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Rp-Hplc Method For Simultaneous Estimation Of Tobramycin And Dexamethasone In Combined Dosage Form

SYED NIZAMUDDIN[1] , APPALA RAJU[2]

[1] Department of Pharmaceutical Analysis, RR College Of Pharmacy, Bangalore, Karnataka-560090

[2] Department of Pharmaceutical Analysis, HKE's College Of Pharmacy, Gulbarga, Karnataka-585105

ABSTRACT

A rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method hasbeen developed for simultaneous estimation of tobramycin and dexamethasone.Chromatographic separation was achieved on reverse phase Hypersil BDS C_{18} column (150 X 4.6 mm,5 μm) using the mobile phase consisting of phosphate buffer pH 6.0 and acetonitrile in the ratio of 70:30, v/v. The mobile phase was pumped at a flow rate of 1.0 ml/min and detection was done by UVdetector at 263 nm. The proposed method was found to be simple, fast, accurate, precise and reproducible and could be applied for routine quality control analysis for simultaneous determination of tobramycin and dexamethasone in pharmaceutical dosage forms.

Keywords: Tobramycin and Dexamethasone,, RP-HPLC, Validation.

INTRODUCTION

Tobramycin is an aminoglycoside antibiotic derived from Streptomyces tenebrarius that is used to treat various types of bacterial infections, particularly Gram-negative infections. It is especially effective against species of Pseudomonas [1]. It was patented in 1965, and approved for medical use in 1974[2]. Like all aminoglycosides, tobramycin does not pass the gastro-intestinal tract, so for systemic use it can only be given intravenously or by injection into a muscle. Eye drops and ointments (tobramycin only, Tobrex, or combined with dexamethasone, sold as Tobradex) and nebulised formulations both have low systemic absorption. The formulation for injection is branded Nebcin. The nebulised formulation (brand name Tobi) is indicated in the treatment of exacerbations of chronic infection with Pseudomonas aeruginosa in people diagnosed with cystic fibrosis.

Tobramycin closely resembles gentamicin in its microbiological and toxicological properties. The two drugs have similar half-lives, peak serum concentrations, lack of protein binding, volumes of distribution, and predominantly renal excretion by glomerular filtration. The main advantage of tobramycin may be its greater intrinsic activity against Pseudomonas aeruginosa. Not all bacterial strains resistant to gentamicin are invariably also resistant to tobramycin. Because of its inherent potential for ototoxicity and nephrotoxicity, renal function and eighth nerve function should be closely monitored. Tobramycin is a 4,6-disubstituted 2-deoxystreptamine (DOS) ring-containing aminoglycoside antibiotic with activity against various Gram-negative and some Gram-positive bacteria. The mechanism of action of Tobramycin has not been unambiguously elucidated, and some

insights into its mechanism rely on results using similar aminoglycosides. In general, like other aminoglycosides, Tobramycin is bactericidal and exhibits both immediate and delayed killing, which are attributed to different mechanisms. Toxicity information regarding Tobramycin is not readily available. Patients experiencing an overdose are at an increased risk of severe adverse effects such as nephrotoxicity, ototoxicity,

neuromuscular blockade, and respiratory failure/paralysis. Symptomatic and supportive measures are recommended; hemodialysis may help clear excess Tobramycin.

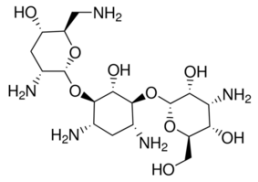

Fig. 1 : Structure Of Tobramycin

DEXAMETHASONE

Dexamethasone is a glucocorticoid medication used to treat rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, eye pain following eye surgery, and along with antibiotics in tuberculosis. It may be given by mouth, as an injection into a muscle, as an injection into a vein, as a topical cream or ointment for the skin or as a topical ophthalmic solution to the eye. The effects of dexamethasone are frequently seen within a day and last for about three days. Long-term use of dexamethasone may result in thrush, bone loss, cataracts, easy bruising, or muscle weakness. Dexamethasone has anti-inflammatory and immunosuppressant effects. Dexamethasone was first synthesized in 1957 by Philip Showalter Hench and was approved for medical use in 1961. It is on the World Health Organization's List of Essential Medicines. In 2017, it was the 321st most commonly prescribed medication in the United States, with more than one million prescriptions. Dexamethasone is a fluorinated steroid that is 9-fluoropregna-1,4-diene substituted by hydroxy groups at positions 11, 17 and 21, a methyl group at position 16 and oxo groups at positions 3 and 20. It is a synthetic member of the class of glucocorticoids. It has a role as an adrenergic agent, an antiemetic, an antineoplastic agent, an environmental contaminant, a xenobiotic, an immunosuppressive agent and an anti-inflammatory drug. It is a fluorinated steroid, a 3-oxo-Delta(1),Delta(4)-steroid, a glucocorticoid, a 20-oxo steroid, an 11beta-hydroxy steroid, a 17alpha-hydroxy steroid and a 21-hydroxy steroid. Literature survey has revealed that co-administration of steroids increases the antiemetic efficacy of 5-HT_3 receptor antagonist. granisetron when combined with dexamethasone has found to be the most effective regimen for prevention of post operative nausea and vomiting and during chemotherapy of cancer[3-5] and only one HPLC method have been reported for the simultaneous estimation of dexamethasone and granisetron[6] and no method was reported for tobramycin and dexamethasone. Hence, the purpose of presented work is to develop and validate a simple, rapid, accurate and precise RP-HPLC method for simultaneous estimation of tobramycin and dexamethasone in a combineddosage form.

