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HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATROPINE SULFATE AND DEXAMETHASONE

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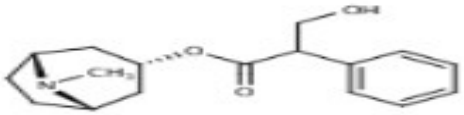
Abstract: -

Analysis of pharmaceutical product is very important as it concerned with quality of life. The objective of present work is to develop sensitive, specific, reproducible, accurate method for determination of Atropine sulfate and Dexamethasone in its combined Liquid dosage form by HPLC method. In HPLC method for Atropine sulfate and Dexamethasone, chromatographic 18 separation was carried out on Thermo Hypersil ,C column (250mm x 4.6mm i.d. 5µm) using mobile phase 0.02M Pottasium dihydrogen ortho Phosphate Buffer pH 4 : Methanol (75:25 v/v), flow rate 1.5 ml/min and detected at 210 nm. Linearity of Atropine sulfate and Dexamethasone found to be 25-75 and 2.5-7.5µg/ml respectively. Developed HPLC method was validated as per ICH guideline Q2 (R1), for its accuracy, precision, LOD & LOQ and the results were found to be satisfactory Developed and validated method was found to be simple, accurate, economical, robust and reproducible & method can be successfully applied for routine QC analysis.

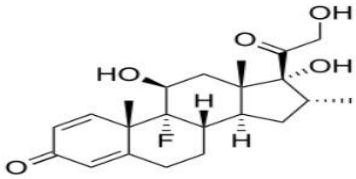
Keywords: - *Atropine sulfate, Dexamethasone, HPLC*

INTRODUCTION:

Atropine Sulfate Atropine binds to and inhibits muscarinic acetylcholine receptors, producing a wide range of Anticholinergic effects. Its Bioavailability & half-life is 25% & 2 hrs respectively. Its Molecular formula & molecular weight is $(C_{17}H_{23}NO_3)_2$, H_2SO_4 & 694.8 gm/mol respectively. Its IUPAC name is benzene acetic acid, alpha- (hydroxyl methyl)-8- methyl-8azabicyclo {3.2.1} oct-3-yl ester endo. Its structure is below:



It is Very soluble in water. 1gm dissolves in 0.4 ml water. 1gm dissolves in 5ml cold alcohol and 2.5 ml boiling alcohol, in 2.5ml glycerol, 420ml chloroform and 3,000 ml ether. Dexamethasone is a glucocorticoid agonist. Unbound dexamethasone crosses cell Membranes and binds with high affinity to specific cytoplasmic glucocorticoid receptors. It's Bioavailability & half-life is 80-90% & 36-54 hrs respectively. Its Molecular formula & molecular weight is C22H29FO5 & 392.47 gm/mol respectively. Its IUPAC name is 9-Fluoro-11β, 17, 21- trihydroxy-16α methylpregna. Its structure is below:



It is practically insoluble in water, sparingly soluble in alcohol, and slightly soluble in chloroform. [1-4] there are some analytical methods done for simultaneous estimation of Atropine sulfate and Dexamethasone.[5-17] so here it's thought to develop HPLC derivative method for simultaneous for same and validated method by ICH guide line.

Materials and methods:

Preparation of standard solutions:

Preparation of buffer pH 4

0.25 gm of Pottasium dihydrogen ortho phosphate was dissolved in 1000ml of HPLC grade water and pH was adjusted to 4 with the help of Sodium hydroxide solution.

Preparation of mobile phase

500 ml of Buffer (pH 4) and 500 ml of Methanol (HPLC grade) were mixed and filtered through 0.45 µm filter, sonicated for 10 minutes to degas and used as mobile phase. Use mobile phase as a diluents.

Preparation of Standard Stock Solution Of Atropine Sulfate

Accurately weighed quantity of ATROPINE SULFATE 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with diluents (mobile phase). This will give a stock solution having strength of 1000 µg/ml.and take 10ml & make up 100ml.(100 µg/ml)

Preparation Of Standard Stock Solution Of Dexamethasone

Accurately weighed quantity of DEXAMETHASONE 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with diluents(mobile phase). This will give a stock solution having strength of 1000 µg/ml.

