. 1.5 mL of FSFB (0.2% w/v) was the optimized conditions for dye solution [Figure 3b]. Instantaneous development of color after mixing of reactants and persistence of color intensity for long hours was witnessed. Dichloromethane was recognized as the best solvent for extraction among the tried solvents (CH_2Cl_2, $CHCl_3$, C_6H_6, $C_6H_5NO_2$, and $C_6H_5NH_2$) [Figure 4]. Persistent higher absorbance was resulted for the addition of 10 mL dichloromethane (organic solvent) to 15 mL of aqueous layer. Hence, contact time was fixed as 2 min

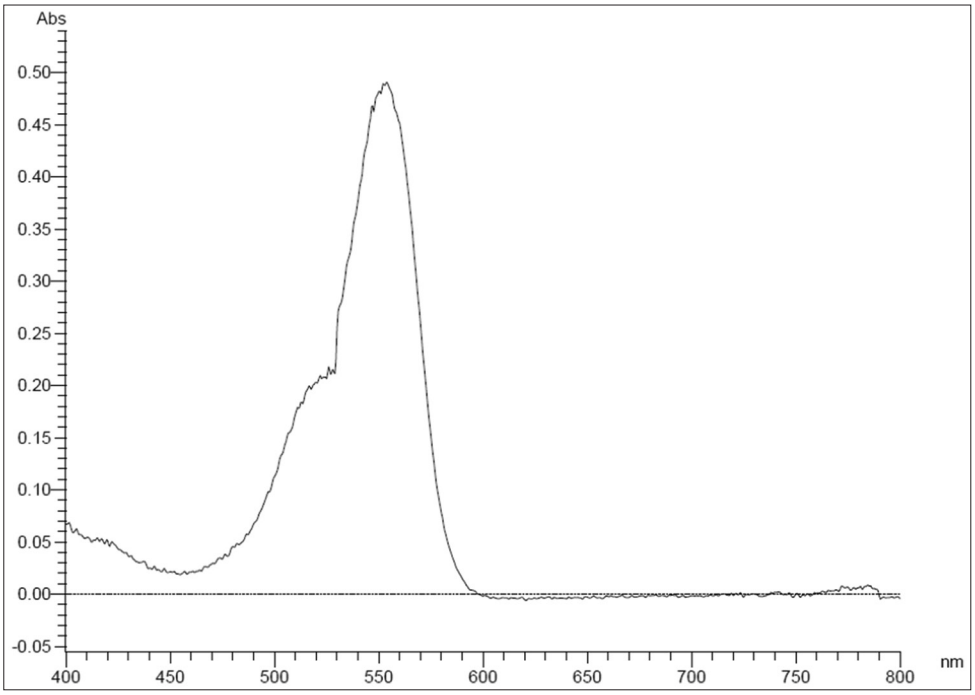


Figure 2: Visible spectrum of mianserin-Fast Sulphon Black F complex

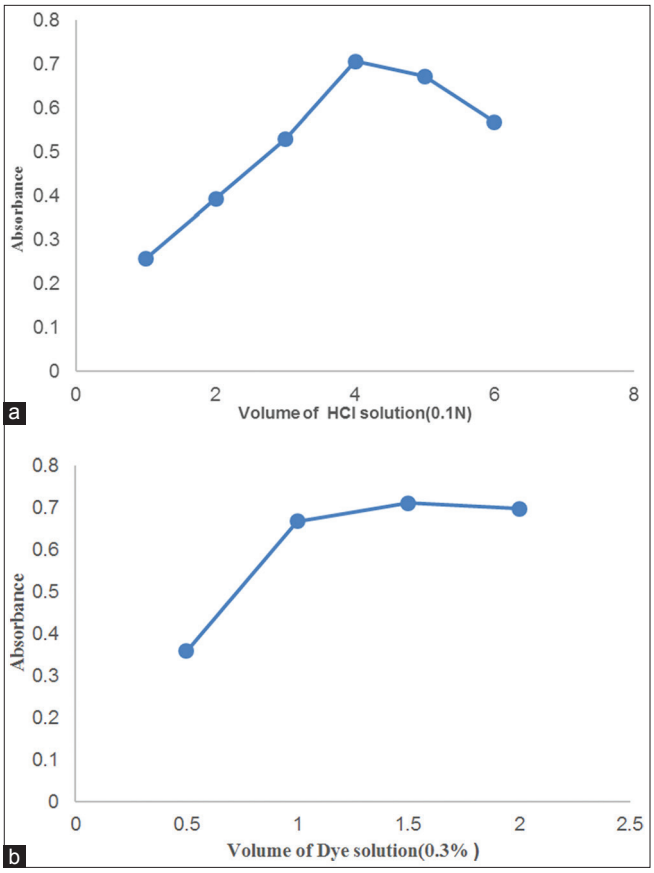


Figure 3: Effect of volumes of (a) acid and (b) FSFB solution

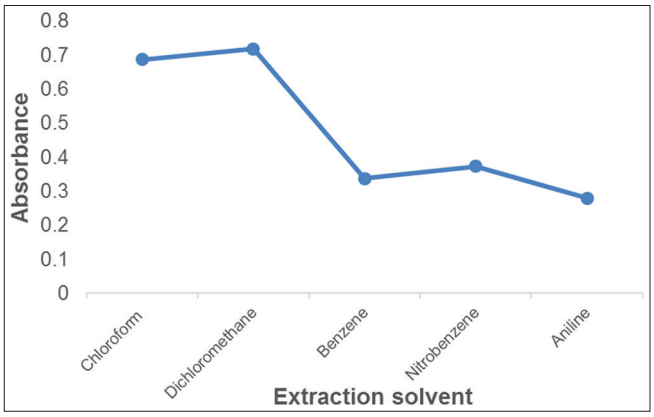


Figure 4: Effect of extraction solvent

between the optimized volumes of organic and aqueous phases (2:3 v/v). Operative successive addition mode of reagents was mianserin, acid and dye solution. Development of 3:1 ion-pair complex between protonated mianserin and FSBF anion was established from Job's continuation method.[20] Scheme 1 demonstrates the formation of colored ion-pair complex between mianserin cation (MH+) and $FSBF^{-3}$ anion.

Optimized method procedure

Appropriate aliquots of the standard solution of mianserin (200 µg/mL) were transferred into an array of separating funnels of 125 mL volume each. Sequential addition of HCl (4.0 mL of 0.1 N conc.) and FSBF solution (1.5 mL of 0.3% concentration) was followed by it. The total volume of aqueous layer was made to 15 mL by the addition of distilled water. After addition of 10 mL of dichloromethane, the contents were shaken for 2 min. Separated the organic layer from aqueous and absorbance values of organic layers was measured.

