Scientific Research Publishing

American Journal of Analytical Chemistry, 2024, 15, 151-164
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

# Robustness Study and Superior Method Development and Validation for Analytical Assay Method of Atropine Sulfate in Pharmaceutical Ophthalmic Solution

**Md. Nazmus Sakib Chowdhury[1*], Sreekanta Nath Dalal[2], Md. Ariful Islam[3], Md. Anwar Hossain[4], Pranab Kumar Das[5], Shakawat Hossain[6], Parajit Das[7]**

[1]Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh
[2]Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka, Bangladesh
[3]Department of Applied Chemistry and Chemical Engineering, University of Chittagong, Chattogram, Bangladesh
[4]Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia, Bangladesh
[5]Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh
[6]Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh
[7]Department of Applied Chemistry and Chemical Engineering, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh
Email: *nscphr@gmail.com

**How to cite this paper:** Chowdhury, Md.N.S., Dalal, S.N., Islam, Md.A., Hossain, Md.A., Das, P.K., Hossain, S. and Das, P. (2024) Robustness Study and Superior Method Development and Validation for Analytical Assay Method of Atropine Sulfate in Pharmaceutical Ophthalmic Solution. *American Journal of Analytical Chemistry*, **15**, 151-164.
https://doi.org/10.4236/ajac.2024.155010

**Received:** April 4, 2024
**Accepted:** May 14, 2024
**Published:** May 17, 2024



## Abstract

**Background:** The robustness is a measurement of an analytical chemical method and its ability to contain unaffected by little with deliberate variation of analytical chemical method parameters. The analytical chemical method variation parameters are based on pH variability of buffer solution of mobile phase, organic ratio composition changes, stationary phase (column) manufacture, brand name and lot number variation; flow rate variation and temperature variation of chromatographic system. The analytical chemical method for assay of Atropine Sulfate conducted for robustness evaluation. The typical variation considered for mobile phase organic ratio change, change of pH, change of temperature, change of flow rate, change of column etc. **Purpose:** The aim of this study is to develop a cost effective, short run time and robust analytical chemical method for the assay quantification of Atropine in Pharmaceutical Ophthalmic Solution. This will help to make analytical decisions quickly for research and development scientists as well as will help with quality control product release for patient consumption. This analytical method will help to meet the market demand through quick quality control test of Atropine Ophthalmic Solution and it is very easy for maintaining (GDP) good documentation practices within the shortest period of time. **Method:** HPLC method has been selected for developing superior method to Compen-

dial method. Both the compendial HPLC method and developed HPLC method was run into the same HPLC system to prove the superiority of developed method. Sensitivity, precision, reproducibility, accuracy parameters were considered for superiority of method. Mobile phase ratio change, pH of buffer solution, change of stationary phase temperature, change of flow rate and change of column were taken into consideration for robustness study of the developed method. **Results:** The limit of quantitation (LOQ) of developed method was much low than the compendial method. The % RSD for the six sample assay of developed method was 0.4% where the % RSD of the compendial method was 1.2%. The reproducibility between two analysts was 100.4% for developed method on the contrary the compendial method was 98.4%.

## Keywords

Robustness, Method Validation, HPLC, Compendial Method, Method Development, GDP, LOQ

## 1. Introduction

Atropine Sulfate is a FDA approved drug and its available as Atropine Sulfate Ophthalmic solution form [1]. The market demand of Atropine Sulfate is tremendously increasing all over the world. It is expected that the compound annual growth rate (CAGR) of Atropine Sulfate will be remarkable during the prognosis of year 2023-2030 [2]. So, the pharmaceutical manufacturing company are producing bulk amount of the Atropine Sulfate ophthalmic preparation to meet the customer demand as well as to serve people. Atropine Sulfate Ophthalmic solution is prescribed for immediate therapy for the myopia progression [3]. However, Atropine Sulfate has various ophthalmic strength like 1% w/v, 0.5% w/v, 0.05% w/v, 0.025% w/v, 0.01% w/v. Low concentration strength like 0.05% w/v, 0.025% w/v, 0.01% w/v are the most suitable for the treatment of myopia progression relative to the high dose strength [4].

Atropine Sulfate ophthalmic solution is a compendial HPLC method where two types of buffer solution containing sodium acetate, triethylamine and organic solvent methanol has been used [5]. The main drawback of this method is time consuming and expensive. The purpose of this study is to develop a cost effective, robust stability indicating validated and reproducible method for Atropine Sulfate Ophthalmic solution.

Atropine is an alkaloids originated from natural plant Atropa belladonna and Atropine sulfate is the polymorphism of Atropine [6]. Alkaloids are basic molecules containing nitrogen groups. Whereas, some of alkaloids also contain acidic molecule [7]. In order to develop the cost effective method pH of the molecule, buffer selection, mobile phase and column has taken into consideration.

