ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DEXAMETHASONE SODIUM PHOSPHATE AND ATROPINE SULPHATE IN EYE DROP

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ABSTRACT

A simple, sensitive, rapid, accurate, precise Absorbance Ratio Method was developed for the simultaneous determination of Dexamethasone Sodium Phosphate and Atropine Sulphate in combined dosage form. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an iso absorptive point and other being the λ_{max} of one of the two components. Dexamethasone Sodium Phosphate and Atropine Sulphate show an iso absorptive point at 228.60 nm in methanol. The second wavelength used is 239.60 nm, which is the λ_{max} of Dexamethasone Sodium Phosphate in methanol. The linearity was obtained in the concentration range of 20-60 μg/ml for Atropine Sulphate (r^2=0.9991) and 2-6 μg/ml for Dexamethasone Sodium Phosphate(r^2=0.9994).LOD is 0.063 and 3.01 μg/ml for Dexamethasone Sodium Phosphate and Atropine Sulphate respectively.LOQ is 0.19 and 9.12 μg/ml for Dexamethasone Sodium Phosphate and Atropine Sulphate respectively. The concentrations of the drugs were determined by using ratio of absorbance at iso absorptive point and at the λ_{max} of Dexamethasone Sodium Phosphate. The method was successfully applied to pharmaceutical dosage form because no interference from the excipients was found. The results of analysis have been validated.

Keywords: Dexamethasone Sodium Phosphate, Atropine Sulphate, Absorbance ratio method, Iso absorptive point.

INTRODUCTION

Atropine is a naturally occuring tropen alkaloid and basic in nature. It is an anticholinergic or parasympatholytic drug. It inhibits the muscarinic actions of acetylcholine [1].Atropine Sulphate is chemically [Bis[(1R,3R,5S)-8-methyl-8-azabicyclo [3,2,1]oct-3-yl(2RS)-3-hydroxy-2-phenylpropanoate] sulphate] and in Indian, British, United States, European Pharmacopoeia[2-5]. In literature, several analytical methods such as Uv-Visible spectroscopy [6-8], HPLC [9-15] and UPLC [16] have been reported for the determination of Atropine Sulphate single and in combination with other drugs in its pharmaceutical formulation.

Dexamethasone Sodium Phosphate is a glucocorticoid and suppresses normal immune response [1]. Chemically 9-Fluoro-11β, 17-dihydroxy-16α-methyl-3, 20-dioxopregna-1,4-dien-21-yl disodium phosphate and Dexamethasone Sodium Phosphate official in Indian,British, European and United States Pharmacopoeia[2-5]. In literature, several analytical methods such as Uv-Visible spectroscopy [17-22], polarographic [23], HPLC [24-36] and HPTLC [37-38] have been reported for the determination of Dexamethasone Sodium Phosphate single and in combination with other drugs in its pharmaceutical formulation.

The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of Dexamethasone Sodium Phosphate and Atropine Sulphate in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for the simultaneous determination of Atropine Sulphate and Dexamethasone Sodium Phosphate in combined dosage form.

Therefore, an objective of this work is to develop spectrophotometric method based on Q-absorbance ratio spectrophotometric method for the simultaneous determination of both these drugs in their combined dosage form. The second objective is to validate the method as per the ICH guidelines.

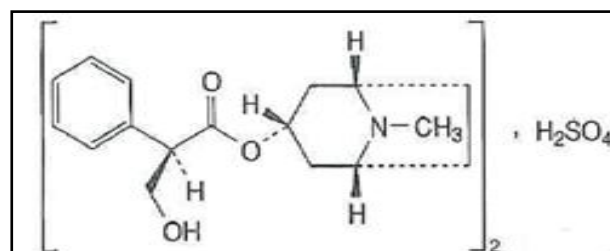The chemical structures of both drugs are as shown in Figure 1 and 2.
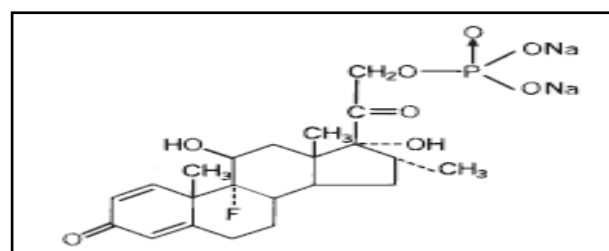

