

Research Article

Sensitive RP-HPLC Method for Estimation of Atropine Sulphate and Dexamethasone Sodium Phosphate in Ophthalmic Formulation.

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ABSTRACT

A simple, accurate and reproducible high performance liquid chromatography method has been developed and validated for Simultaneous quantification of Atropine Sulphate and Dexamethasone Sodium Phosphate in ophthalmic formulation using ODS-BP hyperchrome C₁₈column (250 mm × 4.6 mm id, 5 µm particle size). Optimised mobile phase for chromatographic separation was 0.05 M Potassium dihydrogen phosphate buffer (pH-4): Acetonitrile (60:40%v/v). Flow rate was adjusted to 1.0 ml/min and analytes were monitored at 266 nm. The retention time of Atropine Sulphate was 3.467 minute and Dexamethasone Sodium Phosphate was 5.180 minute. The linearity of Atropine Sulphate and Dexamethasone Sodium Phosphate was in the range of 5-150 µg/ml and 5-15 µg/ml respectively. The developed method was validated in accordance to ICH guidelines. The method was validated and successfully applied to the simultaneous determination of Atropine Sulphate and Dexamethasone Sodium Phosphate in ophthalmic dosage form.

KEYWORDS

Atropine Sulphate, Dexamethasone Sodium Phosphate, RP-HPLC, Validation

1. INTRODUCTION

Dexamethasone Sodium Phosphate (DSP) is highly selective glucocorticoid which is widely used in ocular inflammatory diseases. Its chemical name is Disodium 9 α -fluoro11 β , 17 α -dihydroxy-16 α -methyl-3, 20-dioxo-1, 4-pregnadien-21-yl-phosphate. Atropine Sulphate (AS) is a competitive antagonist for the muscarinic acetylcholine receptor. It is classified as an anticholinergic drug. Its chemical name is (RS)-(1R, 3r, 5S)-3-Tropoyloxytropanium sulphate monohydrate¹. Atropine Sulphate in combination with Dexamethasone Sodium Phosphate is used in eye inflammation such as Uveitis that reduces the inflammation in the iris and ciliary body². Structures of Dexamethasone Sodium Phosphate and Atropine are given in fig.1. and 2 respectively.

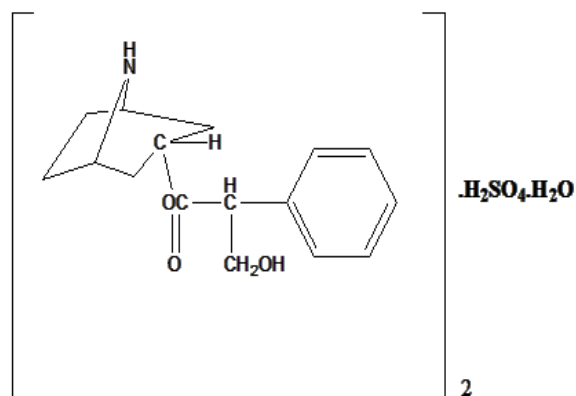


Figure 1: Structure of Atropine.

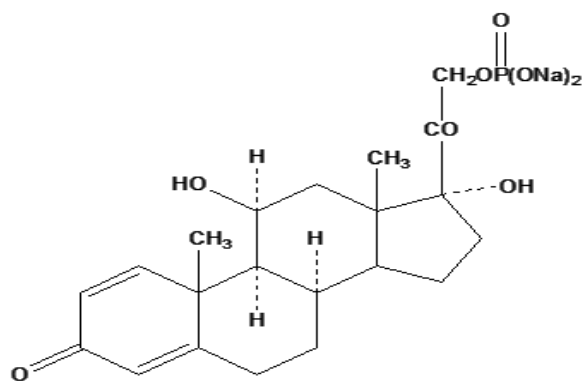


Figure 2: Structure of Dexamethasone Sodium Phosphate.