## 2. Literature Review

Chowdhury reported on his research about superior HPLC method development

and validation of Rifampicin on pharmaceutical solid dosage form to compendial method. He changed the mobile phase ratio and pH for developing his method. He found accurate, precise and reproducible method for assay value determination. However, he did not evaluate the robustness study of his method to prove the method acceptability [8].

Kathrin Koll *et al.* described in their article about method validation of herbal products by high performance thin layer chromatography. They considered solution stability of analyte, selectivity/specificity, robustness and method reproducibility [9].

In order to prove the stability indicating and validation of analytical method LOQ, LOD, accuracy, repeatability, linearity, robustness, specificity/selectivity need to perform [10].

Velusamy B *et al.* narrated about their research. They designed for the analytical method for the quantification of nine impurities of apixaban and they successfully completed the validation. They also ensured the robustness of the analytical method [11].

O. Szerkus *et al.* developed and validated a HPLC-MS method for the quantification of levofloxacin and ciprofloxacin in human prostate biotates and completed the method robustness study [12].

## 3. Purpose

The main goal of this study is to develop a cost effective, robust HPLC method development by QbD approach for Atropine Sulfate Ophthalmic Solution by maintaining good documentation practices. It will be helpful for both the researcher and also useful for quality control scientist of pharmaceutical industry. We will develop the cost effective robust method appropriately considering analytical target profile as per method development guidelines [13].

## 4. Materials and Methods

Analytical assay method for Atropine Sulfate Ophthalmic solution of Compendial method was used for understanding the analytical target profile (ATP). On the other hand superior HPLC method was developed based on pH, pKa, molecular weight, pH dependent solubility of Atropine Sulfate molecule. Atropine Sulfate Ophthalmic solution 0.05% w/v and placebo were taken as a gift from local pharmaceutical company of Bangladesh.

**Study Design**

**Initial Assessment**

**pH of Atropine Sulfate**

pH of 0.0015 molar solution10.0 [14].

**Solubility of Atropine Sulfate**

Atropine Sulfate is very soluble in water, freely soluble in alcohol and even more in boiling alcohol; freely soluble in glycerin [15].

**Molecular Weight of Atropine Sulfate**

289.4 g/mol [16]

**pKa of Atropine Sulfate**

pKa = 9.43 [17]

**Selection of Buffer Solution pH**

The buffer solution is most effective within ±1 pH. However, the buffer solution provides much more buffering capacity within ±2 pH units from the pKa [18]. Based on the buffering capacity the pH of buffer solution for mobile phase may be 7 or 11 (pH = 9.43 ± 2). Based feasibility of stationary phase pH 7.0 has been selected for pH of buffer solution of mobile phase.

**Selection of Buffer** [19]

Phosphate buffer has a buffering capacity from pH 6.2 to pH 8.2 (**Figure 1**). So, phosphate buffer has been selected for mobile phase preparation. 10 mM to 50 mM is the usual concentration for phosphate buffer solution. However, 50 mM will provide sharp peak with good resolving capacity. So, 50 mM concentration has been selected for buffer solution preparation [18].

**Selection of Organic Solvent** [18]

The solubility of phosphate buffer salt is poorer in acetonitrile and worst in tetrahydrofuran. But the solubility is relatively high in methanol. At 80% of organic solvent of acetonitrile, only 5 mM of phosphate buffer is soluble where 15 mM of phosphate buffer is soluble at methanol. For this reason, methanol has been selected for organic solvent.

**Column Characterization** [20]

**Selection of Stationary Phase**

Less than 5000 Molecular Weight is considered solubility. C18 suits water-soluble compounds; other options exist for organic-soluble or chiral molecules.

Higher than 5000 Molecular Weight is Chosen based on solubility; reverse phase chromatography remains a top choice. The molecular weight of Atropine sulfate is 289.4 and the mobile phase is polar. So, reverse phase c18 column has been selected for it's suitability to the intended purpose.

