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FIRST ORDER DERIVATIVE METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATROPINE SULFATE AND DEXAMETHASONE

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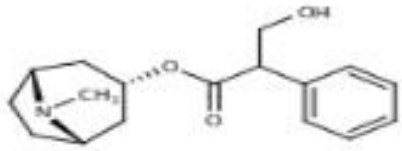
ABSTRACT

Analysis of pharmaceutical product is very important as it concerned with quality of life. The objective of present work is to develop and validate First order Derivative UV Spectrophotometry method for estimation of Atropine sulfate and Dexamethasone in its combined Liquid dosage form. Estimation of Atropine sulfate and Dexamethasone were carried out at λmax 239.60 nm and 246.68 nm for First Order Derivative Spectrophotometry Method, Using Methanol as a solvent. Linearity of Atropine sulfate and Dexamethasone were found to be 100-350 µg/ml and 10-35 µg/ml respectively. UV Spectophometric 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.

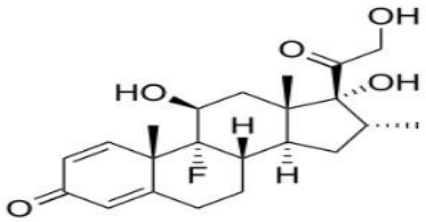
KEYWORDS: Atropine sulfate, Dexamethasone, First order derivative spectrophotometry, Methanol.

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-8- azabicyclo {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.[5-7] so here it's thought to develop first order derivative method for simultaneous for same and validated method by ICH guide line.

MATERIALS AND METHODS

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 Methanol. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of DEXAMETHASONE

100 µg/ml of Dexamethasone solution was prepared by diluting 10 ml of stock solution to 100 ml with Methanol.

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 Methanol. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of DEXAMETHASONE

100 µg/ml of Dexamethasone solution was prepared by diluting 10 ml of stock solution to 100 ml with Methanol.

Determination of wavelength for measurement

1.0 ml of working standard solution of Atropine sulphate (1000µg/ml) and 1.0 ml of working standard solution of Dexamethasone (100µg/ml) was pipette out into two separate 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get 100µg/ml of Atropine Sulphate and 10µg/ml of Dexamethasone. Each solution was scanned between 200-400 nm against methanol as a reagent blank for zero order spectra. The first order derivative spectra of each solution were obtained using smoothing ($\Delta\lambda$ = 2, Scaling Factor = 25). The first order derivative spectra of each solution were obtained. The zero crossing point (ZCP) of Atropine Sulfate at which Dexamethasone is measured and ZCP of Dexamethasone at which Atropine Sulfate is measured, obtained from the overlain spectra of both.

Preparation of Calibration Curve

Calibration curve for the ATROPINE SULFATE (100-350µg/ml)

Calibration curve for ATRO consisted of different concentrations of standard ATRO solution ranging from 100-350 µg/ml. The solutions were prepared by pipetting out 1.5, 2, 2.5, 3 and 3.5 ml of the working standard solution of ATRO (1000µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The curve of each solution against the Methanol was recorded. Absorbance of ATRO was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Calibration curve for the DEXAMETHASONE (10-35 µg/ml)

Calibration curve for DEXA consisted of different concentrations of standard DEXA solution ranging from 10-35 µg/ml. The solutions were prepared by pipetting out 1, 1.5, 2, 2.5,3 and 3.5 ml of the working standard solution of DEXA (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The curve of each solution against the Methanol was recorded. Absorbance of DEXA was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Validation of Proposed Method

Linearity and range

The linearity response was determined by analyzing independent levels of concentrations in the range of 100-350 and 10-30 µg/ml for ATRO & DEXA respectively. Absorbance of each solution was measured at selected wavelength respectively using developed method. Calibration curve of absorbance differences vs. concentration was plotted. The correlation coefficient and regression line equations for ATRO & DEXA were determined.

Precision

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.

Repeatability

A). System Precision was determined from results for six replicates of synthetic mixture. 6 replicates of 100 µg/ml concentrations of ATRO and 10µg/ml of DEXA were prepared and absorbance was measured at selected wavelength respectively. SD and RSD were calculated.

B). Method Precision was determined from results for six replicates of formulation. and absorbance was measured at selected wavelength respectively. SD and RSD were calculated.

Intraday Precision

Standard solutions containing 100,150 and 250µg/ml ATRO and 10, 15 and 20µg/ml DEXA were analyzed 3 times on the same day. The absorbance of solutions was measured at selected wavelength respectively. SD and RSD were calculated.

Interday Precision

Standard solutions containing 100, 150, and 250µg/ml ATRO and 10, 15 and 20µg/ml DEXA were analyzed on 3 different days. The absorbance of solutions was measured at selected wavelength respectively. SD and RSD were calculated.

4.3.5.3 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 ATRO & DEXA by standard addition method.

