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[Research article]

Development and validation of RP – HPLC method for the estimation of Tylosin tartrate in pure and pharmaceutical formulation

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ABSTRACT

A simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the simultaneous determination of tylosin tartrate from pharmaceutical formulation. Chromatographic separation was achieved gradient on a phenomenex c18 column (250 x 4.6 mm, 5 µ particle size) using a mobile phase. Acetonitrile and water in the ratio of 90:10.the flow rate was 1.5ml / min and effluent was detected at 292 nm. The retention time of tylosin tartrate was found to be 2min. linearity was observed in the concentration range of 50 -250µg /ml. The method was validated according to ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed can be used for the routine analysis of tylosin tartrate.

Keywords: RP-HPLC method, Tylosin tartrate.

INTRODUCTION

Tylosin tartrate is chemically [(2*R*,3*R*,4*E*,6*E*,9*R*,11*R*,12*S*,13*S*,14*R*)-12-{[3,6-di deoxy -4-*O*- (2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl) -3- (dimethyl amino)-β-D-glucopyranosyl] oxy}-2-ethyl-14-hydroxy-5, 9,13-trimethyl-8, 16-dioxo-11-(2-oxoethyl) oxacyclo hexadeca- 4,6-dien-3-yl]methyl 6-deoxy-2,3-di-*O*-methyl-β-D-allopyranoside (figure1), is an antibiotic.

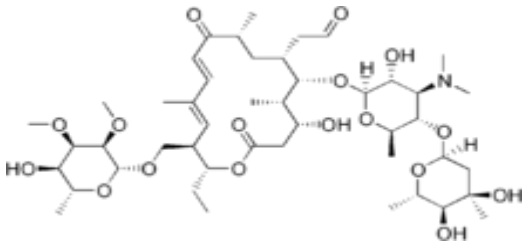

Figure 1: Chemical structure of tylosin tartrate

Several HPLC[5,6,7], GC[8,9] and LC/MS-MS[10-14] methods have been reported for the analysis of tylosin tartrate in plasma that suffer from either undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard. The objective of this study was to

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develop reverse phase high performance liquid chromatography method for the estimation of tylosin tartrate in pure and pharmaceutical dosage form without any derivatization and having short retention time. This method was found to be linear, precise, accurate, sensitive, specific, and robust, and therefore suitable for routine analysis.

MATERIALS AND METHOD

Chemicals and Reagents

Tylosin tartrate was obtained as a gift from vet India, Hyderabad. HPLC grade acetonitrile and, water was obtained from SD Fine Chemicals Ltd, Mumbai.

HPLC Instrumentation and Chromatographic conditions

The analytical separations were carried out on a waters 2487 HPLC system equipped with UV detector. The output of signal was monitored and integrated using LC – solutions 2000 software. The analytical column was phenomenex C_{18} (150 × 4.6 mm, 5 µ). Mobile phase consisted Acetonitrile and water in the ratio of 90:10. Mobile phase was mixed, filtered through 0.45 µ membrane filter and degassed under ultrasonication. The mobile phase was used as diluent. The flow rate was 1.5 ml/min and runtime was 5 minute. The column was maintained at ambient temperature. UV detection was measured at 292 nm and the volume of sample injected was 10 µl.

Preparation of standard stock solution

50 mg of tylosin tartrate was weighed accurately and dissolved in 50 ml of mobile phase to get the concentration of 1000 µg/ml. Resultant solution was filtered through What man filter paper. The standard chromatogram for tylosin tartrate (100µg/ml) was shown in figure 2.

Preparation of working standard solution

Working standard solutions of tylosin tartrate were prepared by accurately transferring the (0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 ml) aliquots of the standard stock solution into a series of six 10 ml volumetric flasks. The volume was made up to mark with mobile phase to obtain concentration range of 10 – 250 µg/ml.

Preparation of sample solutions

0.5 ml of tylosin tartrate injection was taken into 100 mL volumetric flask and then the sample was diluted to 100 ml with mobile phase to get concentration of 100 µg/ml and used for analysis.