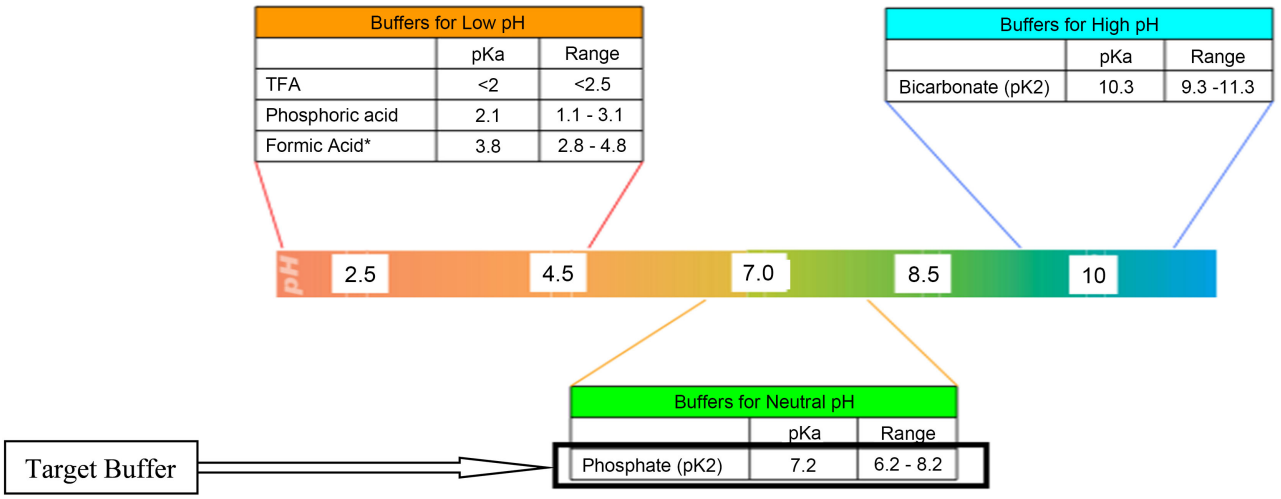


**Figure 1.** Buffering capacity of phosphate buffer.

**Selection of Particle Size**

Lower particle size provides higher efficiency. However, 5 - 10 μm particle size is the common use for analytical chemistry. So, 5 μm particle size has been selected for column.

**Selection of Column Dimension and Length**

Larger internal diameter columns (≥4 mm) is excelled in high sample loading capacity and standard HPLC compatibility but consume more solvent, offer lower sensitivity, and lack LC-MS compatibility. For this reason, 3 mm inner dimension has been selected for column. Lower length of column helps to elute the target molecule quickly. So, 150 mm length of column has been selected.

**Selection of Wavelength**

Compendial wavelength 225 nm was selected

**Method Validation** [21]

**Instrument Information**

Laboratory instruments such as Analytical Balance (Sartorius, Switzerland), Semi micro Balance (Metler Toledo, Switzerland), Micro Balance (Metler Toledo, Switzerland), High Performance Liquid Chromatography with PDA detector (Waters Alliance, USA, pH meter (Metler Toledo, Switzerland), were used for this study.

**Chemical Reagents and Standard**

Potassium dihydrogen phosphate (Sharlau, Spain), Sodium hydroxide pellets (Merck, Germany), Sodium acetate trihydrate (Sharlau, Spain), Acetonitrile (Sharlau, Spain), Methanol (Sharlau, Spain), Distilled water (Ultrapure) were used. All the reagents were used analytical grade. The reference standard of Atropine Sulfate was obtained from a local pharmaceuticals as a gift sample for research. The purity of Omeprazole reference standard was 98.9% on as is basis.

**Chromatographic Condition**

HPLC column (3.0-mm × 150 mm; 5-μm packing C18), injection volume (10 μL), wavelength (225 nm), flow rate (1.0 mL/min) were attributed as instrument parameter for operation.

**Methodology**

**Preparation of Buffer Solution**

Dissolved 6.812 g of Potassium dihydrogen phosphate into 1000 mL of purified water. Adjusted pH 7.02 by diluted sodium hydroxide solution.

Note: Buffer solution prepared at room temperature

**Preparation of Optimized Mobile Phase**

Mixed 400 mL of buffer solution and 600 mL of methanol to make 1000 mL of mobile phase. Degassed mobile phase by ultrasonic water bath. Filtered the mobile phase using 0.2 μm membrane filter.

**Preparation of Standard Stock Solution**

Taken 50.12 mg of Atropine Sulfate standard into 50 mL volumetric flask. Added 30 mL of purified water and sonicated for 5 minutes with intermittent shaking. Cooled to room temperature. Diluted up to the mark with purified water.

**Preparation of Standard Solution**

Taken 10.0 mL of the solution into 100 mL volumetric flask and diluted up to the mark with purified water. Used 0.45 µm PTFE syringe filter for transferring the standard solution into HPLC system.

**Concentration: 0.1 mg/mL as Atropine sulfate**

Note: Standard solution prepared at room temperature

**Preparation of Selectivity/Specificity sample**

**Placebo Preparation**

Taken 2.0 mL of placebo for Atropine sulfate 0.05% w/v into 100 mL volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking. Used 0.45 µm PTFE syringe filter for transferring the placebo solution into HPLC system.

**Spiked Sample Preparation**

Taken 2.0 mL of placebo for Atropine sulfate 0.05% w/v and 1.0 mL of standard stock solution into 10 mL volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking. Used 0.45 µm PTFE syringe filter for transferring the spiked sample solution into HPLC system.