Chromophore formation and chemistry

The three sulfonic acid groups present in FSBF undergo hydrolysis in aqueous medium to form a tribasic anion.[21] Formation of an ion-pair complex with a stoichiometry of 1:1 explains that only one nitrogen of mianserin is protonated out of the available two nitrogen on it. Perhaps, the lone pair of electrons existing on the other nitrogen (of azepine) is engaged in resonance with the neighboring benzene ring. Therefore, lone pair of electrons present on the second nitrogen is not available for protonation. Hence, the second nitrogen is not protonated. Hence, tribasic charged FSBF anion attracts three monoprotonated mianserin cations (formed in acid medium). Electrostatic attraction between these oppositely charged ions helps them to keep together and acts as a single unit. The chemical reactions involved in the formation of colored ion-pair complex are shown in Figure 5.

Validation of method

Linearity and range

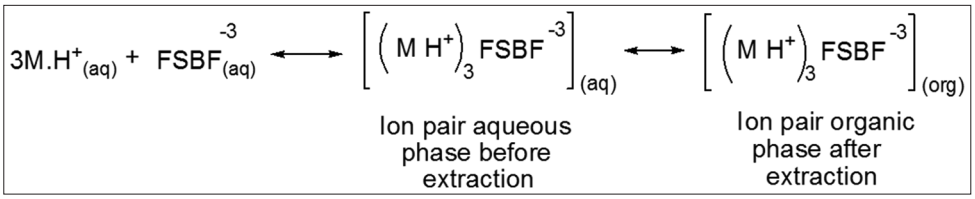
Development of color was carried out with mianserin in a concentration range of 4–14 µg mL^{-1} by adopting the above developed procedure. Thrice measured the absorbance for each concentration of mianserin and their mean value was noted [Table 1]. A linear calibration curve was obtained by plotting mean absorbance values versus concentrations of mianserin [Figure 6]. Linear regression of the data resulted the equation $y = 0.0755x - 0.0147$ with a correlation coefficient >0.9997. Hence, linearity of the proposed analytical method was tested. Table 2 represents key parameters of method development and validation.