Calibration Curve for the Atropine Sulfate (25-75 µg/Ml) & Dexamethasone (2.5-7.5 µg/ml)

Appropriate volume (2.5,3.75,5,6.25,7.5 µg/ml) of aliquots from standard Atropine sulfate and Dexamethasone stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 25, 37.5, 50, 62.5, 75 µg/ml Atropine sulfate and(0.25,0.375,0.5,0.625,0.75 µg/ml) Dexamethasone. Each of these mixed standard solutions was chromatographed for 10 minutes run time using mobile phase at flow rate of 1.5 ml/min. The graphs were plotted for peak area vs. concentration for both the drugs.

Validation

System suitability:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. System suitability test was carried out to verify that the analytical system is working properly to give accurate and precise results. Standard solution (25µg/ml) of Atropine sulfate and 2.5 µg/ml of Dexamethasone) was injected six times and the chromatograms were recorded.

Linearity and Range:
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. The linearity peak area response was determined by analyzing solutions having concentrations in the range of 25-75 µg/ml and 2.5-7.5 µg/ml for Atropine sulfate and Dexamethasone respectively from same solution. Peak area of each solution was measured using developed method. Calibration curve of peak area vs. concentration was plotted. The correlation coefficient and regression line equations for Atropine sulfate and Dexamethasone were determined.

Precision:
Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. It was studied by carrying out System precision and Method Precision

System Precision was determined from results for six replicates of synthetic mixture. 6 replicates of standard mixture solution having Atropine sulfate (50µg/ml) and Dexamethasone (5µg/ml) were prepared and chromatograms were recorded and RSD was calculated.

Method Precision was determined from results for six replicates of formulation. 6 replicates of standard mixture solution having Atropine sulfate (50µg/ml) and Dexamethasone (5µg/ml) were prepared and chromatograms were recorded and RSD was calculated.

Intraday precision: Standard solutions containing 25, 50 and 75µg/ml Atropine sulfate and 2.5, 5 and 7.5µg/ml Dexamethasone were analyzed 3 times on the same day. Chromatogram of each sample was recorded. SD and RSD were calculated.

Interday precision: Standard solutions containing 25, 50 and 75µg/ml Atropine sulfate and 2.5, 5 and 7.5µg/ml Dexamethasone were analyzed on three different days. Chromatogram of each sample was recorded. SD and RSD were calculated.

Accuracy:
Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120%) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Atropine sulfate and Dexamethasone by standard addition method.

LOD & LOQ:
The LOD & LOQ are is estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as;
LOD = 3.3 x (SD / Slope) LOQ = 10 x (SD / Slope)
Where, SD = the standard deviation of Y- intercept of 5 calibration curves. Slope = the mean slope of the 5 calibration curves.

Robustness:
The robustness of an analytical method was carried out to confirm that the method remained unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution was injected five times for each varied conditions of flow, column temperature, pH, and mobile phase ratio and chromatograms were recorded.

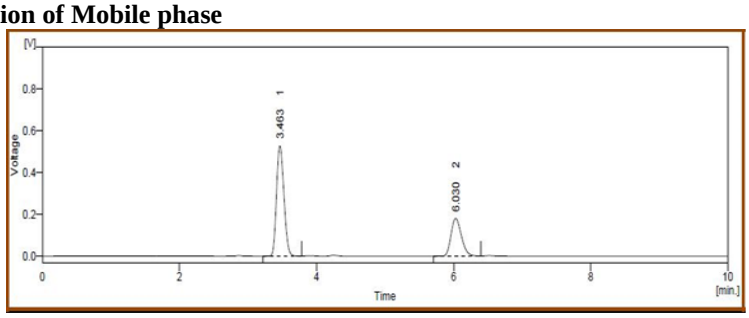
Analysis of marketed formulation: (ASSAY)
An accurately weight equivalent to about 100 mg of Atropine sulfate and 100 mg of Dexamethasone was transferred to 100 ml volumetric flask and the volume was made up to the mark using mobile phase. The solution was sonicated for 20 minutes. The solution was filtered through whatman Filter Paper No.42. First few ml of filtrate was discarded. 0.5 ml of the solution from above filtrate was diluted to 100 ml with mobile phase. The prepared sample solution was chromatographed for 10 minutes run time using mobile phase at 210 nm and a flow rate of 1.5 ml/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated by fitting peak area responses into the equation of the straight line representing the calibration curves for Atropine sulfate and Dexamethasone.