**Concentration: 0.1 mg/mL as Atropine sulfate**

**Preparation of Repeatability (Precision) Sample**

Taken 2.0 mL of Atropine sulfate 0.05% w/v into 10 mL volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking. Used 0.45 µm PTFE syringe filter for transferring the sample solution into HPLC system. Prepared another five sample solution following precision sample.

**Concentration: 0.1 mg/mL as Atropine sulfate**

**Preparation of Linearity Sample**

**50% Linearity Solution**

Taken 1.0 mL of standard stock solution into 10 volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking.

**Concentration: 0.05 mg/mL as Atropine sulfate**

**80% Linearity Solution**

Taken 0.8 mL of standard stock solution into 10 volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking.

**Concentration: 0.08 mg/mL as Atropine sulfate**

**100% Linearity Solution**

Taken 1.0 mL of standard stock solution into 10 volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking.

**Concentration: 0.1 mg/mL as Atropine sulfate**

**120% Linearity Solution**

Taken 1.2 mL of standard stock solution into 10 volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking.

**Concentration: 0.12 mg/mL as Atropine sulfate**

**150% Linearity Solution**

Taken 1.5 mL of standard stock solution into 10 volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking.

**Concentration: 0.15 mg/mL as Atropine sulfate**

**Used 0.45 µm PTFE syringe filter for transferring the Linearity sample solution into HPLC system.**

**Preparation of Accuracy Sample**

**80% Accuracy Sample**

Taken 1.6 mL of placebo for Atropine sulfate 0.05% w/v and 0.8 mL of standard stock solution into 10 mL volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking. Prepared another two sample solution as per 80% accuracy sample.

**Concentration: 0.8 mg/mL as Atropine sulfate**

**100% Accuracy Sample**

Taken 2.0 mL of placebo for Atropine sulfate 0.05% w/v and 1.0 mL of standard stock solution into 10 mL volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking. Prepared another two sample solution as per 100% accuracy sample.

**Concentration: 0.1 mg/mL as Atropine Sulfate**

**120% Accuracy Sample**

Taken 2.4 mL of placebo for Atropine sulfate 0.05% w/v and 1.2 mL of standard stock solution into 10 mL volumetric flask. Dilute up to the mark with purified water. Mixed well by handshaking. Prepared another two sample solution as per 120% accuracy sample.

**Concentration: 0.12 mg/mL as Atropine Sulfate**

**Robustness Study Using Youden's Test** [22]

The robustness evaluation of the chromatographic method for the Atropine Sulfate determination in Pharmaceutical Ophthalmic solution was performed using the method proposed by Youden e Steiner in 1975. Five analytical parameters were identified and small variations were included in the nominal values of the method. Then, ten runs were conducted targeting to determine the influence of each parameter in the final result. Table 1 represents the identified parameters for robustness, actual condition and typical variation condition of the parameters for Atropine Sulfate and Table 2 narrates the design for the factorial combination of identified parameters for 10 run into HPLC system.

**Table 1.** Analytical parameters and variations for the robustness evaluation of atropine sulfate.

| | Parameters | Nominal Condition | | Typical Variation | |
|---|---|---|---|---|---|
| A/a | Methanol concentration in mobile phase | 60% | A | 55% | a |
| A/a' | | | | 65% | a' |
| B/b | Buffer solution pH | 7.00 | B | 6.95 | b |
| B/b' | | | | 7.05 | b' |
| C/c | Column temperature | 25˚C | C | 20˚C | c |
| C/c' | | | | 30˚C | c' |
| D/d | Mobile Phase Flow rate | 1.0 mL/min | D | 0.95 mL/min | d |
| D/d' | | | | 1.05 mL/min | d' |
| E/e | Methanol supplier | Sharlau | E | RCI labscan | e |

**Table 2.** Factorial combination for robustness evaluation of atropine sulfate.

| Parameters | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Methanol concentration in mobile phase | A | A | a | a' | A | A | A | A | A | A |
| Buffer solution pH | B | b | B | B | b' | B | B | B | B | B |
| Column temperature | C | C | C | C | C | c | C | C | C | c' |
| Mobile Phase Flow rate | D | D | D | D | D | D | d | D | d' | D |
| Methanol supplier | E | E | E | E | E | E | E | e | E | E |

