Int. J. Chem. Sci.: 10(4), 2012, 2191-2198
ISSN 0972-768X
www.sadgurupublications.com

METHOD DEVELOPMENT AND VALIDATION FOR ASSAY OF MINOCYCLINE HYDROCHLORIDE IN DOSAGE FORMS BY RP-HPLC

ALKA AGARWAL*, I. J. SINGHVI, S. K. GUPTA, K. C. GUPTA and M. THAKKAR

Pacific College of Pharmacy, UDAIPUR – 313001 (Raj.) INDIA

ABSTRACT

A simple, fast, precise and accurate high performance liquid chromatographic method has been developed for the quantitative estimation of minocycline hydrochloride from powder for oral suspension formulation. The method was developed using acetonitrile : Phosphate buffer in the proportion of 25 : 75 v/v at flow rate of 0.5 mL/min as mobile phase. The separation was carried out on Inertsil ODS 3V C_{18} (250 mm x 4.6 mm, 5 µm) column and the eluents were detected at 280 nm. The linearity was observed in the concentration range of 100 to 900 µg/mL of minocycline hydrochloride. The LOD and LOQ of the method were 0.816 and 2.474 µg/mL, respectively. The system suitability parameters and other validation studies were performed to ensure accurate and precise method for estimation of minocycline hydrochloride.

Key words: Minocycline hydrochloride, RP-HPLC.

INTRODUCTION

Minocycline hydrochloride, chemically, 4, 7-Bis (dimethylamino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 10, 12, 12a-tetrahydroxy-1, 11-dioxo-2-naphthacenecarboxamide monohydrochloride is an tetracycline antibiotic. The molecular mass of minocycline hydrochloride is 493.94 g/mol[1].

For the estimation of minocycline hydrochloride from biological fluid, many HPLC methods have been reported[2-8]. But in most of the method dimethylformamide (DMF) was used as mobile phase, which affect the life period of column, it also affects system suitability parameters of HPLC. So an attempt has been made in the present study to develop such a

*Author for correspondence; E-mail: alkaagarwal2007@rediffmail.com

method, which exclude the use of DMF and prolong column life period and produce the good system suitability parameters.

Minocycline hydrochloride sample was provided as gift sample by Unimark Remedies Limited, Bavla, Gujarat, India. Solvent acetonitrile used was of HPLC grade (Rankem, Mumbai, India). Ortho phosphoric acid used was of AR grade (RFCL Chemicals, Sarkhej, Ahmedabad). The HPLC grade water was used for analysis.

EXPERIMENTAL

Material and method

A Shimadzu delivery module, LC-2010 HT equipped with auto sampler, column thermostat, UV-Visible detector and Diode Array detector were used for present study. The system was controlled by LC solution software.

For HPLC method development, Inertsil C_{18} ODS 3V (5 µ) 250 x 4.6 mm column and phosphate buffer (pH-2.5) : acetonitrile : 25 : 75 as mobile phase were used. Instrumental conditions were : detection at 280 nm, flow rate 0.5 mL/min and run time was 10 min. The column temperature was kept at 40^{o}C. The mobile phase was filtered through 0.45 µm Nylon 6, 6 membrane filter and degassed in ultrasonic water bath. The diluent used for analysis, consists of 50 mg/L di-sodium edentate (EDTA-Na_2) in buffer.

Experimental work

Weighed quantity of 50 mg of minocycline hydrochloride in to 100 mL volumetric flask and about 50 mL of sodium edentate (EDTA-Na_2) is added to it. Sonicated to dissolve completely and diluted to 100 mL with sodium edentate (EDTA-Na_2). The final concentration was 500 µg/mL of minocycline hydrochloride.

Injected the standard solution of minocycline hydrochloride in to the stream of mobile system at a flow rate of 0.5 mL/min. The solution was injected 5 times in to the column and the corresponding chromatograms were obtained. From these chromatograms the area under the peaks and respective retention time of the drug were noted. The retention time of minocycline hydrochloride observed was 7.13 min. A representative chromatogram is shown in Fig. 1.

A commercial brand of powder for oral suspension was chosen for testing suitability of the proposed method to estimate minocycline hydrochloride in powder for oral suspension formulation. The label claim was 50 mg/5 mL. Pipette out 5 mL of

reconstitute sample in 100 mL volumetric flask, added 50 mL disodium edentate and sonicated to complete solubility of drug. The solution was diluted to the volume with disodium edentate, thoroughly mixed and then filtered through 0.45 µm membrane filter. The theoritcal final concentration of the solution was 500 µg/mL minocycline hydrochloride. From this solution, vials for HPLC were filled and kept for auto sampling. The drug content in test preparation was quantified. The results obtained are as shown in Table 1.