RESULTS AND DISCUSSION

HPLC method development and optimization

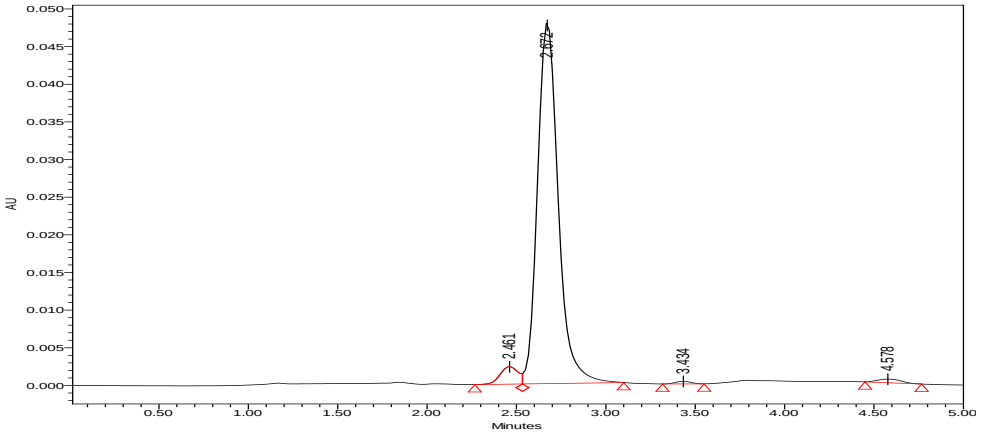
To optimize the chromatographic conditions, different columns, mobile phases, flow rates etc., were tested. Acetonitrile and water in the ratio of 90:10 was preferred as mobile phase because it resulted in a greater response to tylosin tartrate after several preliminary investigatory runs compared with the different mobile phase combinations. The effect of the flow rate was studied in the range 0.9 to 2.0 ml/min and 1.5 ml/min was preferred to be effective. Under these conditions, the analytic peak obtained was well-defined and free from tailing. The retention time (RT) was found to be 2.672 min.

The optimized chromatographic parameters were listed in table 1.

Table 1: Optimized chromatographic parameters

Optimized Chromatographic	parameters
Elution	Gradient
Mobile phase	Acetonitrile :water (9.:10)
Column	Phenomenex c18 column
Flow rate	1.5 ml/min
Detection	292 nm
Injection volume	10 µl
Temperature	Ambient
Retention time	2.672 min
Run time	5.0 min
Concentration	10 – 250 µg/ml

Graph 2. Optimized chromatogram



Validation of the method

When method development and optimization are complete, it is necessary to accomplish method validation. The validation studies include linear range (correlation coefficient), method precision (RSD, %), method accuracy (% recovery and RSD, %), sensitivity studies (LOD & LOQ), and robustness.

System suitability studies

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), tailing factor (T), and peak asymmetry (AS), resolution (RS) were evaluated. The system suitability test was performed using five replicate injections of standards before analysis of samples. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was <2.0%. System suitability parameters were shown in table 2.

Table 2: System suitability parameters

Parameters	Values
Retention time	2.672min

Linearity

The linearity of the method was evaluated by preparing six series of standard solutions of tylosin tartrate in the range of 10 – 250 µg/ml in mobile phase and injecting the solutions into the HPLC system. Excellent correlation between tylosin tartrate peak area and concentration was observed with R^2 = 0.999 (Figure.3). The regression equation was found to be Y = 1652x +30311. Statistical data are presented in table 3 and the calibration curve was shown in figure 3.