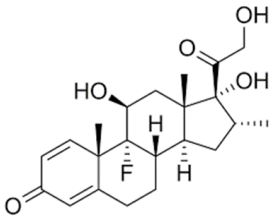

Fig. 2 : Structure Of Dexamethasone

EXPERIMENTAL

Chromatographic Conditions

The chromatographic separation was achieved on Shimadzu LC isocratic system Shimadzu LC isocratic HPLC system with Isocratic solvent delivery pump PDA-SPD M-10AVP photo diode array detector Precision loop injector - RHEODYNE, LC Solution data station was applied for data collecting and processing, analytical column such as, Phenomenex C18 (250mm X 4.6mmI.D, 5µ), flow rate 1.0 ml/min, wave length 250 nm, at Room temperature, injection volume10 µl .

Chemicals and Solvents

The working standards of tobramycin and dexamethasone were provided as giftsamples from Chandra Labs, Hyderabad, India.Tobramycin and dexamethasonetablets were purchased from local market. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC gradeacetonitrile was purchased from E. Merck (India)Ltd., Mumbai, India. HPLC grade water obtainedfrom Milli Q water purification system was usedthroughout the study.

Preparation of mobile phase and diluents

700 mL of phosphate buffer pH 6.0 (1.6 g of potassium dihydrogen phosphate and 0.3g of dipotassium hydrogen phosphate wasdissolved in 1000 ml water, adjusted pH 6.0±0.1 with orthophosphoric acid) was mixed with 300 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter undervacuum. The same mobile phase was used as diluent.

Preparation of standard stock solution

Accurately weighed and transferred 100 mg ofdexamethasone and 10 mg of tobramycin working standards into 100 ml volumetric flask,about 60 ml of diluent was added, sonicated to dissolve it completely and made volume up tothe mark with the same solvent.

Preparation of standard solution

Pipetted 10 ml of the standard stock solution into 100 ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution

Five containers of ophthalmic suspension, containing tobramycin 3.0 mg/ml, were shaken gently and transferred to a glass beaker and mixed. About 5.0 gm of ophthalmic suspension was weighed accurately into a 25 ml volumetric flask, about 10 ml of diluent was added, shaken to disperse the sample, the solution was filtered through a 0.45 µm membrane filter and diluted to volume with diluent and mixed. From this aliquot amount transfer to 10 ml volumetric flask to get concentration of 25 µg/ml of dexamethasone and 12 µg/ml tobramycin. UV spectra's of tobramycin and dexamethasone Absorbance maxima of tobramycin anddexamethasone were detected at 200 nm and 250 nm , respectively. The UV spectrum of tobramycin anddexamethasone were shown in Figure 3.

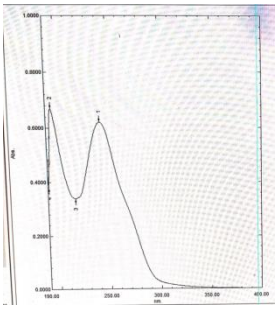

Figure 3: UV Spectra of Tobramycin and dexamethasone in sample

Method validation

The developed analytical method was validatedas per ICH guidelines[7] for the parameters like linearity, accuracy, precision, ruggedness, specificity and system suitability.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of drug in the samples within a given range. The linearity graphs were plotted between the absorbance versus concentration to obtain the calibration curve. Linearity graphs for tobramycin and dexamethasone for the concentration 25-150 µg/ml and 50-175 µg/ml were shown in Figure 5 and 6. The response obtained for tobramycin and dexamethasone was found to be linear. The correlation coefficient observed for tobramycin and dexamethasone compounds was not <0.99 and also statistical values for these compounds were shown in Table 1 and 2.

