DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF OXYTETRACYCLINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Abstract: A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of oxytetra cycline HCl in pharmaceutical dosage form. Chromatographic separation of oxytetra cycline HCl was achieved on Waters Alliance -2695, by using Luna C18 (250mm x 4.6mm, 5µm) column and the mobile phase containing 0.1%OPA+ACN in the ratio of 30:70 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 245 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for oxytetra cycline HCl were NLT 2000 and should not more than 2 respectively. The linearity of the method was excellent over the concentration range 10-150 µg/ml for oxytetra cycline HCl respectively. The correlation coefficient was 0.999. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of oxytetra cycline HCl and study of its stability.

Keywords: RP-HPLC, Oxytetra cycline HCl, Validation.

Introduction: Oxytetracycline inhibits cell growth by inhibiting translation[1]. It binds to the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome[2]. The binding is reversible in nature. Oxytetracycline is lipophilic and can easily pass through the cell membrane or passively diffuses through porin channels in the bacterial membrane[3].

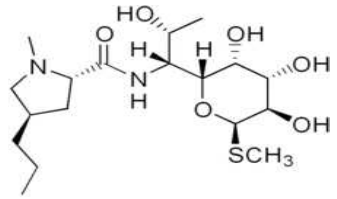

Fig.1: Structure of octatetracycline.

Many researchers have developed useful methods estimation of Oxytetracycline using HPLC, for example, Many researchers have developed useful methods estimation of Oxytetracycline using HPLC, for example, Pavagada J. Ramesh[4] Development and validation of RP-HPLC method for the

determination of doxycycline hyclate in spiked human urine and pharmaceuticals. G.Violeta Tauber[5] development and validation of a HPLC method for the determination of metronidazole, oxytetracycline and furazolidone in veterinary formulations. Elena Pătruț[6] Development and validationof an HPLC method for the determination of oxytetracycline and enrofloxacin in veterinary formulations. Cezary Kowalski[7] Rapid And Validated Hplc Assay For The Determination Of Oxytetracycline In Biological Material. Patrícia Penido Maia[8], Determination of oxytetracycline in tomatoes by HPLC using fluorescence detection. Uday A. Deokateet.Al[9], Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Doxycycline Hyclate And Tinidazole In Bulk And Tablet Dosage Form. Chilumuru Rama Mohana Rao[10], Quantitative Analysis of Oxytetracycline Residues in Honey by High Performance Liquid Chromatography. Konstantina I. Nikolaidou[11], Development and Validation of an HPLC Method for the Determination of Seven Tetracycline Antibiotics Residues in Chicken Muscle and Egg Yolk According to 2002/657/EC. M.Akiful[12]Original Article Method Development and Validation for the Simultaneous Estimation of Doxycycline and Ornidazole in Bulk and Pharmaceutical Dosage Form by Using RP-HPLC Method.

Results and Discussion:

Linearity:

Linearity of detector response for Oxy Tetra Cycline Hcl:

Table 1: Linearity of Detector Response for Oxy Tetra Cycline Hcl

S.No.	Conc.(µg/ml) of Oxy Tetra Cycline Hcl	Area Oxy Tetra Cycline Hcl	Acceptance criteria
1	25.00	383813	Squared co relation coefficient should be not less than0.999.
2	62.50	757627	
3	125.00	1455255	
4	250.00	2810510	
5	312.50	3578137	
6	375.00	4265765	

Linearity graphs of Oxy Tetra Cycline Hcl:

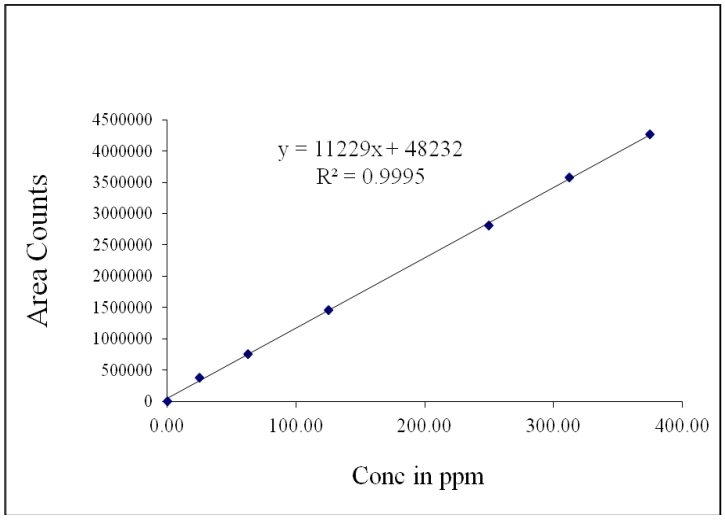

Fig 2: linearity of Detector Response Graphs for Oxy Tetra Cycline Hcl

Table No 2: Linearity of Detector Response Graphs for Oxy Tetra Cycline Hcl

S.No	Linearity Parameters	Aceclofenac
1.	Linearity range	10.0-150.0 µg/ml
2.	Correlation coefficient	0.999
3.	Y intercept	28337x+16612

Robustness:
Flow rate and Oraganic variation:

Table 3: Flow rate and Organic phase variations

Parameters	Oxy Tetra Cycline Hcl		%RSD
Flow rate	Retention Time	Tailing factor	
0.8ml/min	4.397	1.14	1.52
1.0ml/min	3.284	0.28	0.75
1.2ml/min	2.654	0.84	0.62
Organic phase			
77:23	4.169	1.18	1.02
70:30	3.289	1.12	0.84
63:37	2.050	0.26	0.46

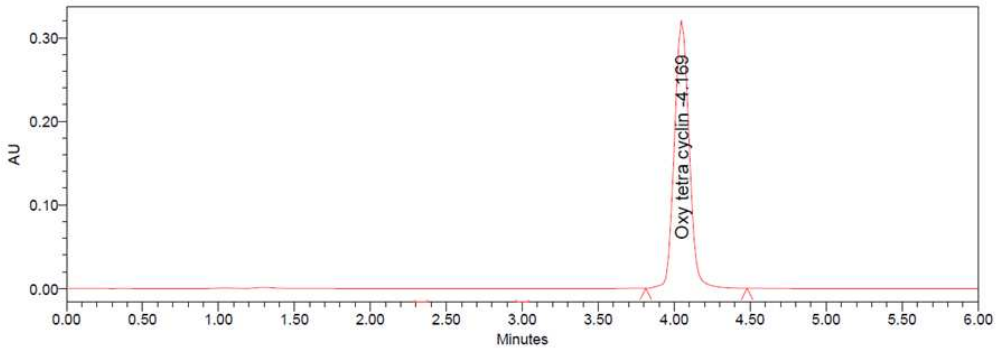

Fig 3: Typical Chromatogram of Robustness (Organic minus 77:23)

Analytical method validation of oxy tetra cycline Hcl:-
System Suitability:

Table 4: Results for System Suitability of Oxy Tetra Cycline Hcl

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.314	2848089	4952	1.14
2	3.318	2844302	4833	1.11
3	3.317	2846510	4813	1.12
4	3.318	2840506	4922	1.16
5	3.314	2848749	4835	1.17
6	3.282	2843079	4859	1.14
Mean	3.313	2844563	4884	1.12
SD	0.012	4257.57	--	--
%RSD	0.46	0.5834	--	--

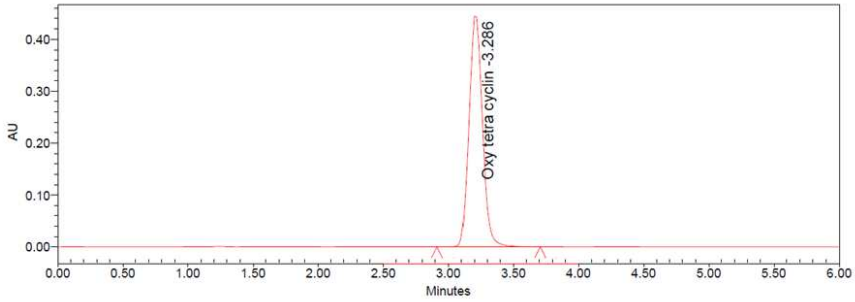

Fig 4: Typical Chromatogram of Accuracy 150%

Conclusion: In conclusion a validated RP-HPLC method has been developed for determination of Oxytetracycline the bulk and tablet dosage forms. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of Oxytetracycline Capsule dosage form. Several analytical procedures have been proposed for the quantitative estimation of Oxytetracycline separately and in combination with other drugs. So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Oxytetracycline in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

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