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Analytical Method Development and Validation for Estimating of Sodium Thiosulfate in Ophthalmic Dosage Form

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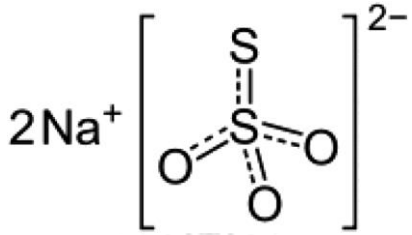
ABSTRACT

A robust HPLC method for estimation of Sodium Thiosulfate was developed and validated in ophthalmic solution. The system consisted of Agilent technologies, USA. Zorbax Eclips XBD-C8150 x 4.6 mm, 5µ and detection was performed at UV 210 nm for estimation of Sodium Thiosulfate. The mobile phase consisted of 0.01 M phosphate buffer pH adjusted to 7.1 ± 0.05 with sodium hydroxide solution and methanol (85:15) containing 1.698 g/L of Tetrabutylammonium hydrogen sulphate. The flow rate maintained as 1.0 mL/min and column temperature is 25°C. The standard concentration for Sodium Thiosulfate estimation was prepared about 150 µg/mL. The calibration curve was linear from 25 % to 150% of standard concentration with r > 0.99. Accuracy (mean recovery 99.41 %) and precision were found to be satisfactory for Sodium Thiosulfate. All the placebo peaks were not interfered with Sodium Thiosulfate, thus the methods can be considered as a specific method. The proposed method is suitable for routine quantification of Sodium Thiosulfate in ophthalmic dosage form.

INTRODUCTION

Sodium Thiosulfate (Sodium Thiosulfate) is an inorganic compound with the formula $Na_2S_2O_3.5H2O$. Typically it is available as the white or colourless pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$. The solid is an efflorescent (loses water readily) crystalline substance that dissolves well in water. [1]

Sodium Thiosulfate is used in gold mining, water treatment, analytical chemistry, the development of silver-based photographic film and prints, and medicine. The medical uses of Sodium Thiosulfate include treatment of cyanide poisoning and pityriasis. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system.



Molecule Formula: $Na_2S_2O_3 \cdot 5H_2O$

Thiosulfuric acid (as Sodium Thiosulfate) has the chemical name Thiosulfuric acid, disodium salt, pentahydrate. The chemical formula is $Na_2S_2O_3 \bullet 5H_2O$ and the molecular weight is 248.17. Sodium Thiosulfate Injection is a cyanide antidote which contains one 50 mL glass vial containing a 25% solution of Sodium Thiosulfate Injection.

It has been indicated as antidote for cyanide poisoning. It is also used as adjunct agent for patients taking cisplatin chemotherapy.

Beside the above indications, Sodium Thiosulfate could be used as ingredient in the following: agricultural chemicals (non-pesticidal), fillers, intermediates, laboratory chemicals, oxidizing/reducing agents, process regulators, processing aids, and solids separation agents [2].

This paper describes a simple, precise, accurate and robust, specific reversed phase HPLC method for the determination of Sodium Thiosulfate in Lifitegrast Ophthalmic solution. The proposed HPLC methods utilizes economically available common solvents, reagents system and

HPLC columns, well separated peaks from each other's and from main peak, good retention time, sharp and symmetrical peak shapes. The method was validated as per International Conference on Harmonization (ICH) [3] suggestions.

MATERIALS AND METHODS

Chemicals, samples and reference standards

Analytical reagent grades of Potassium dihydrogen orthophosphate, Sodium hydroxide, Tetrabutylammonium hydrogen sulphate, Sodium chloride solution (0.9%), HPLC grade of Methanol. Potassium dihydrogen orthophosphate, Sodium hydroxide, Tetrabutylammonium hydrogen sulphate was procured from Merck, India. Sodium chloride solution (0.9%) manufactured by Baxter, USA was procured from market. Methanol was obtained from Merck KGaA, Germany. A Milli-Q purification system (Millipore, Bedford, MA, USA) was used to further purify demineralized water.