Accuracy

The proposed method's accuracy was tested by studying percentage recovery studies and the obtained results were noted in Table 3. To perform this, various quantities of mianserin (range of 50–150%) were supplemented to constant amount of drug to maintain total concentration within linearity range. The obtained recovery percentages are in the range of 98.25–101.40. The proposed method is found to be accurate because SD as well as percentage relative standard deviation (RSD) values are <1%.

Precision

Both precisions of this method were checked by choosing three different concentrations in the linearity range



Scheme 1: Colored ion-pair complex formation

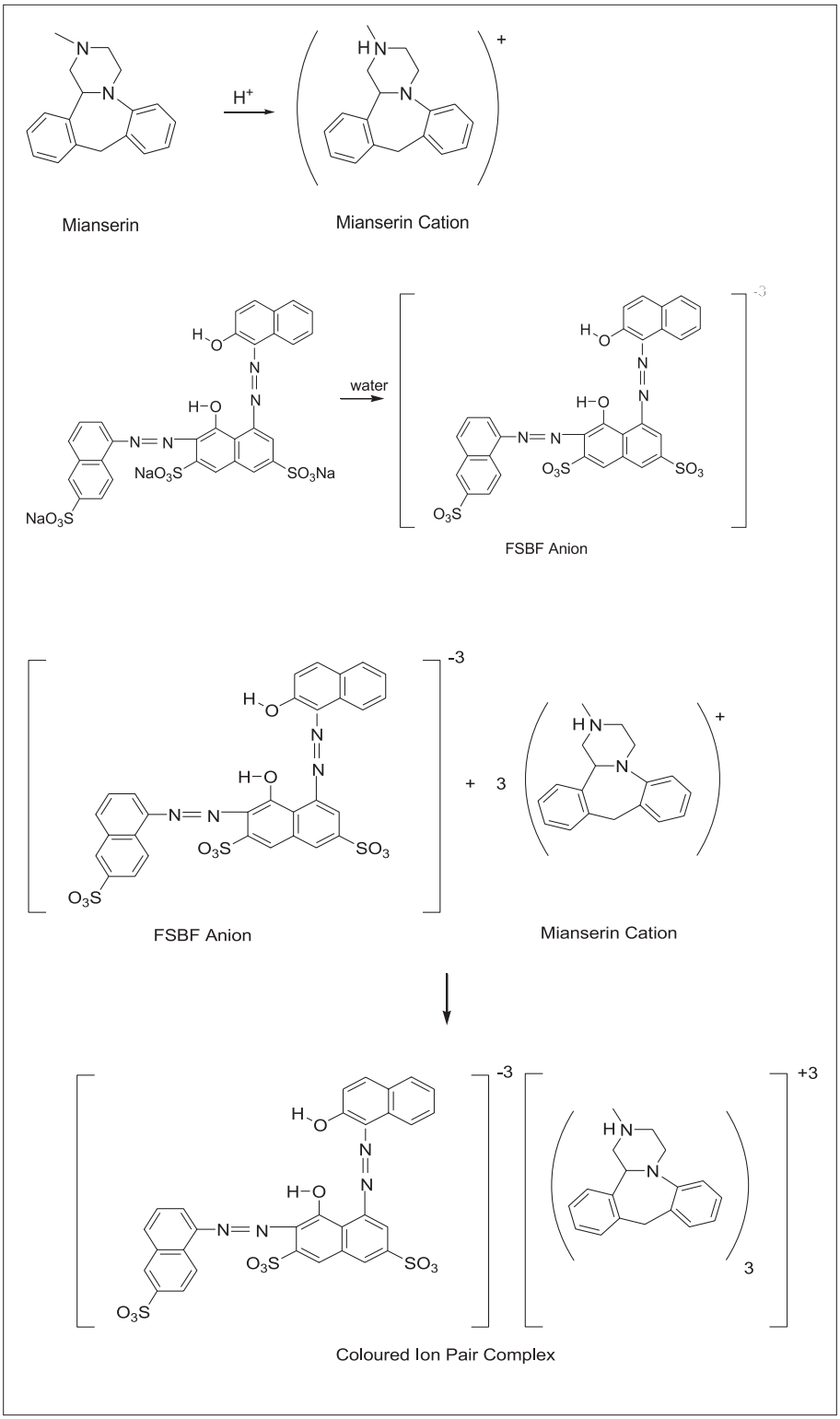


Figure 5: Reaction of mianserin with Fast Sulphon Black F

(4–24 µg/mL). A sequence of six independent analyses was performed for each concentration on 6 concurrent days [Table 4]. Precision studies of the current method were found to be satisfied because percentage RSD values for interday and intraday were in the range of 1.88–1.97 and 1.57–1.98, respectively.

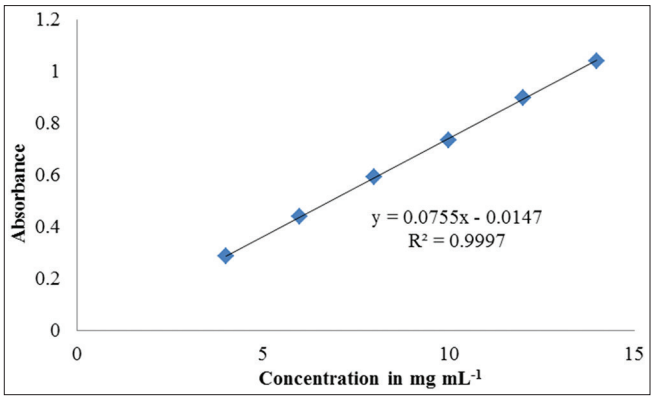


Figure 6: Calibration graph of mianserin

Table 1: Calibration curve values

Concentration of mianserin (µg/mL	Absorbance*
4	0.286
6	0.439
8	0.592
10	0.734
12	0.898
14	1.039

*Average of three determinations

Table 2: Key parameters of method development and validation

Parameter	Observation
Optical characteristics	
Apparent molar absorptivity	1.94×10^4 L/mol/cm
Sandell's sensitivity	0.0136 µg/cm/A
Regression analysis	
Slope	0.0755
Intercept	–0.0147
Regression coefficient (*r*)	0.9997
Validation parameters	
λ_{maxa}	554 nm
Linearity (Beer's law limit)	4–14 µg/mL
Limit of detection	0.60 µg/mL