Result and Discussion:
Selection of Detection wavelength
Atropine sulfate and Dexamethasone were scanned in UV as well different trails were taken in RP-HPLC at different wavelength in which both Atropine sulfate and Dexamethasone show reasonably good response at 210 nm.

Selection & Optimization of Mobile phase

Different mobile phases were tried in order to find the best conditions for the separation of both the drugs. It was found that 0.02M Pottasium dihydrogen ortho phosphate pH 4: Methanol (75:25 v/v) gives satisfactory results as compared to other mobile phases

Figure No 1: Optimization of Mobile phase



Optimized criteria fulfilled for method development

Table 1: Optimized criteria fulfilled for method development

PARAMETER	ATROPINE SULFATE	DEXAMETHASONE
Retention Time (min.)	3.463 min	6.030 min
Asymmetry Factor (AS)	1.321	1.359
Theoretical Plates (N)	4369	7252
	10.416	

System suitability

Figure 2: Chromatogram of Standards for System Suitability

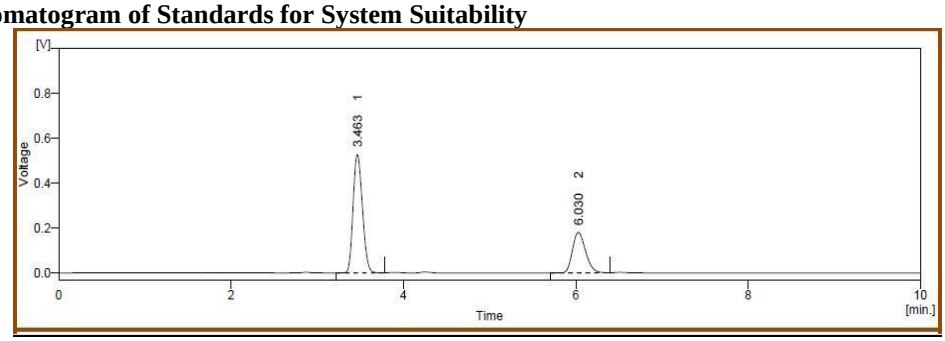


Table 2: System suitability data for Atropine sulfate & Dexamethasone

Sr. No.	STANDARD RESPONSE (MAU*S)	
	Atropine sulfate (25 µg/ml)	Dexamethasone (2.5µg/ml)
1	2005.151	938.084
2	2005.150	930.024
3	2104.175	935.085
4	2003.150	940.444
5	2026.200	950.278
6	2046.387	952.328
Average	2031.702	941.0405
SD	39.30251	8.702494
%RSD	1.93	0.92
Tailing Factor	0.09	0.25
Resolution	10.416	

Linearity and Range

Table No 3: Linearity data for ATROPINE SULFATE and DEXAMETHASONE

ATROPINE SULFATE		DEXAMETHASONE	
Concentration (µg/ml)	**Area**	**Concentration (µg/ml)**	**Area**
25	2005.151	**25**	93.084
37.5	2960.063	**3.75**	1384.8
50	4046.815	**5**	1893.199
62.5	4982.488	**6.25**	2230.987
75	6062.048	**7.5**	2835.999