A Small amount of Sodium Thiosulfate was procured from sigma Aldrich. Lifitegrast ophthalmic solution 5% was purchased from market. All the reagents and chemicals were procured from Merck KGaA, Germany. Standard solution of Sodium Thiosulfate and samples solutions were prepared in 0.9% sodium chloride. All the stock solutions were stored at 2-8°C and used for all the research work.

HPLC system and chromatographic conditions

The HPLC Agilent (Agilent Technologies, USA) 1260 Infinity II LC System, consisted of quaternary pump which can be operates at pressures up to 400 bar and flow rates up to 10 mL/min, High performance degasser is designed for low-flow and analytical LC up to 5 mL/min, reducing baseline noise and quenching effects, Vial sampler injects from up to 132 standard 2 mL vials and has a pressure rating of 600 or 800 bar and time-programmable wavelength switching provides optimum sensitivity and selectivity for your applications. Analysis was done using a high pH-resistant column, the Zorbax Eclips XBD-C8150 x 4.6 mm, 5µ from Agilent (Agilent Technologies, USA) estimation of Sodium Thiosulfate from ophthalmic dosage form. The column was designed to resist a pH up to 9.0. Column temperature was adjusted at 25°C and maintained constant using inbuilt column thermostat. Several trials were carried out to separate

all the possible placebo peaks from main peak as well from main Lifitegrast peak. At finally a simple isocratic method with Mobile phase consisted of 0.01 M phosphate buffer pH adjusted to 7.1 ± 0.05 with sodium hydroxide solution and methanol (85:15) containing 1.698 g/L of tetrabutylammonium hydrogen sulphate. The flow rate maintained 1.0 mL/min and injection volume is 20 µL for ophthalmic dosage form. Before use, the mobile phase was degassed by sonication for about 10.0 min.

Method validation

The stated study is aimed to method development for estimation of Sodium Thiosulfate from Lifitegrast ophthalmic dosage form. We have focused validation efforts toward necessary test parameters for estimation of Sodium Thiosulfate, such as precision, accuracy, linearity, and selectivity. Sensitivity has been determined with crucial importance since the interference of placebo peaks with peak of interest for formulation product. The analytical method validation was performed as per the recommended guidelines provided by the International Conference on Harmonization (ICH) guidelines [3]. Accuracy was also validated as this method was developed for estimation of Sodium Thiosulfate from formulated drug product. The developed and validated method is well suitable for estimation of Sodium Thiosulfate in ophthalmic dosage pharmaceutical formulations.

Precision

Repeatability and intermediate precision should be evaluated for assessment of precision. Repeatability was determined by six repetitive sample preparations of formulation product. The relative standard deviations (RSD) would be calculated for the assay of Sodium Thiosulfate in commercially available formulation product [4]. For intermediate precision, assay of Sodium Thiosulfate estimation was done on different day on same HPLC equipment using the respective standard to make daily one-point calibration. Samples were prepared in such a way to obtain similar concentration equivalent to standard solution concentration i.e. 150 µg/mL solution for formulation product.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

In the assay estimation method, it is important to be able to selectively determine the concentration of the analyte compound without interferences from the expected related impurity substances and from placebo peaks. Therefore, resolution between the Sodium Thiosulfate peak and from excipient peaks was investigated. Separation of peaks from each other and from main peak was our goal.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.[3]

The accuracy of the assay of Sodium Thiosulfate method for formulation product was evaluated, by spiking main known concentration of Sodium Thiosulfate standard solution to placebo preparation at three concentration levels i.e. 50%, 100% and 150% of label claim of the product.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample preparation. For the evaluation of linearity for Sodium Thiosulfate, the stock solutions were diluted with diluent to a standard concentration of 37.4 to 224.4 µg/mL. This range was sufficiently large as ICH guidelines usually prescribe a range for assay estimation.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

For the robustness evaluation of the analytical method for the estimation of assay of Sodium Thiosulfate, the optimized HPLC conditions set for this method have been slightly modified to evaluate the method robustness. The small changes include: The effect of column temperature at 25° ± 2C i.e. 23°C and 27°C instead of 25°C. The effect of flow rate was at 0.9 and 1.1mL/min instead of 1.0mL/min.