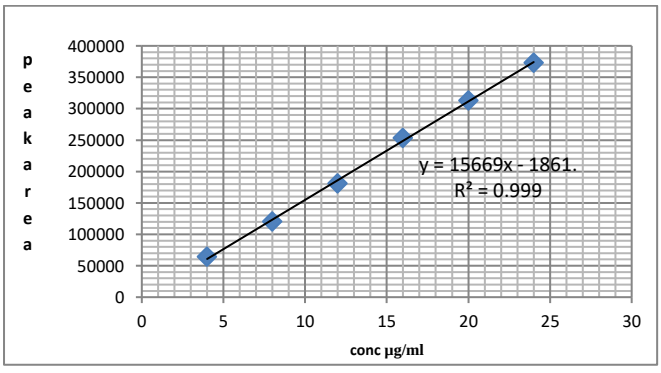

Figure 5. Calibration curve of Tobramycin

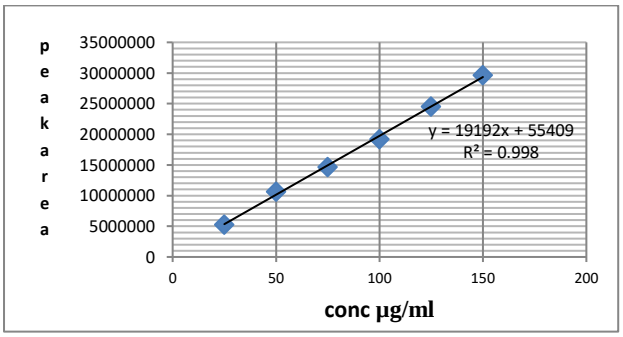

Figure 6. Calibration curve of Dexamethasone

Table 1: Calibration data of Tobramycin

Conc (µg/ml)	Peak area (n=6)	%RSD
4	64189	0.374
8	120593	0.142
12	180916	0.632
16	253455	0.135
20	312745	0.911
24	373118	0.116

Table 2: Calibration data of Dexamethasone

Conc (µg/mL)	Peak area (n=6)	%RSD
25	5307956	0.53
50	10669245	0.15
75	14687167	0.77
100	19213842	0.15
125	24543476	1.71
150	29665676	0.16

Accuracy

Accuracy was performed, and % recovery was found to be within 99-102% at all threelevels. This indicates that the dexamethasone (Table 3and 4) and tobramycin can be recoveredsuccessfully in presence of excipients. It was concluded that the developed method is capablefor the estimation of tobramycin and dexamethasone drug substances and isadequate for routine analysis.

Table 3: Accuracy data of Tobramycin (n=3).

Level of recovery	Sample Conc. (µg/ml)	Conc. of Std added (µg/ml)	Total Conc. (µg/ml)	Peak Area	Mean Peak Area1	Amt. Recovered (µg/ml)	% Recovery
80%	12	9.6	21.6	335806 335703 334815	335441	21.51	99.58

100%	12	12	24	373118 372216 373225	372853	24.23	100.95
120%	12	14.4	26.4	410429 411324 410426	410726	26.32	99.67

[1]Mean area of n=3.

Table 4: Accuracy data of Dexamethasone (n=3).

Level of recovery	Sample Conc. (µg/ml)	Conc. of Std added (µg/ml)	Total Conc. (µg/ml)	PeakArea	Mean Peak Area[1]	Amt. Recovered (µg/ml)	% Recovery
80%	25	20	45	9602320 9612210 9601421	9605317	44.98	99.95
100%	25	25	50	10659245 10667144 10679242	10668543	49.79	99.58
120%	25	30	55	11736167 11726178 11746269	11736204	55.34	100.61

[1]Mean area of n=3.

Precision

The precision of an analytical procedure expresses the closeness of agreement betweena series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions. Sample stock Solution containing 25, 75 and 125 µg/ml of tobramycin and 50, 100 and 150 µg/ml of dexamethasone was prepared from their respective solution. Analysis was performed in triplicate; the result of intra-day precision studies was shown in Table 5.

Table 5: Intra-day precision data of Dexamethasone and Tobramycin.

Sl.No	Dexamethasone			Tobramycin		
	Peak area			Peak area		
	Sample -1	Sample -2	Sample -3	Sample -1	Sample -2	Sample -3
1	5307956	14687167	24543476	64189	180916	312745
2	5316852	14387162	24342474	64176	181912	312638
3	5327953	14587126	24453353	64298	180725	313643
Average	5317587	14553818.33	24446434.33	64221	181184.333	313008.6667
SD	10018.741	152750.779	100679.4515	67	637.372	551.947
% RSD	0.18840	1.04955	0.41183	0.10432	0.35178	0.176336

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision three replicates of a

different concentration of sample solution are used for each determination. First day: 3 replicates, on a second day: 3 replicates, then on third day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions. The result of interlay reproducibility and Percentage amount obtained was shown in Table 6.