Figure 3: Chromatograms of Atropine sulfate and Dexamethasone for Linearity

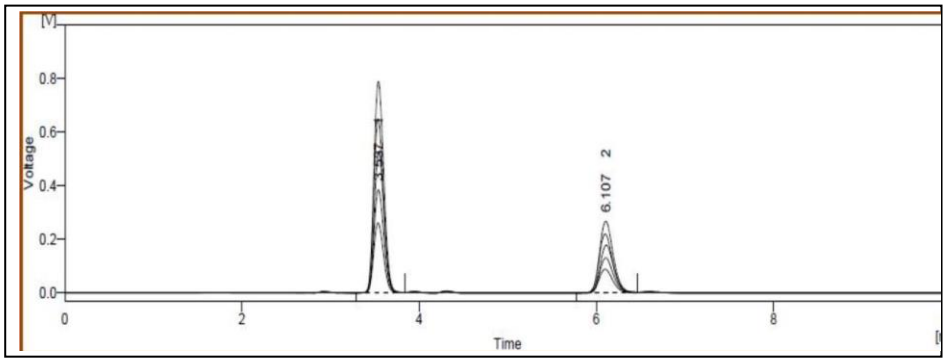


Figure 4: Calibration curve of Atropine sulfate

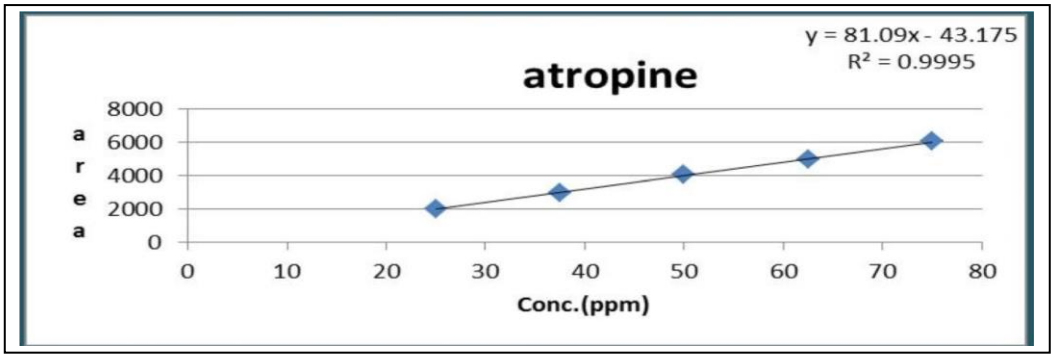


Figure 5: Calibration curve of Dexamethasone

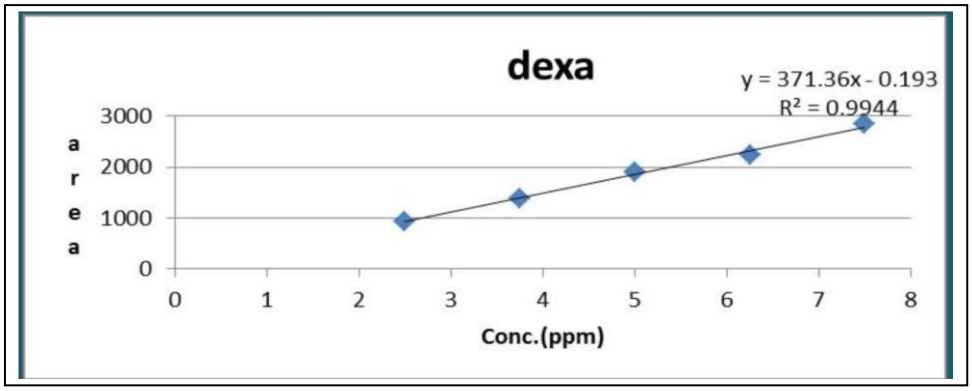


Table 4: Repeatability data for Atropine sulfate and Dexamethasone

Atropine sulfate		Dexamethasone	
Concentration (µg/ml) at 100%	**Peak Area (mAU*S)**	**Concentration (µg/ml) at 100%**	**Peak Area (mAU*S)**
50	4029.734	**5**	1885.653
50	3974.092	**5**	1889.375
50	4046.759	**5**	1831.745
50	4054.879	**5**	1896.985
50	4038.618	**5**	1889.435
50	4046.727	**5**	1893.162
Mean	**4031.8015**	**Mean**	**1881.059**
SD	**29.53030302**	**SD**	**24.46389**
%RSD	**0.732434447**	**%RSD**	**1.300538**