Stability of stock solutions

This study was performed on Bench top for 72 hours for sample solution preparation. This solution was injected into the HPLC system and chromatogram was recorded. Sample solution was injected at room temperature at periodic time interval.

RESULTS

Method development

As part of method development, literatures were referred for method development of estimation of Sodium Thiosulfate in Lifitegrast ophthalmic solution. Sodium Thiosulfate is inorganic compound. All the referred literatures were stating that the maxima wavelength for Sodium Thiosulfate would be about 210 nm [5,6]. To confirm wavelength maxima, 100µg/mL of Sodium Thiosulfate was prepared in purified water. From the above solution spectrum, the maxima were found at 210 nm. The same wavelength was utilised for the further development and validation actives for estimation of Sodium Thiosulfate in ophthalmic dosage formulation.

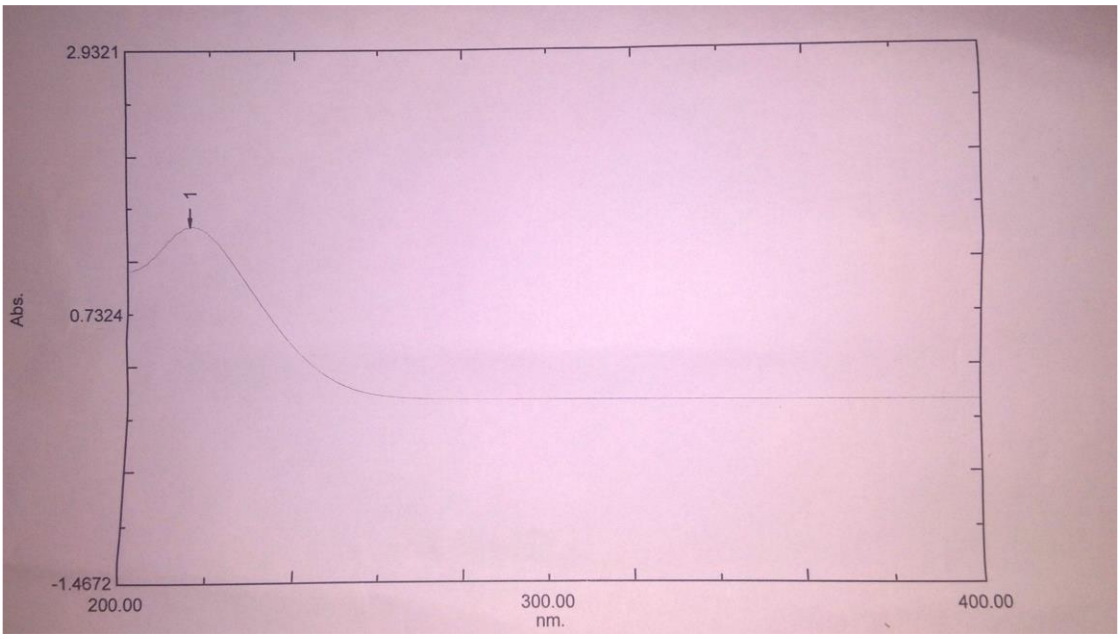


Figure No. 1: UV spectrum of 100 µg/mL of Sodium Thiosulfate in purified water

Initially, the assay method development was attempted with reference method of Sodium Thiosulfate [5]. The mobile phase consisted of 0.01 M phosphate buffer pH adjusted to 7.1 ± 0.05 with sodium hydroxide solution and methanol (85:15) containing 1.698 g/L of Tetrabutylammonium hydrogen sulphate. The flow rate maintained as 1.0 mL/min and column temperature is 25°C. The standard concentration for assay of Sodium Thiosulfate estimation was prepared about 150 µg/mL. The proposed reference method is working good for the estimation of Sodium Thiosulfate in the Lifitegrast Ophthalmic solution. The same method was used in for the validation of analytical method for the determination of assay of Sodium Thiosulfate.