In the literature, there are methods described for the individual estimation of Atropine Sulphate and Dexamethasone Sodium Phosphate in aqueous samples and biological fluids by liquid chromatography³⁻⁶. A few methods have also been described for the simultaneous determination of Dexamethasone Sodium Phosphate with other drugs such as Chloramphenicol⁷, Ciprofloxacin⁸, Moxifloxacin⁹, Gatifloxacin¹⁰, Lomefloxacin¹¹, and Ofloxacin¹².

Methods were also described for simultaneous determination of Atropine Sulphate with other drugs such as Obidoxime Chloride, Diphenoxylate Hydrochloride¹³⁻¹⁴. But simultaneous determination of Atropine Sulphate and Dexamethasone Sodium Phosphate has not been reported earlier in the literature. So an attempt was made to develop a HPLC method for the estimation of these drugs available as eye drops.

The purpose of the present study was to develop a simple, sensitive and economical HPLC method for simultaneous determination of Atropine Sulphate and Dexamethasone Sodium Phosphate in bulk and pharmaceutical formulations. The developed method has been validated¹⁵⁻¹⁶ by evaluation of the system suitability, specificity, linearity, limit of detection and quantification, precision, accuracy and recovery. The validated method was applied to the commercially available pharmaceutical formulations containing both the drugs.

2. MATERIALS AND METHODS

2.1. Materials

Dexamethasone Sodium Phosphate was obtained as a gift samples from Merck Bioscience Ltd., Gujarat, India and Atropine Sulphate was obtained as a gift samples from Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. HPLC grade Acetonitrile was purchased from Chemdyes Corporation, Rajkot. Triple distilled water was used during the study. The Pharmaceutical formulations containing 10mg/ml of Atropine Sulphate and 1mg/ml Dexamethasone Sodium Phosphate (DEXAPIN eye drops, Ophtho remedies Pvt. Ltd., India.) was purchased from local market.

2.2. Instrumentation

A high performance liquid chromatography (Analytical Technologies) equipped with pump (Model-S 1122) and 2203UV-Visible detector, Ultrasonic bath (Toshcon, Toshniwal process instrument Pvt. Ltd., Ajmer).

2.3. Chromatographic conditions

For chromatographic analysis, ODS-BP hyperchrome C₁₈ column (250 mmx4.6 mm i.d, 5µm particle size) was used. Separation was carried out by isocratic elution. The mobile phase consisting of a mixture of 0.05 M Potassium dihydrogen phosphate buffer and Acetonitrile in the ratio of (60:40, v/v, pH adjusted to 4 with 1% Orthophosphoric acid) was used. Mobile phase was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 30 min before passing through the instrument. The injection volume was 20µl and the flow rate was 1ml/min. UV detection was carried out at 266 nm. Chromatographic separations were carried out at room temperature (25-30°C).

2.3.1. Selection of wavelength

Standard stock solutions of Atropine Sulphate 100µg/ml and Dexamethasone Sodium Phosphate 10µg/ml were prepared for the selection of wavelength and it was found that both drugs showed

reasonably good response at 266 nm. So 266 nm was selected as a wavelength for estimation. (Figure.3).

2.4. Preparation of standard solution

Stock standard solutions of AS and DSP were prepared in the mobile phase at a concentration of 1000 µg /ml and 100µg/ml. working standard solutions was prepared by serial dilution of stock solutions with the mobile phase. Chromatogram of the standard is shown in Figure.4.

2.5. Preparation of sample

Sample solutions of AS and DSP were prepared at a concentration of 1000 µg /ml and 100 µg/ml. From this 1 ml was taken and diluted to 10 ml to get a concentration of 100 µg /ml and 10 µg /ml of AS and DSP respectively. Chromatogram of the standard is shown in Figure.5.