## 5. Results and Discussion

It was observed that no peak was eluted at the elution zone of Atropine Sulfate for blank and placebo solution and the purity angle of Atropine sulfate was lower than the purity threshold. So, the peak is pure and the method is specific for Atropine sulfate identification (Table 3). The developed method was precise enough to provide reproducible results. The % RSD for the six sample solutions is 1.3 (Table 4). It is very important to ensure the accuracy of an analytical method to confirm the method's accuracy for target molecule quantification. The accuracy results of nine sample solutions were satisfactory and the 95% confidence interval of the nine sample solution was between 98.97% and 101.10% (Table 5). It ensures that whatever the number of analysis for the assay quantification used, the method's accuracy would be contained between this confidence interval limits. Linearity is another parameters for a method analytical target profile (ATP). It disclosed the minimum and maximum range of concentration for an analytical method for a molecule. If the concentration result out of limit of both the minimum and maximum concentration then the outcome will be unacceptable. So, linearity study is vital part for an analytical method. The linearity study of Atropine Sulfate developed method was performed from 50% to 150% of working concentration and found satisfactory results. The correlation coefficient value for linearity study of Atropine Sulfate was 0.999 (Table 6). Both compendial method and developed method was used for Atropine Sulfate repeatability and other analytical target profile (ATP) parameters. It was found satisfactory for both method but the developed method was superior (Table 7). However, the retention time of developed method is 2.345 minutes and the retention of compendial method is 11.175 minutes. Though, both method provides satisfactory results, the developed method is superior to compendial method due to least amount reagent in mobile phase (cost effective) and short time for operation. Figure 2 and Figure 3 represents the specimen chromatogram for developed method and compendial method.

Robustness is the integral part of method development and validation. Five parameters were taken into consideration for robustness study and ten analysis were performed with the variation of five parameters. All of the the ten runs is found satisfactory. But the assay value for condition number 8 found low relative

to others (97.4%) (Table 8). Table 9 disclosed that the average value of typical variation for the robustness study was subtracted from the original condition for the assay, retention time, tailing factor and theoretical plate of Atropine Sulfate. The typical maximum difference value for % assay for the supplier variation of methanol was high 1.67%. Moreover, the maximum typical difference value for retention time, tailing factor and theoretical plate was for 0.67%, 0.03 and 150. So, the developed method is robust in terms of mentioned design study.

**Table 3.** Specificity results.

| Sample Name | RT in minutes (Atropine Sulfate) | Purity Angle | Purity Threshold |
|---|---|---|---|
| Blank | - | - | - |
| Placebo | - | - | - |
| Spiked Solution | 2.345 | 0.101 | 0.235 |

**Table 4.** Repeatability results of atropine sulfate in ophthalmic solution.

| Sample Name | % Assay (Atropine Sulfate) | Average (%) | % RSD |
|---|---|---|---|
| 1 | 98.912 | 99.529 | 1.3 |
| 2 | 97.421 | | |
| 3 | 101.234 | | |
| 4 | 100.245 | | |
| 5 | 99.234 | | |
| 6 | 100.128 | | |

**Table 5.** Accuracy results of atropine sulfate in ophthalmic solution.

| Sample Name | % Recovery | Average (%) | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower limit | Upper Limit |
| 80% Accuracy-1 | 99.51 | 98.94 | 98.97 | 100.10 |
| 80% Accuracy-2 | 98.43 | | | |
| 80% Accuracy-3 | 98.89 | | | |
| 100% Accuracy-1 | 99.41 | 99.15 | | |
| 100% Accuracy-2 | 99.31 | | | |
| 100% Accuracy-3 | 98.74 | | | |
| 120% Accuracy-1 | 101.21 | 100.51 | | |
| 120% Accuracy-2 | 100.44 | | | |
| 120% Accuracy-3 | 99.87 | | | |

**Table 6.** Linearity results of atropine sulfate in ophthalmic solution.

| Sample Name | Concentration (mg/mL) | Concentration (%) | Area (AU) | Co relation coefficient, R |
|---|---|---|---|---|
| 1 | 0.05 | 50 | 500,240 | |
| 2 | 0.08 | 80 | 815,123 | |
| 3 | 0.1 | 100 | 1,001,240 | 0.999 |
| 4 | 0.12 | 120 | 1,204,532 | |
| 5 | 0.15 | 150 | 1,545,623 | |

**Table 7.** Comparison between compendial method and USP Results.

| **Parameters** | **Compendial Method** | **Developed Method** |
|---|---|---|
| Specificity by RT | 11.175 minutes | 2.345 minutes |
| Repeatability(average assay) | 98.461 | 99.529 |
| Linearity (R) | 0.999 | 0.999 |
| Accuracy (average) | 99.812 | 99.534 |
| Accuracy (% RSD) | 1.2 | 0.9 |