Preparation of sample solution for % recovery

An accurately weighed powder equivalent to about 1000 mg of ATRO and 100mg of DEXA was transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as solvent and aliquate them to make final concentration. The resulting solution was

filtered through Whatman filter paper. and take 1ml and make up 10ml. Absorbance of sample solutions was measured at selected wavelength of ATRO and DEXA and concentration is calculated which is known as pre-analyzed sample. In pre-analyzed sample 80, 100 and 120% of ATRO and DEXA was spiked. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated.

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The LOD and LOQ are estimated from the set of 6 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 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Analysis of marketed formulation (Assay)

Content accurately. An accurately weighed powder equivalent to about 100 mg of to 100 ml volumetric flask and the volume was made up to the mark using Metanol as solvent. The solution was sonicated for 20minutes. The solution was filtered through whatman Filter Paper No.42. First few ml of filtrate were discarded. 1.5 ml of the solution from above filtrate was diluted to 100 ml with Methanol. Resulting solution further diluted to 100 ml using same solvent. Find out the Conc. of both drugs by putting the values of absorbance at selected wavelength in the Equation. The above solution in triplicates were analysed and concentration was found out.

RESULT AND DISCUSSION

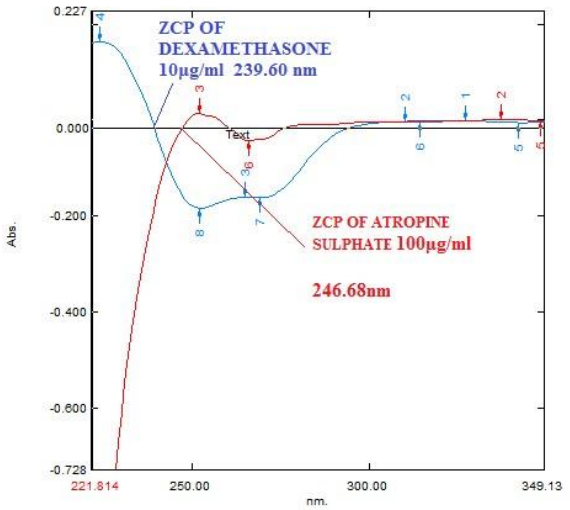


Figure No: 1 Selection of wavelength for simultaneous estimation of Atropine Sulphate and Dexamethasone

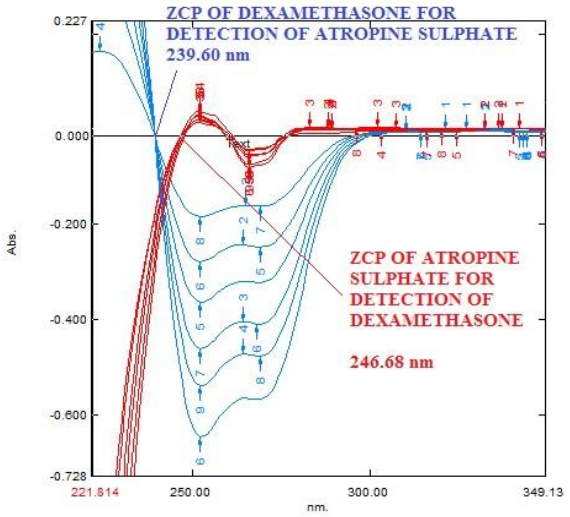


Figure 2: Linearity first order UV spectra of Atropine Sulfate and Dexamethasone.

Validation of Proposed Method

Linearity and Range

Table 1: Linearity data for Atropine Sulfate and Dexamethasone.

Atropine Sulfate at 239.60 nm		Dexamethasone at 246.68 nm	
Concentration (µg/ml)	**D^1Absorbance Mean* ± S.D.**	**Concentration (µg/ml)**	**D^1Absorbance Mean* ± S.D.**
100	-0.110 ±0.001378	10	-0.155 ±0.0008944
150	-0.141 ±0.001264	15	-0.232 ±0.001366
200	-0.173 ±0.0008944	20	-0.300 ±0.001751
250	-0.198 ±0.001048	25	-0.382 ±0.001414
300	-0.231 ±0.001378	30	-0.446 ±0.001414
350	-0.260 ±0.001751	35	-0.531 ±0.001505

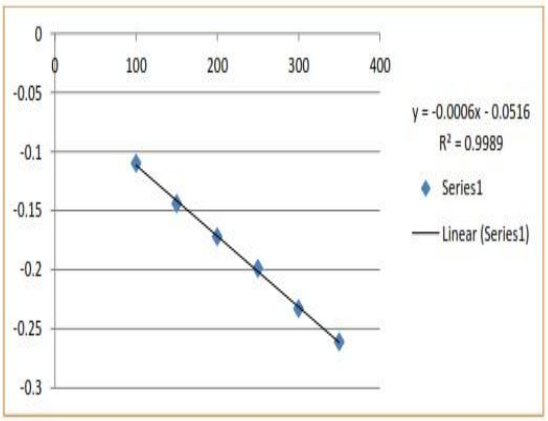


Figure 3: Calibration curve First order UV of Atropine Sulfate.