Table 5: Intraday precision data for estimation of Atropine sulfate and Dexamethasone

Atropine sulfate Concentration (µg/ml)	**Peak Area Mean (mAU*S)**	**%RSD**	**Dexam. Concentration (µg/ml)**	**Peak Area Mean (mAU*S)**	**%RSD**
25	1992.7536	0.3388558	**2.5**	930.131	0.738302
50	3994.9246	0.7869067	**5**	1876.9783	0.752931
75	6016.8513	0.2525370	**7.5**	2815.2333	0.551645

Table 6: Interday precision data for estimation of Atropine sulfate and Dexamethasone

Atropine sulfate Concentration (µg/ml)	Peak Area Mean (mAU*S)	%RSD	Dexam. Concentration (µg/ml)	Peak Area Mean (mAU*S)	%RSD
25	1987.002	0.980591	**2.5**	930.33566	0.844997
50	4024.336	0.386472	**5**	1876.9446	0.632838
75	6019.712	0.625808	**7.5**	2810.631	0.694105

Table 7: Accuracy (%Recovery) data for Atropine sulfate (n=3)

Level of recovery	Sample amt. µg/ml	Std. amt. of Atropine sulfate added µg/ml	Mean % recovery	Mean % RSD
80 %	20	16	99.754	0.999
100 %	20	20	99.378	0.340
120 %	20	24	99.559	0.395

Table 8: Accuracy (%Recovery) data for Dexamethasone (n=3)

Level of recovery	Sample amt. µg/ml	Std. amt. of Atropine sulfate added µg/ml	Mean % recovery	Mean % RSD
80 %	2.5	2	99.889	1.141
100 %	2.5	2.5	99.602	0.664
120 %	2.5	3	99.609	0.478

LOD & LOQ

Table 9: LOD & LOQ data for Atropine sulfate and Dexamethasone

PARAMETERS	ATROPINE SULFATE	DEXAMETHASONE
Mean Slope (n=5)	81.09	371.3
SD (n=5)	42.7172	63.6715
LOD	1.738	0.565
LOQ	5.267	1.714

Table 10: Result of Change in Flow Rate

Sr. No	Flow rate 1.7 ml/min (+0.2ml/min)		Flow rate 1.3 ml/min (-0.2ml/min)	
	Atropine sulfate Area (mAU*S)	Dexamethasone Area (mAU*S)	Atropine sulfate Area (mAU*S)	Dexamethasone Area (mAU*S)
Mean	3946.666 ± 29.14373	1836.772 ± 24.92923	4192.206 ± 23.36439	1947.772 ± 28.13728
% RSD	0.738439	1.357231	0.557329	1.444588

Table 11: Result of Change in pH

Sr. No	pH (+2)		pH (2)	
	Atropine sulfate Area (mAU*S)	Dexamethasone Area (mAU*S)	Atropine sulfate Area (mAU*S)	Dexamethasone Area (mAU*S)
Mean	3855.13 ± 44.94767	1804.993 ± 13.80097	4141.604 ± 30.23082	1933±22.9375
% RSD	1.165919	0.764599	0.72993	1.186627

Table 12: Result of Change in M.P.

Sr. No	M.P. (+2)		M.P. (-2)	
	Atropine sulfate Area (mAU*S)	Dexamethasone Area (mAU*S)	Atropine sulfate Area (mAU*S)	Dexamethasone Area (mAU*S)
Mean	3935.19± 44.79152	1840.076 ± 16.91332	4135.259± 37.93544	1928.658±10.99112
% RSD	1.13823	0.919164	0.917365	0.569884

Analysis of marketed formulation (ASSAY):

Table 13: Analysis of marketed formulation

BRAND NAME	DRUGS	LABEL CLAIM	AMOUNT FOUND	% ASSAY
DEXAPINE	Atropine Sulfate	1 W/V	1.01 % W/V	101 %
	Dexamethasone	0.1 W/V	0.098 % W/V	98 %

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