**Table 8.** Results for 10 analyses performed for robustness evaluation of Atropine Sulfate.

| Condition | Area | | Retention Time | | Tailing Factor | | Theoretical Plate | | % Assay |
|---|---|---|---|---|---|---|---|---|---|
| | Sam | Std | Sam | Std | Sam | Std | Sam | Std | |
| 1 | 1,011,240 | 1,031,445 | 2.34 | 2.32 | 1.01 | 1.02 | 6123 | 6012 | 99.8 |
| 2 | 1,023,241 | 1,071,049 | 2.11 | 2.10 | 1.01 | 1.03 | 6250 | 6125 | 99.5 |
| 3 | 1,001,241 | 1,091,247 | 3.12 | 3.33 | 1.01 | 1.00 | 5124 | 5945 | 99.2 |
| 4 | 1,061,242 | 1,011,543 | 1.94 | 1.99 | 1.06 | 1.04 | 5874 | 5812 | 98.5 |
| 5 | 1,091,242 | 1,051,741 | 4.16 | 4.25 | 1.05 | 1.01 | 5324 | 5463 | 98.6 |
| 6 | 1,071,245 | 1,041,440 | 2.41 | 2.38 | 1.08 | 1.02 | 6124 | 5945 | 99.1 |
| 7 | 1,061,277 | 10,918,242 | 3.42 | 3.40 | 1.09 | 1.03 | 5712 | 5842 | 99.9 |
| 8 | 1,041,210 | 1,071,544 | 2.37 | 2.35 | 1.06 | 1.02 | 5924 | 5621 | 97.4 |
| 9 | 1,071,211 | 1,081,346 | 2.01 | 2.00 | 1.08 | 1.04 | 5824 | 6231 | 98.7 |
| 10 | 1,031,288 | 1,021,848 | 2.27 | 2.25 | 1.05 | 1.02 | 5128 | 5765 | 98.3 |

**Table 9.** Effect of analytical parameters in content for the robustness evaluation of atropine sulfate considering RT, N, assay value.

| Parameters | % Assay* | RT* | Tailing factor* | N* |
|---|---|---|---|---|
| Methanol concentration<br>A = 60%; a = 55%, a' = 65% | 98.91 − 99.2 = 0.2 | 2.63 − 3.23 = −0.6 | 1.04 − 1.03 = 0.01 | 5838 − 5688 = 150 |
| Buffer solution pH | 98.86 − 99.05 = −0.19 | 2.49 − 3.16 = −0.67 | 1.04 − 1.03 = 0.01 | 5813 − 5791 = 22 |
| Column temperature | 98.95 − 98.70 = 0.25 | 2.70 − 2.33 = 0.37 | 1.04 − 1.04 = 0.00 | 5825 − 5740 = 85 |
| Mobile Phase Flow rate | 98.80 − 99.30 = −0.5 | 2.61 − 2.71 = −0.10 | 1.03 − 1.06 = − 0.03 | 5785 − 5775 = 10 |
| Methanol supplier | 99.07 − 97.4 = 1.67 | 2.66 − 2.36 = 0.3 | 1.04 − 1.04 = 0.00 | 5812 − 5773 = 39 |

*Average values of the nominal condition - average value of typical variation.