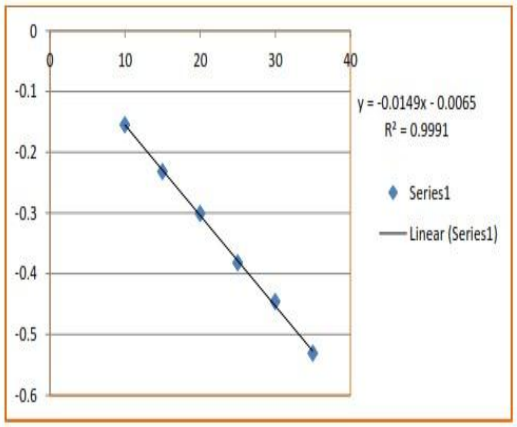


Figure 4: Calibration curve First order UV of Dexamethasone.

PRECISION REPEATABILITY

Table 3: Repeatability data for Atropine Sulfate and Dexamethasone.

ATRO at 239.60 nm		DEXA at 246.68 nm	
Concentration (µg/ml)	**D^1 Absorbance**	**Concentration (µg/ml)**	**D^1 Absorbance**
100	-0.113	10	-0.155
100	-0.111	10	-0.154
100	-0.112	10	-0.156
100	-0.113	10	-0.155
100	-0.11	10	-0.154
100	-0.109	10	-0.156
Mean	-0.11133	**Mean**	-0.155

Intraday precision

Table 4: Intraday precision data for estimation of Atropine Sulfate and Dexamethasone.

ATRO Conc. (µg/ml)	D^1Absorbance* ±S.D.	%RSD	DEXA Conc. (µg/ml)	D^1Absorbance* ±S.D.	% RSD
100	-0.111 ±0.001527	1.37	10	-0.154 ±0.001155	0.74
150	-0.140 ±0.000577	0.4	15	-0.230 ±0.000577	0.25
200	-0.172 ±0.001155	0.67	20	-0.301 ±0.000577	0.19

Table 5: Interday precision data for estimation of Atropine Sulfate and Dexamethasone.

ATRO Conc (µg/ml)	Absorbance* ±S.D.	%RSD	DEXA Conc. (µg/ml)	Absorbance* ±S.D.	%RSD
100	-0.111 ±0.001528	1.37	**10**	-0.157 ±0.001	0.63
150	-0.142 ±0.001	0.7	**15**	-0.231 ±0.001528	0.66
200	-0.172 ±0.002	1.16	**20**	-0.302 ±0.001528	0.5

***n=3**

ACCURACY

Concentration of Pre analyzed sample of ATRO: 177.54µg/ml

Table 6: Accuracy (%Recovery) data for Atropine Sulfate.

Level of recovery	Sample amount (mcg/ml)	amount added (mcg/ml)	Total amt of Atro (µg/ml)	Amt of Atro found (µg/ml)	Amount of Atro Recovered (µg/ml)	% Recovery	Mean % recovery ± SD
80%	100	80	180	180.08	78.7	98.38	99.09±0.69
	100	80	180	180.19	79.8	99.75	

	100	80	180	180.14	79.3	99.13	
100%	100	100	200	200.02	98.1	98.17	98.53±0.31
	100	100	200	200.08	118.2	98.5	
	100	100	200	200.03	90.7	98.71	
120%	100	120	220	220.03	118.2	98.5	98.86±0.34
	100	120	220	220.08	118.7	98.92	
	100	120	220	220.11	119	99.17	

Concentration of Pre analyzed sample of ATRO: 15.54µg/ml

Table 7: Accuracy (%Recovery) data for Dexamethasone.

Level of recovery	Sample amount (mcg/ml)	Amount added (mcg/ml)	Total amt of Dexa (µg/ml)	Amt of Dexa Found (µg/ml)	Amount of Dexa recovered (µg/ml)	% Recovery	Mean % recovery ± SD
80%	10	8	18	17.89	8	100	100.92 ±0.8
	10	8	18	18	8.11	101.38	
	10	8	18	18	8.11	101.38	
100%	10	10	20	19.95	10.06	100.6	99.37± 1.1
	10	10	20	19.74	9.85	98.5	
	10	10	20	19.79	9.9	99	
120%	10	12	22	22	12.11	100.92	100.03 ± 0.88
	10	12	22	21.89	12	100	
	10	12	22	21.79	11.90	99.16	

LOD & LOQ

Table 8: LOD & LOQ data for Atropine sulfate and Dexamethasone.

PARAMETERS	ATROPINE SULFATE	DEXAMETHASONE
Mean Slope (n=6)	0.0295	0.074
SD (n=6)	0.0011	0.0009
LOD (µg/ml)	0.1231	0.0401
LOQ (µg/ml)	0.3729	0.1216

Analysis of marketed formulation (ASSAY)

Table 10: Analysis of marketed formulation.

BRAND NAME	DRUGS	LABEL CLAIM	AMOUNT FOUND	% ASSAY
DEXAPINE	Atropine Sulfate	1 W/V	0.98 % W/V	98 %
	Dexamethasone	0.1 W/V	0.099% W/V	99 %

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