3. RESULTS AND DISCUSSION

3.1. Mobile phase optimization

Chromatographic parameters were optimized to develop a HPLC method for simultaneous determination of AS and DSP with short analysis time (<10 min), and acceptable resolution ($R_s > 2$). Various compositions of mobile phases like methanol: buffer and ACN: buffer in different ratios were tried. But with mixed 0.05 M phosphate buffer and ACN in the ratio of (60:40 v/v, pH adjusted to 4 with 1% Orthophosphoric acid) at a flow rate of 1ml/min, symmetrical peaks with good resolution were obtained. The optimum wavelength for detection was set at 266 nm at which better detector response for both drugs was obtained. The retention times were 3.4 and 5.2 min for AS and DSP respectively.

3.2. Method validation

The developed analytical method was validated as per ICH and USP guidelines for the parameters like Linearity, Limit of detection (LOD), Limit of quantification (LOQ), Precision, Specificity, Accuracy, Robustness, and System suitability. Calibration graphs were constructed by plotting the peak area versus their corresponding concentrations. Good linearity was obtained in the range of 50-150 µg/ml and 5-15 µg/ml for AS and DSP. The results are shown in table 1. Limit of detection (LOD) and limit of quantification (LOQ) were calculated from the slope and standard deviation of y-intercepts of the regression line of the calibration curve. For AS it was found to be 0.15 and 0.46 µg/ml and for DSP 0.0081 and 0.024 µg/ml respectively. The precision of the method and instrument precision was evaluated and relative standard deviation (RSD) values were calculated. The RSD values for AS and DSP showed that the precision of the method was satisfactory. The accuracy of the method was determined by recovery studies. The recoveries were close to 100% for AS and DSP. Developed method was found to be robust when the mobile phase ratio and flow rate was changed from 60:40± 2% and 1ml/min to 1±0.1ml/min.

3.2.1. Linearity

Five working standard solutions of each analyte in the concentration range of 50-150 µg/ml for AS and 5-15 µg/ml for DSP were prepared in triplicate and injected. Calibration curves were constructed by plotting concentration versus mean peak area. Table 1.

3.2.2. Precision

The precision of the method was evaluated in terms of intermediate precision i.e., intra-day and inter-day precision. For intra-day precision three different concentrations of AS and DSP in the linearity range was prepared in triplicate and was analyzed during the same day. For inter-day precision the same concentrations were analyzed on three consecutive days and RSD values were calculated. RSD values were calculated from the peak areas and retention times of AS and DSP. Table.2.

3.2.3. Accuracy

Accuracy of the method was determined by recovery studies. These studies were carried out by addition of known amounts of AS and DSP to a sample solution of known concentration and comparing calculated and measured concentrations. A sample solution containing AS and DSP (100 and 10 mg/ml, respectively) was prepared by diluting 1 ml of the ophthalmic solution to 25 ml in a volumetric flask, and make up the solution with the mobile phase. Samples (0.5ml) of the filtered solution were transferred to 10 ml volumetric flasks containing 0.4, 0.5, and 0.6 ml of AS and DSP standard solution, and analyzed. Table 3.

3.2.4. System suitability

System suitability was established in order to determine the adequate resolution and reproducibility of the proposed method. Suitability parameters including retention factor, resolution, asymmetry factor, plate number were investigated. Table 4.

3.2.5. Robustness

Robustness of the method was evaluated by deliberately varying method parameters such as mobile phase composition and flow rate; and flow rate was changed from 1ml/min to 1 ± 0.1 ml/min. Effect of these changed parameters was studied by injecting the sample in to the system. Table 5.

3.3. Assay of the marketed formulation

According to ICH in the case of assay, demonstration of specificity requires that the procedure is unaffected by the presence of impurities or excipients. The assay value of the marketed formulation was found to be within the limits. The low RSD value indicated suitability of this method for routine analysis of AS and DSP in pharmaceutical dosage forms. Chromatogram of the sample shows that there was no interference from the excipients present in the formulation (Fig. 3); this indicates the specificity of the method. The results are shown in Table 6.