Table 3: Linearity results for tylosin tartrate

S.no	Concentration	Area
1	10	50826
2	50	108340
3	100	198556
4	150	273494
5	200	360050
6	250	446214

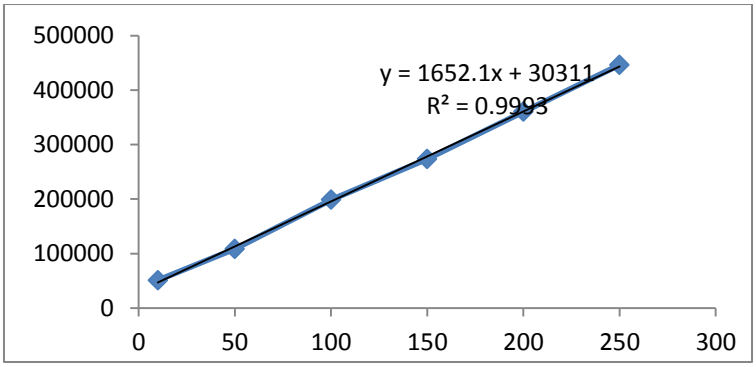


Figure 3: Calibration curve of tylosin tartrate

Precision

System precision: (Repeatability)

To study precision, five replicate standard solutions of tylosin tartrate (100µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated. Results of system precision studies were shown in table 4.

Table 4: Results of system precision for tylosin tartrate

S.no	Retention time(min)	Area (mv.Sec)
1	2.164	108564
2	2.183	1902567
3	2.207	2036987
4	2.241	2326987
5	2.173	2569874
6	2.244	2698745
Mean	2.202	1940620.667
Standard deviation	0.03451377	47620.365
% RSD	1.56738262	1.453872919

Method precision: (Reproducibility)

The intraday and inter-day precision of the proposed method was determined by analyzing the corresponding responses 6 times on the same day and on different days for concentration of sample solutions of 100µg/ml. The result was reported in terms of relative standard deviation (% RSD). Results of method precision studies were shown in table 5.

Table 5: Results of Method precision for tylosin tartrate

S.no	Peak area	%labelled claim
1	105896	90.236
2	123698	101.235
3	165987	102.325
4	206598	106.325
5	215694	123.36
6	223654	125.365
Mean	173587.833	108.141
SD	49996.3476	9.36985456
% RSD	28.8017579	0.4569854

Intermediate precision

The intermediate precision of the proposed method was determined by performing the method by two analysts (Analyst 1 and Analyst 2) for concentration of sample solutions 100 µg/ml. The percent relative standard deviation (% RSD) for peak responses was calculated. The results for intermediate precision were shown in table 6.

Table 6: Results of Intermediate precision for tylosin tartrate

	ANALYST 1		ANALYST 2	
S.NO	**RT (MIN)**	**AREA (MV.SEC)**	**RT (MIN)**	**AREA(MV.SEC)**
1	2.193	1389879	2.241	1385965
2	2.736	1397256	2.661	1377715
3	2.601	1372689	2.594	1366121
4	2.606	1377661	2.693	1345688
5	2.643	1388821	2.619	1366127
6	2.493	1401127	2.514	1399910
Mean	2.54533333	1387905.5	2.553667	1373587.667
SD	0.18949899	10986.0312	0.165023	17092.40841
% RSD	7.44495764	0.791554699	6.462207	1.244362397

ACCURACY

Accuracy of the method was confirmed by the standard addition method, which was carried out by performing recovery studies at 3 different concentrations 100%, 150% and 200% of these expected, in accordance with ICH guidelines, by replicate analysis (n=3). Known amount of standard drug solution (100 µg/ml) was added to a pre analyzed sample solution (100, 150, 200 µg/ml) and percentage drug content was measured. The closeness of obtained value to the true value indicates that the proposed method is accurate. Recovery studies were shown in table 7.

% Recovery = [(Ct –Cpa)/ Cs] × 100.

Where,

Ct = Total concentration of analyte

Cpa = Concentration of pre-analysed sample

Cs = Concentration of standard added to pre-analysed sample.