Fig.1: Structure of Atropine Sulphate

Fig.2: Structure of Dexamethasone Sodium Phosphate

MATERIALS & METHODS

Materials

Spectroscopic Analysis was carried out on a UV/VISIBLE 2450 (Shimadzu) double beam UV-Visible spectrophotometer with software of UV Probe version 2.34. The zero order absorption spectra were recorded over the wavelength range of 200-400 nm, against solvent blank, in quartz cuvettes with 1 cm diameter. A Semi micro analytical balance (Sartorius CD2250, Germany) was used for weighing purpose. Atropine Sulphate obtained as gift samples from Yarrow Chem. Products Mumbai-India and Dexamethasone Sodium Phosphate from Enzal Chemicals (I) Ltd. Panoli.The commercial fixed dose combination product was procured from the local market.

Methanol(Rankem).

Methods

Preparation of Standard Solutions

A 10 mg of standard Dexamethasone Sodium Phosphate and Atropine Sulphate were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100µg/ml each of Dexamethasone Sodium Phosphate and Atropine Sulphate.

Methodology

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an iso absorptive point and other being the λ_{max} of one of the two components. From the overlay spectra of two drugs, it is evident that DSP and ATR show an iso absorptive point at 228.60 nm. The second wavelength used is 239.60 nm, which is the λ_{max} of DSP (Figure 3). Working standard solutions having concentration 2,3,4,5 and 6 µg/ml for DSP and 20,30,40,50,and 60 µg/ml for ATR were prepared in methanol and the absorbance at 228.60 nm (iso absorptive point) and 239.60 nm (λ_{max} of DSP) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/ax_1$ _____ (1)

$C_Y = [(Q_M - Q_X) / (Q_Y - Q_X)] \times A_1/ay_1$ _____ (2)

Where, A_1 and A_2 are absorbance of mixture at 228.60 nm and 239.60 nm; ax_1 and ay_1 are absorptivities of DSP and ATR at 228.60 nm; ax_2 and ay_2 are absorptivities of DSP and ATR respectively at 239.60 nm; $Q_M = A_2 / A_1$, $Q_X = ax_2 / ax_1$ and $Q_Y = ay_2 / ay_1$.
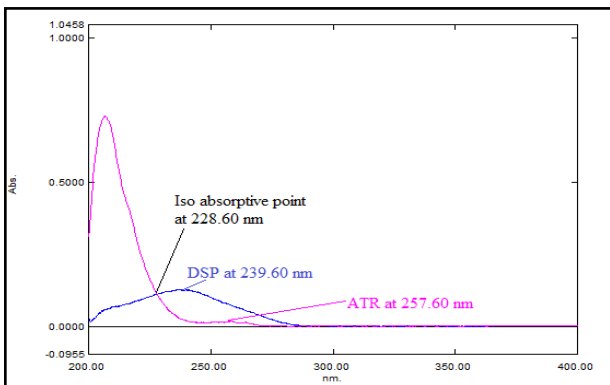
Figure 3: Overlain absorption spectra of DSP (239.60 nm) and ATR (257.60 nm) showing iso absorptive point (228.60 nm) in methanol.

VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity (Calibration Curve)

The calibration curves were plotted over a concentration range of 2-6 µg/ml for DSP and 20-60 µg/ml ATR. Appropriate aliquots from the standard stock solutions of DSP and ATR were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of DSP (2-6 µg/ml). Aliquot from the stock solution of DSP (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 2-6 µg/ml (0.2, 0.3, 0.4, 0.5 and 0.6 ml). Series B consisted of varying concentrations of ATR (20-60 µg/ml). Appropriate volume of the stock solution of ATR (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol to get final concentration in range of 20-60 µg/ml (2.0,3.0,4.0,5.0 and 6.0 ml). The absorbances of

solution were then measured at 239.60nm and 228.60nm.(Table 1) The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.(Figure 4 and 5)