4. CONCLUSION

The method described in this paper for the simultaneous estimation of AS and DSP was found to be simple, sensitive, accurate, precise, rapid, robust and economical. The analytical conditions and the solvent system developed provided good resolution within a short analysis time. The RSD for all parameters was found to be within the limits, which indicates the validity of method and assay results obtained by this method are in fair agreement. Thus the developed method can be proposed for routine analysis of AS and DSP in laboratories and for quality control purposes.

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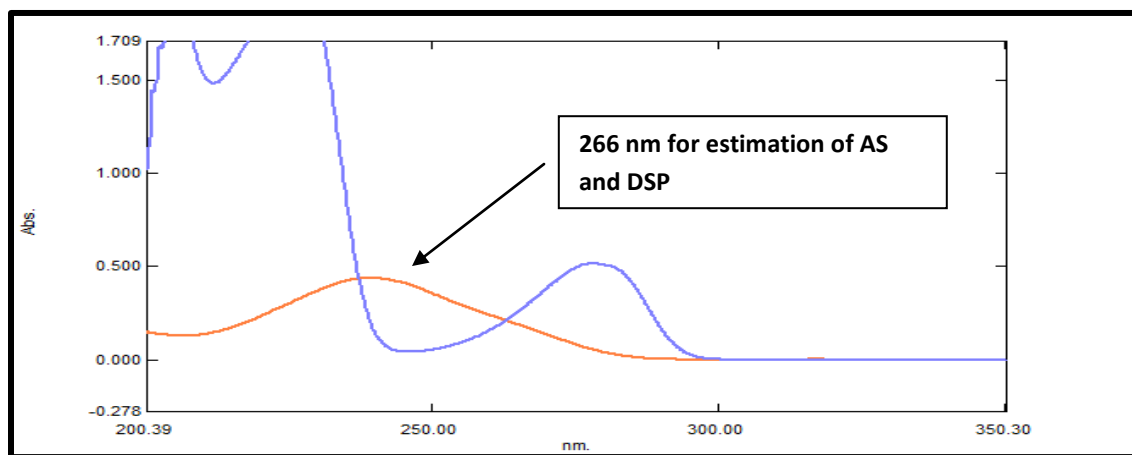


Figure 3: Typical chromatogram for the sample solution of AS and DSP.