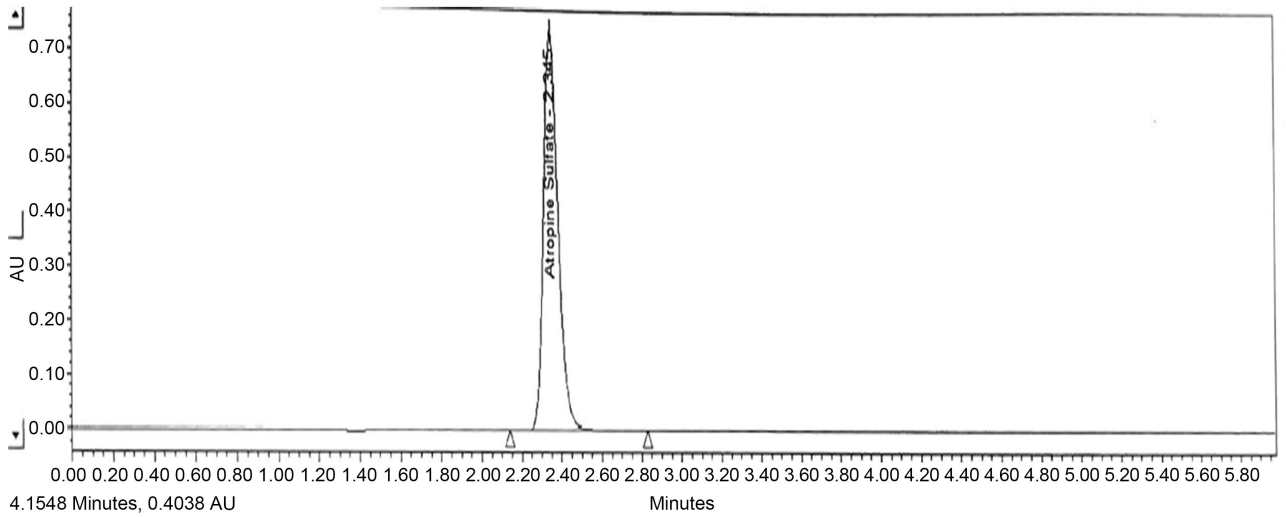


**Figure 2.** Specimen chromatogram of developed method.

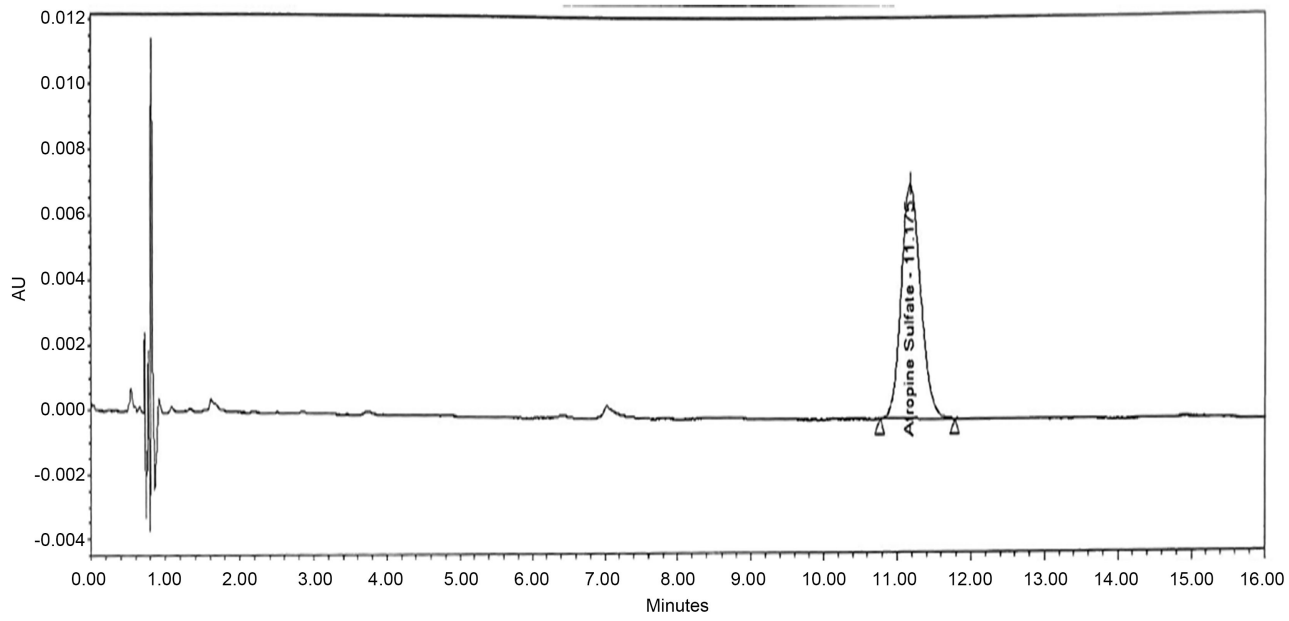


**Figure 3.** Specimen chromatogram of compendial method.

## 6. Conclusions

In order to provide a cost effective, least time, robust and superior method development and validation, this research was performed. This developed robust method will be helpful for the pharmaceutical manufacturing company, quality control scientist and researchers. It can also be used as a study material for method development for the students.

The data summarizes that the developed method is superior to the compendial method based on time consuming and cost. This information will also help for drug control authority about the nature of this developed method. This method is developed only for Atropine Sulfate. It will be better if the method was developed for combined product with Atropine Sulfate.

## Funding

This research was conducted from the self fund for purchasing and collecting sample, reagent and standard

## Acknowledgements

The research proposal and plan was initiated by Md Nazmus Sakib Chowdhury and the maximum amount of publication fees were provided by Sreekanta Nath Dalal, Md Ariful Islam, Anwar Hossain, Pranab Kumar Das, Shakawat Hossainand Parajit Das.

## Authors' Contribution

This research was designed by Md Nazmus Sakib Chowdhury and performed by Md Nazmus Sakib Chowdhury, Md Ariful Islam, Anwar Hossain, Pranab Kumar Das, Shakawat Hossain and Parajit Das. Sreekanta Nath Dalal was observed the analysis and reviewed the article. All authors drafted the article.

## Conflicts of Interest

The authors disclose no conflicts of interest regarding the publication of this paper.