**Table 1: Absorbance for DSP and ATR at 239.60nm and 228.60 nm, respectively**

DSP			ATR		
Conc. µg/ml	At 228.60nm	At 239.60nm	Conc. µg/ml	At 228.60nm	At 239.60nm
2	0.0779±0.00016	0.0853±0.00068	20	0.0778±0.00062	0.0155±0.00012
3	0.1093±0.0020	0.1213±0.00059	30	0.1091±0.00096	0.0221±0.00016
4	0.1428±0.0024	0.1618±0.0071	40	0.1425±0.0021	0.0292±0.00014
5	0.1767±0.0018	0.1986±0.0083	50	0.1769±0.0038	0.0359±0.00021
6	0.2054±0.0019	0.2332±0.0046	60	0.2053±0.0033	0.0433±0.00034

y = 0.03732x + 0.01075 for DSP at 239.60nm_____ (3)

y = 0.00323+0.01320 for ATR at 228.60nm_____ (4)
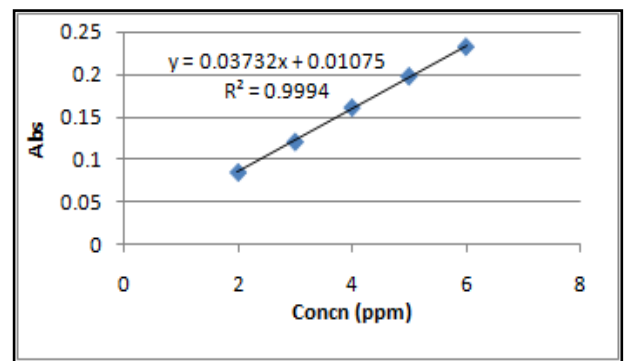
Fig.4: Calibration graph of Dexamethasone Sodium Phosphate at 239.60 nm
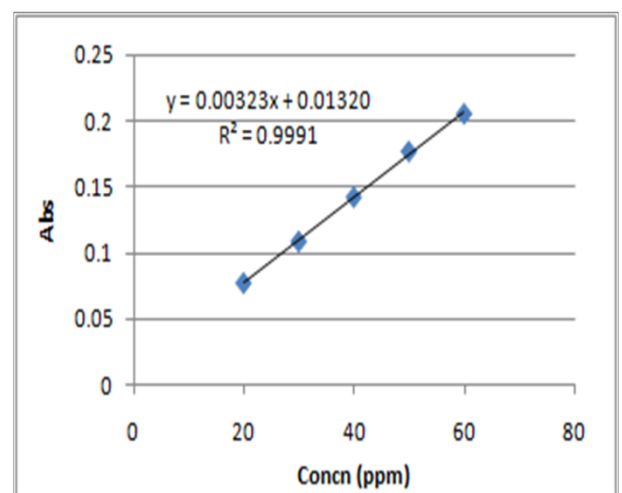
Fig.5: Calibration graph of Atropine Sulphate at 228.60 nm

Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3

different concentrations of standard solutions of DSP and ATR (2,4,6 µg/ml for DSP and 20, 40, 60µg/ml for ATR). The result was reported in terms of relative standard deviation (Table no 2 and 3)

Table 2: Intraday precision data for estimation of DSP and ATR *(n=3)

Conc. (µg/ml)		Abs. ± SD * DSP	% RSD	Abs. ±SD* ATR	% RSD
DSP	ATR				
2	20	0.0875±0.00030	0.34	0.1425±0.00035	0.24
4	40	0.1677±0.00055	0.33	0.2457±0.00070	0.28
6	60	0.2524±0.00056	0.22	0.3683±0.00085	0.23

Table 3: Interday precision data for estimation of DSP and ATR *(n=3)

Conc. (µg/ml)		Abs. ±SD* DSP	% RSD	Abs. ±SD* ATR	% RSD
DSP	ATR				
2	20	0.0868±0.00035	0.40	0.1420±0.00075	0.52
4	40	0.1682±0.00050	0.29	0.2472±0.00090	0.36
6	60	0.2522±0.00065	0.26	0.3680±0.00075	0.20

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of DSP and ATR by the standard addition method. Known amounts of standard solutions of DSP and ATR were at added at 80, 100 and 120 % level to pre quantified sample solutions of DSP and ATR (2µg/ml for DSP and 20µg/ml for ATR). The amounts of DSP and ATR were estimated by applying obtained values to the respective regression line equations.Data given in table 4 and 5.

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and

S = slope of the calibration curve.