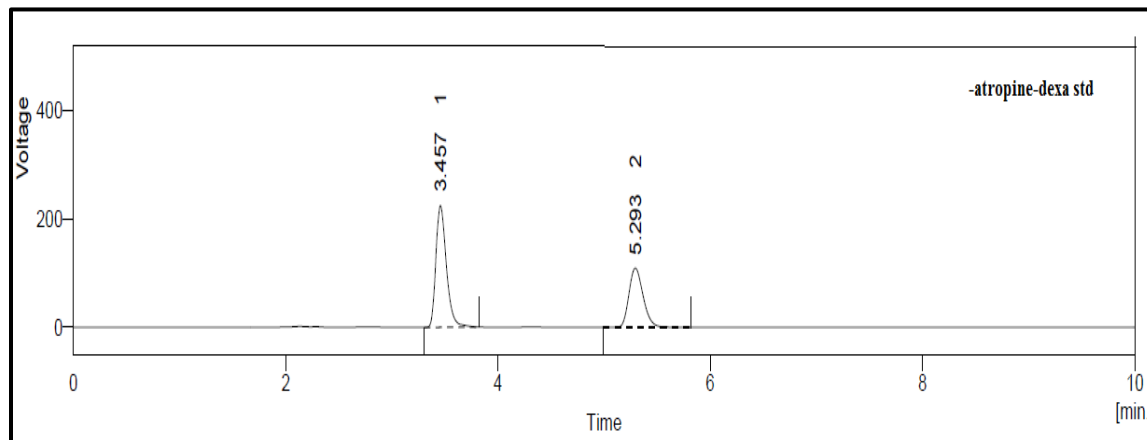


Figure 4: Typical chromatogram for the standard solution of AS and DSP

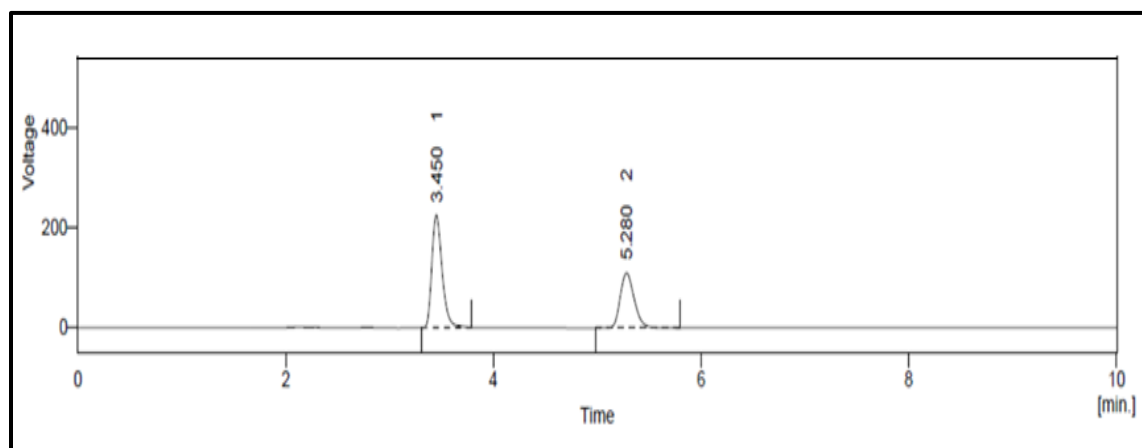


Figure 5: Typical chromatogram for the sample solution of AS and DSP.

Table 1: Linearity by regression analysis (n=5)

Substance	R ²	Slope	Conc. range (µg/ml)
AS	0.9995	14.09	50-150
DSP	0.9994	99.87	5-15

'n' is number of determinations

Table 2: Precision expressed as %RSD

Parameters	AS	DSP
Intra-day precision (n=3)	0.30	0.15
Inter-day precision (n=3)	0.20	0.51
Injection repeatability for peak area (n=6)	0.23	1.91

'n' is number of determinations and RSD is relative standard deviation

Table 3: Recovery studies (n=3)

Drug	%of std added	Amount recovered (µg/ml)	%recovery	%RSD
AS	80%	90.88	101.24	0.79
	100%	100.82	101.18	0.56
	120%	120.13	99.74	0.78
DSP	80%	9.17	101.24	0.57
	100%	10.19	101.67	0.40
	120%	11.12	101.39	0.25

'n' is number of determinations and RSD is relative standard deviation

Table 4: System suitability parameters

Parameters	AS	DSP
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Retention time (R_t)	3.46	5.18
Asymmetry factor	1.54	1.39
Resolution	8.065	
Number of Theoretical Plates	5852	7236
LOD (µg/ml)	0.15	0.0081
LOQ (µg/ml)	0.46	0.024

'n' is number of determinations

Table 5: Robustness study (n=3)

Drug	Flow rate	Mean area	%RSD
AS	0.8	1614.78	0.58
	1.2	1511.5	0.38
DSP	0.8	1050.02	0.31
	1.2	991.16	0.66
Drug	Mobile phase ratio	Mean area	%RSD
AS	58.4 : 41.6	1597.57	0.68
	61.4 : 38.6	1521.16	0.61
DSP	58.4 : 41.6	1038.83	0.37
	61.4 : 38.6	990.82	0.35

'n' is number of determinations and RSD is relative standard deviation

Table 6: Assay of eye drops (n=5)

Drug	Label claim µg/ml	Amt found µg /ml	Mean %recovery	%RSD
AS	100	101.10	101.10	0.28
DSP	10	10.03	100.36	0.41

Dexapin eye drops containing 100 µg /ml AS and 10µg /ml DSP