Limit of quantitation	1.8 µg/mL
Stability period	18 h

Ruggedness

Under the optimized conditions, the current proposed method's ruggedness was appraised by carrying out mianserin assay by two different analysts on different days at three different quantities (4, 16, and 24 µg/mL). The obtained results are reproducible as there is no significant difference in the values produced by different analysts [Table 5]. Hence, the ruggedness of this method was confirmed.

Quantification and detection limits

To estimate the present method's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) values were calculated as per the ICH guidelines (2005) using formulae (3.3 × σ/S) and (10 × σ/S), respectively,[22,23] where S (calibration curve slope) and σ (SD of the response). The resultant obtained values are given below.

LOD = 0.60 µg/Ml and

LOQ = 1.8 µg/mL

Analysis of pharmaceutical formulations

Chromophore was generated with the extracts of mianserin tablets (Deipnon®) by following the above developed procedure and measured the absorbance values to determine API quantity in the tablet formulation by considering average weight as basis [Table 6]. The above developed method can be extended successfully to determine the mianserin amount present in of Deipnon, tablet formulation due to excellent recovery values of API. It shows the non-interference of common excipients in this method. Spectrophotometry is the best selected analytical technique in quality control laboratories of developing and underdeveloped countries.[24-31] Therefore, the above developed method involving ion-pair formation by mianserin using a chromogen (FSBF) can be extended to determine its quantity in pure and tablet formulations.