Validation

Precision

As discussed, repeatability and intermediate precision have been evaluated. Six consecutive sample preparations which is equivalent to Sodium Thiosulfate in the sample is about 150 µg/mL prepared and analysis has been performed. The % RSD of Sodium Thiosulfate was calculated and found below 2.0% in formulation product. This analysis was performed during two different days on same HPLC systems and the % RSD calculated on the obtained averages for each day

was below 2.0%. These results show a sufficient intermediate precision of the assay estimation method. The results were tabulated below table. (Table – 1)

Table No. 1: Precision and Intermediate Precision data for assay of Sodium Thiosulfate in Lifitegrast Ophthalmic Solution.

S. No	Sample Description	Assay	
		Precision (Day-1)	**Intermediate Precision (Day-2)**
1	Preparation - 1	97.5	98.1
2	Preparation – 2	99.2	99.1
3	Preparation – 3	98.3	100.8
4	Preparation – 4	99.1	97.6
5	Preparation – 5	97.9	99.4
6	Preparation - 6	98.2	98.3
Average:		98.4	98.9
% RSD		0.68	1.16
Cumulative % RSD			0.95

Linearity

The adjusted method yielded a linear calibration curve over the chosen range for Sodium Thiosulfate concentration. The regression equation obtained was y = 24097.7029x+18966.6400, with the correlation coefficient (r^2) being 0.9992. The % bias is -0.53%. Since the correlation coefficient of curves is greater than 0.980, a good linear relationship between the detector response and the concentration of analyte could be concluded [7]. For the Sodium Thiosulfate compound, zero is included in the 95% confidence interval of the intercept allowing one-point calibration. A residual plot was produced to assess the appropriateness of linear regression to fit the data. Since the points were distributed randomly around the horizontal axis, it was concluded that linear regression is suitable for these data. The data were tabulated below in Table – 2.

Table No. 2: Linearity data of Sodium Thiosulfate

S. No	% of Nominal Concentration	Concentration (µg/mL)	Avg. Peak Area
1.	25	37.4	924624
2.	50	74.8	1706998
3.	75	112.2	2738309
4.	100	149.6	3556245
5.	125	187.0	4480869
6.	150	224.4	5405492
Correlation coefficient (r^2)			**0.9992**
Slop			**24097.7029**
Intercept			**18966.64**
% bias			**-0.53**

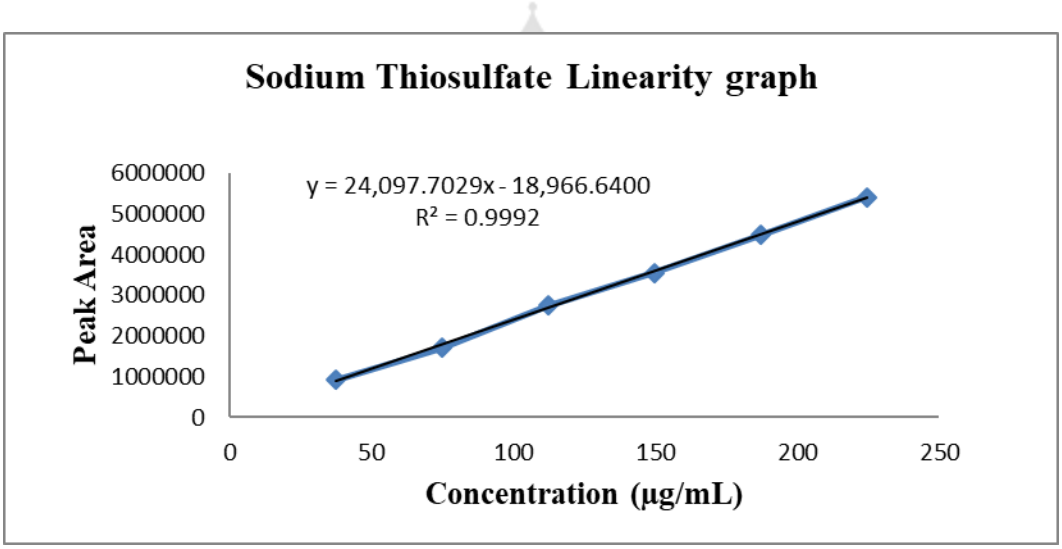


Figure No. 2: Linearity Graph

Accuracy

Accuracy of analytical method was evaluated by spiking the known concentration of Sodium Thiosulfate standard solution to placebo of Sodium Thiosulfate prepared with Lifitegrast and all other excipients. The results were tabulated below in Table No. 3.