## References

[1] U.S. Food and Drug Administration (2024) Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations. https://www.accessdata.fda.gov/scripts/cder/ob/results_product.cfm?Appl_Type=N&Appl_No=213581#41631

[2] Reports, V.M. (2024) Low Concentration Atropine Eye Drop Market Size, Share, Growth & Industry Trends [2030] Verified Market Reports® | Get Market Analysis and Research Reports. https://www.verifiedmarketreports.com/product/low-concentration-atropine-eye-drop-market/

[3] Yam, J.C., *et al.* (2019) Low-Concentration Atropine for Myopia Progression (LAMP) Study. *Ophthalmology*, **126**, 113-124. https://doi.org/10.1016/j.ophtha.2018.05.029

[4] Ha, A., Kim, S.J., Shim, S.R., Kim, Y.K. and Jung, J.H. (2022) Efficacy and Safety of 8 Atropine Concentrations for Myopia Control in Children. *Ophthalmology*, **129**, 322-333. https://doi.org/10.1016/j.ophtha.2021.10.016

[5] United States Pharmacopeia (2024) Atropine Sulfate Ophthalmic Solution. Rockville. https://doi.org/10.31003/USPNF_M6430_02_01

[6] National Center for Biotechnology Information (2024) PubChem Compound Summary for, Atropine. https://pubchem.ncbi.nlm.nih.gov/compound/Atropine

[7] Dey, P., *et al.* (2020) Analysis of Alkaloids (Indole Alkaloids, Isoquinoline Alkaloids, Tropane Alkaloids). In: Silva, A.S., Nabavi, S.F., Saeedi, M. and Nabavi, S.M., Eds., *Recent Advances in Natural Products Analysis*, Elsevier, Amsterdam, 505-567. https://doi.org/10.1016/B978-0-12-816455-6.00015-9

[8] Chowdhury, N.S. (2023) A Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Quantification for Assay of Rifampicin in Pharmaceutical Solid Dosage Form. *International Journal of Pharmaceutical Chemistry and*

*Analysis*, **10**, 55-58. https://doi.org/10.18231/j.ijpca.2023.010

[9] Koll, K., Reich, E., Blatter, A. and Veit, M. (2003) Validation of Standardized High-Performance Thin-Layer Chromatographic Methods for Quality Control and Stability Testing of Herbals. *Journal of AOAC INTERNATIONAL*, **86**, 909-915. https://doi.org/10.1093/jaoac/86.5.909

[10] Chowdhury, N.S. (2023) A Review on Specific and Sensitive Analytical Method Development and Validation. *International Journal of Pharmaceutical Chemistry and Analysis*, **10**, 28-30. https://doi.org/10.18231/j.ijpca.2023.005

[11] Subramanian, V.B., Katari, N.K., Dongala, T. and Jonnalagadda, S.B. (2020) Stability-Indicating RP-HPLC Method Development and Validation for Determination of Nine Impurities in Apixaban Tablet Dosage Forms. Robustness Study by Quality by Design Approach. *Biomedical Chromatography*, **34**, e4719. https://doi.org/10.1002/bmc.4719

[12] Szerkus, O., *et al.* (2017) Robust HPLC-MS/MS Method for Levofloxacin and Ciprofloxacin Determination in Human Prostate Tissue. *Journal of Pharmaceutical and Biomedical Analysis*, **132**, 173-183. https://doi.org/10.1016/j.jpba.2016.10.008

[13] European Medicines Agency (2024) ICH Guideline Q14 on Analytical Procedure Development. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q14-analytical-procedure-development-step-2b_en.pdf

[14] O'Neil, M.J., *et al.* (2024) The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Merck, New Jersey. http://catdir.loc.gov/catdir/toc/wiley041/2003267062.html

[15] United States Pharmacopeia (2012) Reference Tables: Description and Solubility-A.

[16] National Center for Biotechnology Information (2024) Compound Summary for CID 3661, Atropine Sulfate. https://pubchem.ncbi.nlm.nih.gov/compound/3661

[17] National Center for Biotechnology Information (2024) PubChem Compound Summary for, Atropine Sulfate. https://pubchem.ncbi.nlm.nih.gov/compound/Atropine-Sulfate

[18] https://www.hplc.eu/Downloads/ACE_Guide_BufferSelection.pdf.

[19] Science Unfiltered (2024) Buffers in Mobile Phase—Their Significance of Choosing the Correct Ones. https://phenomenex.blog/2020/04/08/buffers-mobile-phase/

[20] Separation Science in Collaboration with PerkinElmer (2024) LC Learning Series: Mastering the Art of Column Choice. https://www.sepscience.com/lc-learning-series-mastering-the-art-of-column-choice/

[21] ICH Expert Working Group (2006) Q2(R1) Validation of Analytical Procedures: Text and Methodology.

[22] Da Costa César, I. and Pianetti, G.A. (2009) Robustness Evaluation of the Chromatographic Method for the Quantitation of Lumefantrine Using Youden's Test. *Brazilian Journal of Pharmaceutical Sciences*, **45**, 235-240. https://doi.org/10.1590/S1984-82502009000200007

## Abbreviation

ATP: Analytical target profile
HPLC: High performance liquid chromatography
RT: Retention time
N: Theoretical plate
RSD: Relative standard deviation
mg/mL: Milligram per milliliter
g: Gram
PTFE: Ploy tetrafuluro ethylene
µm: Micro metre
LOD: Limit of detection
LOQ: Limit of quantitation
nm: Nano metre
Sam: Sample
Std: Standard