CONCLUSIONS

The proposed method comprising FSBF as an ion-pair forming agent is simple due to no requirement to maintain intricate reaction conditions (such as elaborate procedure for sample treatment and maintenance of critical optimum pH). Moreover, it is not necessary of using high-end costly instruments. These profits inspire the adaptation of this method in quality control divisions for mianserin routine analysis in tablet formulation as well as bulk drug.

Table 3: Recovery of mianserin

Level of recovery (%)	Amount of drug recovered (µg/mL) (practical)	Statistical evaluation		% Recovery=Practical×100/ Theoretical
50	5.97	Mean	5.92	99.50
	5.87	SD	0.041	97.83
	5.93	% RSD	0.694	98.83
100	7.86	Mean	7.88	98.25
	7.88	SD	0.020	98.50
	7.91	%RSD	0.260	98.87
150	10.14	Mean	10.05	101.40
	10.05	SD	0.073	100.50
	9.96	%RSD	0.731	99.60

Nominal concentration used (a): 4 µg/mL. Amount of drug added (b): 2, 4, and 6 µg/mL, respectively, for 50%, 100%, and 150% recovery levels. Theoretical amount: Total amount of drug (a+b)=6, 8, and 10 µg/mL, respectively, for 50%, 100%, and 150% recovery levels

Table 4: Intraday and interday precision readings

Concentration of mianserin (µg/mL)	Concentration*			
	Intraday Mean±SD (µg/mL)	% RSD	Interday Mean±SD (µg/mL)	% RSD
4	3.956±0.075	1.91	3.956±0.062	1.57
10	9.916±0.196	1.97	9.916±0.196	1.98
14	13.877±0.261	1.88	13.88±0.261	1.98

* 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	3.969±0.062	1.56
10	9.916±0.196	1.98
14	13.890±0.234	1.69

*Average of six determinations

Table 6. Estimation of mianserin from its formulation

Formulation	Labeled amount (mg)	Amount found* (mg)	% Drug recovered	% RSD
Deipnon®	30	29.709±0.184	99.03	0.62

*Average of three determinations

REFERENCES

1. Hamadjida A, Nuara SG, Gourdon JC, Huot P. The effect of mianserin on the severity of psychosis and dyskinesia in the parkinsonian marmoset. Prog Neuropsychopharmacol Biol Psychiatry 2018;81:367-71.
2. Yang M, Liu S, Hu L, Zhan J, Lei P, Wu M, *et al.* Effects of the antidepressant, mianserin, on early development of fish embryos at low environmentally relevant concentrations. Ecotoxicol Environ Saf 2018;150:144-51.
3. Manikowska K, Mikołajczyk M, Mikołajczak PŁ, Bobkiewicz-Kozłowska T. The influence of mianserin on TNF-α, IL-6 and IL-10 serum levels in rats under chronic mild stress. Pharmacol Rep 2014;66:22-7.
4. Üçel Uİ, Can ÖD, Demir Özkay Ü, Öztürk Y. Antihyperalgesic and antiallodynic effects of mianserin on diabetic neuropathic pain: A study on mechanism of action. Eur J Pharmacol 2015;756:92-106.
5. Farag RS, Afifi MS, Abd-Rabow MM. Extractive spectrophotometric determination of mianserin hydrochloride by acid-dye complexation method in pure and in pharmaceutical preparations. Int J Pharm Sci Res 2011;2:1197.
6. Han IU, Aman T, Kazi AA, Khan ZA. Spectrophotometric determination of mianserin in pure and pharmaceutical preparations. J Chem Soc Pak 2002;24:114-8.
7. Devani MB, Pandya SS, Shah SA. Spectrophotometric