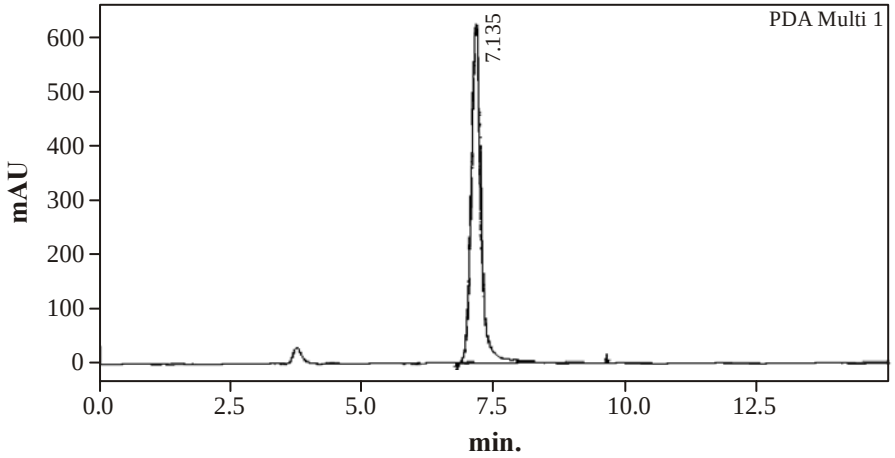

Fig. 1: Chromatogram of minocycline hydrochloride

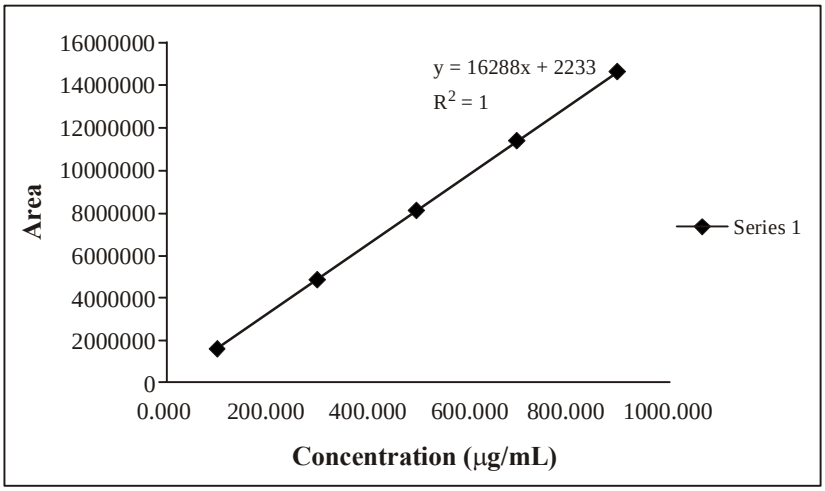

Fig. 2: Linearity of minocycline hydrochloride

Table 1: Assay of commercial sample

Parameters	Minocycline hydrochloride (500 µg/mL)
Standard area	8099982
	8050985
	8086015
	8076914
	8082782
Mean area	8083406.4
Sample area	8081415
	8083917
Mean area	8082666
% Assay (w/w)	99.70

Table 2: Linearity of minocycline hydrochloride

Con. (µg/mL)	Mean area
100	1630485
300	4882475
500	8138345
700	11385474
900	14648721
Correlation coefficient (r^2)	1.0000
Slope of regression line	16288
Y-intercept	2233
LOQ	2.474
LOD	0.816

Table 3: Accuracy study (Recovery)

MNC	Area	Con. (µg/mL)	% w/w Added	% w/w Found	% Recovery	Avg. %	SD	% RSD
50% of 500	4046145	251	50.22	49.79	99.10	99.7	0.55	0.6
	4052784	250	50.02	50.08	100.10			
	4039757	249	49.82	49.81	100.00			
100% of 500	8047215	501	100.24	99.03	98.80	99.5	0.61	0.6
	8109623	499	99.84	99.80	100.00			
	8094518	500	100.04	99.61	99.60			
150% of 500	12165548	752	150.47	149.71	99.50	100.0	0.76	0.8
	12176545	751	150.27	149.85	99.70			
	12302456	750	150.07	151.40	100.90			

Table 4: Precision

Concentration (µg/mL)	Area (mAU/sec.)
500	8036459
500	8132476
500	8069543
500	8189327
500	8102471
500	8124585
Avg.	8109144
SD	53119
%RSD	0.70

To achieve sharp peaks with good resolution under isocratic conditions, mixture of buffer and acetonitrile in different proportion were tested as mobile phase on a C18

stationary phase. The mixture of buffer and acetonitrile in the proportion (25 : 75 v/v) proportion was proved to be the most suitable for estimation. Since the chromatographic peaks were better defined, resolved, and free from tailing with this system, under the above mentioned chromatographic conditions, the retention time obtained for minocycline hydrochloride was 7.13 min. The method was validated for specificity, linearity, accuracy, precision, as per ICH Q2 (R1) guidelines[9,10].