Table 6: Inter-day reproducibility data of Dexamethasone and Tobramycin.

	Assay (% labeled amount)					
Sample No	Dexamethasone			Tobramycin		
	DAY-1	DAY-2	DAY-3	DAY-1	DAY-2	DAY-3
Sample -1	99.95	99.92	99.46	99.58	101.76	100.02
Sample -2	99.58	99.92	98.43	100.95	99.46	100.34
Sample -3	100.61	100.32	100.22	99.67	99.69	99.28
Average	100.04	100.05	99.37	100.06	100.30	99.88
SD	0.52175	0.23094	0.89838	0.76631	1.2667	0.5437
%RSD	0.52151	0.23081	0.90408	0.76580	1.2629	0.5443

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of dexamethasone and tobramycin found to be 7.3 µg/ml and 0.88 µg/ml respectively. The LOQ of dexamethasone and tobramycin found to be 22.2 µg/ml and 2.69 µg/ml respectively.

System suitability test

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition at a 100% level to verify the precision of the chromatographic system. The purposed RP-HPLC method permits the determination of tobramycin and dexamethasone in sample drug have different retention times. Data are given in Table 7. The chromatogram of tobramycin and dexamethasone was shown in Figure 4. There was clear resolution between tobramycin and dexamethasone with retention time of 3.12 and 5.90 minutes; respectively.

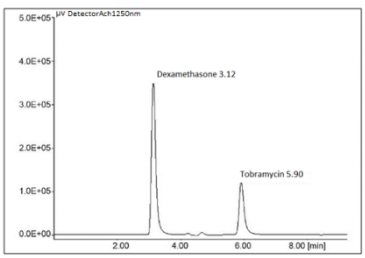

Figure 4. Developed chromatogram of Dexamethasone and Tobramycin

Table 7. System suitability was assessed using a sample as six injections

Drug Name	Retention Time	Area	Tailing Factor	Theoretical Plate
Dexamethasone	3.12	14687167	1.272	3587.132
Tobramycin	5.90	180916	0.832	3602.006

Estimation of Tobramycin and Dexamethasone in ophthalmic dosage forms

Commercial formulation of eye drops was chosenfor testing the suitability of the proposed method toestimate tobramycin and dexamethasone in ophthalmic. The results of the assay were found to be within of label claim for formulations containing dexamethasone and tobramycin, as shown in Table 8.

Table 8: Assay data Dexamethasone and Tobramycin (n=3).

Drug	Label Claim (mg)	Sample Conc. (µg/mL)	Peak Area
Dexamethasone	1	25	5317946 5306934 5307755
%Assay ± SD			99.82±6134
%RSD			0.11550874
Tobramycin	3	12	181916 182912 180523
%Assay ± SD			100.0833±1199
%RSD			0.660117139

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drugs tobramycin and dexamethasone in a combined dosage form. Hypersil BDS C_{18} column in isocratic mode, with mobile phase phosphate buffer pH 6.0 and acetonitrile (70:30 v/v) (pH was adjusted to 6.0 with orthophosphoric acid) resulted in peak with good shape and resolution. The flow rate was 1ml/min and tobramycin and dexamethasone were measured with UV detector. Linearity was assessed by plotting concentration vs area within the range of concentration 25-150 µg/ml and 50-175 µg/ml for tobramycin and dexamethasone with correlation coefficient of Y=19192x + 55409, r^2 =0.998 and tobramycin Y=15669x + 1861, r^2 =0.999. The % recovery was found to be was found to be within 99-102% at all threelevels within limits of the acceptance criteria for tobramycin and for dexamethasone. The high percentage ofrecovery indicates that the proposed method is highly accurate. The %RSD for intra-day and inter-day precision is less than 2% for tobramycin and dexamethasone. The detection limit of the proposed method was 7.3 µg/ml and 0.88 µg/ml respectively and the quantification limit was 22.2 µg/ml and 2.69 µg/ml for tobramycin and dexamethasone respectively which indicate thesensitivity of the method. The assay procedureswere repeated for three trials and the results werefound to give 99.82±6134 and 100.0833±1199. The number of theoretical plates calculated was 3602.006 and

3587.132 for tobramycin and dexamethasone respectively, which indicates efficient performance of the column. No interfering peakswere found in the chromatogram of the formulation within the run time indicating that excipients used in formulations did not interfere with the simultaneous estimation of the drugs tobramycin and dexamethasone by the proposed HPLC method.

CONCLUSION

A simple, specific, sensitive, rapid, accurate andprecise RP-HPLC method has been developed for simultaneous estimation of tobramycin and dexamethasone. The result of the research work follows the protocol of ICH guidelines andit can be successfully applied for the simultaneous estimation of the marketed products of tobramycin and dexamethasone and in combined tablet formulations.

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