determinations of mianserin hydrochloride with -3-methyl-2-benzothiazolinonehydrozone. J Pharm Pharm 1990;52:123-4.
8. Xu P, Chen BM, Ma N, Yan M, Zhu YG. Determination of mianserin in human plasma by high performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI/MS): Application to a bioequivalence study in Chinese volunteers. J Pharm Biomed Anal 2008;47:994-9.
9. Prasad GG, Nadh RV. Determination of mianserine using Fe3+-phenanthroline by visible spectrophotometry. Res J Pharm Tech 2018;12(1):1-4.
10. Łukaszkiewicz J, Piwowarska J, Skarzyńska E, Łojewska MS, Pachecka J. Development, validation and application of the HPLC method for determination of mianserin in human serum. Acta Pol Pharm 2007;64:103-7.
11. Hefnawy MM, Aboul-Enein HY. Fast high performance liquid chromatographic analysis of mianserin and its metabolites in human plasma using monolithic silica column and solid-phase extraction. Anal Chim Acta 2004;504:291-7.
12. Sun LL, Si TM, Shu LA, Zhang HY, Tian CH. HPLC determination of mianserin in human plasma. Zhongguo Xinyao Zazhi 2002;11:714-6.
13. Grodner B, Pachecka J. A simpler and faster capillary electrophoresis method for determination of mianserin enantiomers in human serum. Acta Pol Pharm 2006;63:9-14.
14. Martínez MA, Sánchez de la Torre C, Almarza E. A comparative solid-phase extraction study for the simultaneous determination of fluvoxamine, mianserin, doxepin, citalopram, paroxetine, and etoperidone in whole blood by capillary gas-liquid chromatography with nitrogen-phosphorus detection. J Anal Toxicol 2004;28:174-80.
15. Andersen S, Halvorsen TG, Pedersen-Bjergaard S, Rasmussen KE. Liquid-phase microextraction combined with capillary electrophoresis, a promising tool for the determination of chiral drugs in biological matrices. J Chromatogr A 2002;963:303-12.
16. Wang F, Khaledi MG. Capillary electrophoresis chiral separation of basic pharmaceutical enantiomers with different charges using sulfated beta-cyclodextrin. J Microcolumn Sep 1999;11:11-21.
17. Ishii A, Kurihara R, Kojima T, Sano T, Mizuno Y, Yamakawa Y, *et al.* Sensitive determination of mianserin and setiptiline in body fluids by gas chromatography with surface ionization detection (GC-SID). Leg Med (Tokyo) 2000;2:115-8.
18. Lewis J, Cairncross KD. A simplified method for the estimation of mianserin in plasma. Br J Clin Pharmacol 1981;12:583-5.
19. Kumar KK, Nadh RV, Nagoji KE. Extractive spectrophotometric determination of nicergoline through ion-pair complexation reaction. Orient J Chem 2013;29:263-9.
20. Job P. Job's method of continuous variation. Ann Chim 1928;9:113-203.
21. Ch VR, Acharyulu ML, Reddy TS, Srinivas BV, Rao PV. Determination of mirtazapine using fast sulphone black F as chromogenic reagent. Int J Res Chem Environ 2013;3:107-12.
22. Sethi PD. HPLC quantitative Analysis of Pharmaceutical Formulations. India: CBS Publications; 2001.
23. ICH Guidelines, Validation of Analytical Procedures. Text and Methodology. Q2 (R1); 2015. p. 8-13.
24. Sudhir MS, Nadh RV. Diazo-coupling a facile mean for the spectrophotometric determination of rasagilinehemitartrate. Orient J Chem 2014;29:1507-14.
25. Kumar KK, Nadh RV, Nagoji KE. Determination of bendamustine hydrochloride in pure and dosage forms by ion-associative complex formation. Orient J Chem 2014;30:905-10.
26. Prasad GG, Nadh RV. Oxidative coupling: A tranquil approach for determination of selexipag by visible spectrophotometry. Orient J Chem 2018;34:3112-7.
27. Prasad GG, Nadh RV. Determination of mianserine using tropaeoline-OOO by ion pair formation. Int J Appl Pharm 2019;11:168-73.
28. Prasad GG, Nadh RV. Extractive spectrophotometric determination of ulipristal acetate using naphthol blue black. Res J Pharm Technol 2019;12:1-4.
29. Prasad GG, Nadh RV, Kumar KK. Piperacillin estimation by ion-associative complex formation. Asian J Pharm Clin Res 2019;12:1-5.
30. Prasad GG, Nadh RV, Kumar KK. Ion-associative complex formation for estimation of piperacillin. Int J Res Pharm Sci 2019;10:33-7.
31. Prasad GG, Nadh RV. Diazocoupling for determination of selexipag by visible spectrophotometry. Int J Green Pharm 2018;12 Sup 3:485.

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