Robustness and Ruggedness

Different Instrument and different stock solution was used for Robustness and Ruggedness No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.(Given in table 6)

Table 4: Recovery data of DSP *(n=3)

Conc. of DSP from formulation (µg/ml)	Amount of Std.DSP added (µg/ml)	Total amount of DSP (µg/ml)	Total amount of DSP found (µg/ml)* Mean ± SD	% Recovery	% RSD
2	1.6	3.6	3.58±0.016	99.67	0.45
2	2	4	4.02±0.007	100.61	0.18
2	2.4	4.4	4.39±0.015	99.83	0.34

Table 5: Recovery data of ATR *(n=3)

Conc. of ATR from formulation (µg/ml)	Amount of Std.ATR added (µg/ml)	Total amount of ATR (µg/ml)	Total amount of ATR found (µg/ml)* Mean ± SD	% Recovery	% RSD
20	16	36	36.04±0.147	100.11	0.46
20	20	40	39.88±0.056	99.71	0.33
20	24	44	43.95±0.070	99.89	0.18

Table 6: Robustness and Ruggedness data of DSP and ATR *(n=3)

Conc. (ppm)	Dexamethasone Sodium Phosphate (Mean abs. ±% RSD)			
	Instrument 1	Instrument 2	Stoke – 1*	Stoke – 2*
2	0.0867±0.59	0.0877±0.45	0.0878±0.28	0.0877±0.52
3	0.1278±0.29	0.1274±0.43	0.1278±0.35	0.1277±0.43
4	0.1662±0.30	0.1667±0.24	0.1657±0.36	0.1652±0.46
Atropine Sulphate (Mean abs. ±% RSD)				
20	0.1413±0.39	0.1416±0.52	0.1426±0.42	0.1415±0.45
30	0.1969±0.40	0.1967±0.38	0.1970±0.38	0.1977±0.30
40	0.2446±0.22	0.2448±0.22	0.2442±0.26	0.2436±0.17

Assay procedure

From the formulation 1ml transferred in 100 ml volumetric flask and made up to the mark with the Methanol. Stock solution contained 10µg/ml DSP and 100µg/ml ATR and from that pipette out 2 ml and transferred in 10 ml volumetric flask and made up to the mark with the Methanol. The absorbance of the sample solution i.e. A_1 and A_2 were recorded at 228.60 nm (iso absorptive point) and 239.60 nm (λ_{max} of DSP) respectively. (Given in table 7)

RESULTS AND DISCUSSION

The overlain UV absorption spectra of DSP (239.60 nm) and ATR (257.60 nm) showing iso absorptive point (228.60 nm) in methanol is shown in Figure 3. Accuracy was determined by calculating the recovery and the mean was determined (Table 4 and 5). The method was successfully used to determine the amounts of DSP and ATR present in dosage forms. The results obtained were in good agreement with the corresponding labeled amount (Table 7).Precision was calculated as intra and inter day variations (% RSD) for both the drugs. Summary of validation parameters for method is given in Table 8. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of DSP and ATR in dosage form. The method utilizes easily available and cheap solvent

for analysis of DSP and ATR hence the method was also economic for estimation of DSP and ATR from dosage form. % recoveries greater than 99 % shows that method is free from the interference of excipients used in the formulation

Table7: Analysis data of formulation *(n=3)

Sr. No	Drug	Formulation (µg/ml)	% Assay ± SD*
1	DSP	2	100.12±0.80
2	ATR	20	100.64±0.61

Table 8: Regression Analysis Data and Summary of Validation Parameters for DSP and ATR by Absorbance Ratio Method

PARAMETERS	Absorption Ratio Method	
	DSP	ATR
Concentration range(µg/ml)	2-6	20-60
Regression equation	y= 0.03732x + 0.01075	y = 0.00323x + 0.01320
Correlation Coefficient(r2)	0.9994	0.9991
Accuracy(%Recovery) (n=3)	100.04	99.90
Intra-day Precision (%RSD) (n=3)	0.22-0.34	0.23-0.28
Inter-day precision (%RSD) (n=3)	0.26-0.40	0.20-0.52
LOD(µg/ml)	0.063	3.01
LOQ(µg/ml)	0.19	9.12
Ruggedness and Robustness	0.24-0.59	0.17-0.52

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