Table No. 3: Accuracy of Sodium Thiosulfate.

Accuracy Level	(mg) added of Sodium Thiosulfate	(mg) found of Sodium Thiosulfate	% Accuracy	Mean Accuracy
50%	0.0753	0.0743	98.67	98.72
		0.0734	97.48	
		0.0753	100.00	
100%	0.1505	0.1506	100.07	99.98
		0.1495	99.34	
		0.1513	100.53	
150%	0.2258	0.2245	99.42	99.53
		0.2262	100.18	
		0.2235	98.98	

Robustness

As per ICH guidelines [3], the robustness of an analytical method is its ability to withstand small but deliberate changes in the experimental variables. In this study, the robustness was evaluated by an experimental design examining the simultaneous influence of flow rate variation and column temperature variation on the peak area as the response variable as well peak RT. There is no significant effect on the RT (RT of main peak is about 5.0 min) and peak response with flow rate alteration and column temperature. This means that the method was found to be robust with respective to flow rate and column temperature.

Robustness data

S. No	Robustness condition	% Assay
1	Control conditions	98.2
2	Flow rate variation (0.9 mL/min)	98.6
3	Flow rate variation (1.1 mL/min)	99.1
4	Column Temperature variation (24 °C)	98.3
5	Column Temperature variation (26 °C)	97.6

Stability of stock solutions

Sodium Thiosulfate stock solutions was prepared and kept at room temperature and in the refrigerator for 1 week. The stock solution remained stable in the refrigerator during the whole period of test procedure and remained stable at room temperature for 3 days. At room temperature, stability is guaranteed for 3 days and 1 week at refrigerator.

Stability of standard solution at 25°C.

S. No	Sample Description	Standard Area	Differences (%)
1.	Initial	3459632	NA
2.	Day -1	3425946	0.97
3.	Day - 3	3393875	1.90

Stability of standard solution at 2- 8 °C.

S. No	Sample Description	Standard Area	Differences (%)
1.	Initial	3459632	NA
2.	Day - 3	3419946	1.15
3.	Day - 7	3396945	1.81

DISCUSSION

During method development, different options were evaluated to optimize sample preparations, peak detection parameters, placebo peak interference from main peak and chromatography. The mobile phase consisted of 0.01 M phosphate buffer pH adjusted to 7.1 ± 0.05 with sodium hydroxide solution and methanol (85:15) containing 1.698 g/L of Tetrabutylammonium hydrogen sulphate. The flow rate maintained as 1.0 mL/min and column temperature is 25°C. The standard concentration for assay of Sodium Thiosulfate estimation was prepared about 150 µg/mL. The proposed method was validated as per the ICH guidelines for its precision, linearity, specificity, accuracy and robustness. No interference peaks were observed in the chromatogram of placebo solution, and diluents at the retention time of Sodium Thiosulfate. The method is very robust, simple and specific, as all the peaks were well separated from each other and placebo peaks which makes it especially suitable for routine quality control analysis.

CONCLUSION

The present methods are specific, rapid, precise, linear, accurate and robust with respective to flow rate and column temperature. The mobile phase was easy to prepare and all the chemicals and solvents are easily available in the market. Application of these methods for the analysis of Sodium Thiosulfate estimation in ophthalmic formulations reveals that neither the degradation products nor the excipients interfere with the analytical determination. This indicates that the proposed method could be used for the determination of Sodium Thiosulfate in pharmaceutical formulations (ophthalmic solution). Therefore, this method could easily be used in a wide range of analytical laboratories.

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