Table 7: Results of recovery studies for tylosin tartrate

S.no	Level	Std	Amount added	Total recovery	Recovered	% Recovery
1	50	100	50	149.254	49.254	98.508
2	50	100	50	151.062	51.062	102.365
3	50	100	50	148.971	48.971	98.265
4	100	150	100	205.421	55.421	55.421
5	100	150	100	206.036	56.036	56.326
6	100	150	100	209.919	59.919	56.398
7	100	150	150	250	100	66.66
8	100	150	150	253.473	103	68.66
9	100	150	150	249.523	99	66

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength

- Change in the detection wavelength by ± 2nm (294nm and 290nm)
- Change in flow rate by ± 0.1 ml/minute (1.6 ml/min and 1.4 ml/minute)

After each change, sample solution was injected and % assay with system suitability parameters were checked. Robustness values were given in table 8

Table 8: Results of Robustness for tylosin tartrate

Parameter	Rt(min)	Area(mvsec)
Flow rate(ml/min)1.7	2.229	389654
1.3	2.569	386954
Wavelength(nm)	2.204	1896.369
	2.18	1852.369

Limit of Detection and Quantitation

Detection and Quantitation limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula.

$$\text{Limit of Detection} = \sigma \times 3.3/S$$

$$\text{Limit of Quantitation} = \sigma \times 10/S$$

Where σ = The standard deviation of the response.

S = The slope of the calibration curve (of the analyte).

Results of LOD & LOQ were shown in table 9.

Table 9: Results of LOD, LOQ for tylosin tartrate

S.No	LOD	LOQ
1	0.099	0.301

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions were compared in order to provide an indication of specificity of the method.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine tylosin tartrate in their pharmaceutical dosage form And the % Assay results were shown in table 10.

Table 10: Results of % assay

S.No	Amount Found	%Assay
1	197.876	98.39
2	198.044	99.022
3	191.501	95.75

CONCLUSION

A simple, rapid, accurate, and precise RP-HPLC method for the analysis of tylosin tartrate in pure and in pharmaceutical dosage forms had been developed and validated in accordance with ICH guidelines. The RP-HPLC method developed is cost-effective due to short retention time which enabled analysis of tylosin tartrate samples with a small amount of mobile phase. From the % RSD values of precision and recovery studies the method was found to be precise and accurate. The low detection and quantification limits achieved indicate the method is very sensitive. The robustness data gathered during method validation showed that the method is not susceptible to small changes in chromatographic conditions. The proposed RP-HPLC method developed by the author is suitable for routine analysis and quality assessment of tylosin tartrate in pharmaceutical products.

Table 12: Summary of validated parameters for proposed method

Parameter	Result
Linearity range	10 – 250 µg/ml
Regression equation	Y =1652. X +30311
Slope	1652
Intercept	30311
Correlation coefficient	0.999
System precision (% RSD, n=5)	1.453
Method precision (% RSD, n=5)	0.456
Intermediate precision (% RSD, n=5)	1.244
LOD (µg/ml)	0.099
LOQ (µg/ml)	0.301
% Recovery(Accuracy =3)	102.365%
% Assay (% Assay, n=3)	98.365%

REFERENCES

[1]. Parsons CG, Danysz W, Quack G. Memantine is a clinically well Tolerated N-methyl-D-aspartate (NMDA) receptor antagonist-a review of preclinical data. Neuropharmacology. 38; 1999: 735-767.

[2]. Sonkusare SK, Kaul CL, Ramarao P. Dementia of Alzheimer's disease and other neurodegenerative disorders-memantine. Pharmacol Res. 51; 2005: 1-17.

[3]. Erickson CA, Posey DJ, Stigler KA, Mullett J, Katschke AR, Mc Dougle CJ. A retrospective study of memantine in children and adolescents with pervasive developmental disorders. Psychopharmacology. 191; 2007: 141-147.

[4]. Zdanys K, Tampi RR. A systematic review of off-label uses of memantine for psychiatric disorders.Prog Neuropsychopharmacol Biol Psychiatry. 32; 2008: 1362-1374.

[5]. R F Suckow, M F Zhang, E D Collins, M W Fischman, T B Cooper. Sensitive and selective liquid chromatographic assay of memantine in plasma with fluorescence detection after pre-column derivatization. J Chromatogr B Biomed Sci Appl. 729 (1-2); 1999: 217-224

[6]. Afshin Zarghi¸ Alireza Shafaati, Seyed Mohsen Foroutan,Arash Khoddam, and Babak Madadian. Sensitive and Rapid HPLC Method for Determination of Memantine in Human Plasma Using OPA Derivatization and Fluorescence Detection. Application to Pharmacokinetic Studies. Sci Pharma. 78(4); 2010: 847–856.