The recovery study was carried out by adding known amount of minocycline hydrochloride and analyzing by the proposed HPLC method. Accuracy was determined in terms of recovery study and the recoveries were done at three levels i.e. 50%, 100% and 150%. Calculated amount of was added in placebo to attain 50%, 100% and 150% of sample concentration of minocycline hydrochloride (500 µg/mL). Each sample was prepared in triplicate at each level and injected each preparation in duplicate. The data shown that the proposed method was accurate. From the amount found, percentage recovery was calculated. The mean percentage recovery found was 99.7%, 99.5% and 100.0% and % RSD was 0.6, 0.6 and 0.8, respectively.

Linearity at five levels over the range of 50% to 150% with respect to the sample concentration of minocycline hydrochloride was performed. Dilutions of the solution ranging from 100 µg/mL to 900 µg/mL for minocycline hydrochloride were prepared and linearity was checked. The solutions were injected each in the stream of mobile phase, at a flow rate of 0.5 mL/min. Each of these dilutions of different concentration was injected in duplicate in to the column and corresponding chromatograms were obtained. From these chromatograms, the areas under the peaks of the drug were noted. Linearity has been obtained in the concentration range of 100 µg/mL to 900 µg/mL minocycline hydrochloride against the absorbance. Based on the calibration curve, value of r^2 is 1.000, which is in acceptance range. Slope is 16288, intercept is 2233. LOD is 0.816 and LOQ is 2.474.

Placebo solutions (mixture of excepients), diluent used for preparation of standard solution and sample solution were injected in the chromatographic system and checked for interference at retention time corresponding to the retention time of minocycline hydrochloride. There was no interference found from blank and placebo at retention time of minocycline hydrochloride, so the method is specific.

The system suitability was carried out by injecting standard solution of minocycline hydrochloride (500 µg/mL) in to the chromatographic system to check reproducibility of peak areas (% RSD). The % RSD observed was 0.70 for minocycline hydrochloride.

RESULTS AND DISCUSSION

In the present study, an attempt has been made to develop a simple, sensitive, accurate, precise HPLC method for estimation of minocycline hydrochloride. The proposed method for determination of minocycline hydrochloride from pharmaceutical dosage forms is specific, accurate, rapid, and precise. So, the proposed method can be used in routine analysis and have financial benefits, since it excludes the use of dimethylformamide (DMF) from the mobile phase, which affects the chromatographic parameters as well as column.

REFERENCES

1. N. J. O. Maryadele, The Merck Index, An Encyclopedia of Chemicals, Drugs and Biological, Merck Research Laboratories, Division of Merck and Co. Inc. Whitehouse Station; New Jersey, NJ, 14th Edition (2006).

2. V. Orti, M. Audran, P. Gibert, G. Bougard and F. Bressolle, High-Performance Liquid Chromatographic Assay for Minocycline in Human Plasma and Parotid Saliva, J. Chromatogr. B: Biomedical Sci. Appl., **738(2)**, 357-385 (2000).

3. W. R. Wrightson, S. R. Myers and S. Galandiuk, Analysis of Minocycline by High Performance Liquid Chromatography in Tissue and Serum, J. Chromatogr. B. Biomedical Sci. Appl., **706(2)**, 358-361 (1998).

4. V. F. Samanidou and K. I. Nikolaidou, Development and Validation of an HPLC Confirmatory Method for the Determination of Tetracycline Antibiotics Residues in Bovine Muscle According to the European Union regulation, J. Separation Sci., **28(17)**, 2247-2258 (2005).

5. H. Zhou and Wang, Determination of Minocycline Hydrochloride for Injection by RP-HPLC, The First Clinical Hospital, China Medical University, J. Pharmaceut. Biomed. Anal., **23(9)**, 515-516 (2003).

6. P. Vinas and N. Balsalobre, Liquid Chromatography with Ultraviolet Absorbance Detection for the Analysis of Tetracycline Residues in Honey, J. Chromatogr., **1022(1-2)**, 125-129 (2004).

7. P. Bryan, D. Stewart and T. James, Chromatographic Analysis of Selected Tetracycline from Dosage Forms and Bulk Drug Substance using Polymeric Columns with Acidic Mobile Phases, J. Pharmaceut. Biomed. Anal., **12(5)**, 675-692 (1994).

8. G. K. Jain, N. Jain, Z. Iqbal, S. Talegaonkar, F. J. Ahmad and R. K. Khar, Development and Validation of an HPTLC Method for Determination of Minocycline in Human Plasma, ACTA Chromatographica, **19**, 197-205 (2007).

9. ICH-Q2A, Guidelines for Industry: Text on Validation of Analytical Methods, March (1995).

10. ICH-Q2B, Validation of Analytical Procedures : Methodology, International Conference on Harmonization, Nov. (1996).

Accepted : 30.10.2012