[7]. Belen Puente, Esther Hernandez, Susana Perez, Luis Pablo, Esther Prieto, Maria Angeles Garcia, And Miguel Angel Bregante. Determination of memantine in plasma and vitreous humour by HPLC with precolumn derivatization and fluorescence detection. Journal of Chromatographic Science. 49; 2011: 745-752

[8]. Leis HJ, Fauler G, Windischhofer W. Quantitative analysis of memantine in human plasma by gas chromatography/negative-ion chemical ionization/mass spectrometry. J Mass Spectrom 37; 2002: 477-480.

[9]. K. Siddappa, Metre Mallikarjun, Tambe Mahesh, Kote Mallikarjun, Reddy Chandrakanth. Development and validation of a gas chromatographic method for the assay of memantine hydrochloride in pure and tablet dosage forms. Physics, Chemistry and Technology. 9(1); 2011: 1 - 8

[10]. Koeberle MJ, Hughes PM, Wilson, Skellern GG. Development of a liquid chromatography-mass spectrometric method for measuring the binding of memantine to different melanins. J Chromatogr B Analyt Technol Biomed Life Sci. 787; 2003: 313-322.

[11]. Almeida AA, Campos DR, Bernasconi G, Calafatti S, Barros FAP,Eberlin MN. Determination of memantine in human plasma by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 848; 2007: 311-316.

[12]. Liu MY, Meng SN, Wu HZ, Wang S, Wei MJ. Pharmacokinetics of single-dose and multiple-dose memantine in healthy Chinese volunteers using an analytical method of liquid chromatography-tandem mass spectrometry. Clin Ther. 30; 2008: 641-653.

[13]. Pan RN, Chian TY, Kuo B, Pao LH. Determination of memantine in human plasma by LC–MS–MS. Application to a pharmacokinetic study. Chromatographia. 70; 2009: 783-788.

[14]. Sarita Karthikeyan, Anju Aji , Sarabjit Singh And Shivanand P. Puthli . An LC-MS/MS method for the quantification of Memantine in human plasma: Development, validation and application to a pharmacokinetic study. International Journal of Pharmacy and Biological Sciences. 3(2); 2013: 343-354

[15]. Jagathi V, Anupama B, Praveen PS, Rao GD. Developed two simple, sensitive and reproducible colorimetric methods for the estimation of Memantine (MEM) in bulk and in pharmaceutical formulations. Int J Current Pharma Res. 2; 2010: 17-18.

[16]. Praveen PS, Jagathi V, Rao GD, Aparna A . Developed two simple and sensitive extractive spectrophotometric methods for the estimation of Memantine (MEM) in pure and pharmaceutical dosage forms. Res J Pharma Biological Chemical Sci. 1; 2010: 222-225.

[17]. Karim Michail, Hoda Daabees, Youssef Beltagy, Magdi Abdelkhalek, Mona Khamis*. Spectrophotometric And Spectrofluorimetric Determination of Memantine Hydrochloride in Bulk and Pharmaceutical Preparations. International Journal of Pharmacy and Pharmaceutical Sciences. 3(3); 2011:180-185

[18]. Bhavil Narola, A.S. Singh, P. Rita Santhakumar, and T.G. Chandrashekhar. A Validated Stability-indicating Reverse Phase HPLC Assay Method for The Determination of Memantine Hydrochloride Drug Substance With UV-detection Using Pre column Derivatization Technique. Anal Chem Insights. 5; 2010: 37–45

[19]. kishore kumar Hotha, Satti Phani Kumar Reddy, V. Kishore Raju, L.K. Ravindranath., Forced degradation studies: Practical approach-overview of regulatory guidance and literature for the drug products and drug substances. Int. Res. J. Pharm. 4(5); 2013: 78-85.

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