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# Development and validation of stability-indicating RP-HPLC method for the simultaneous estimation of xylometazoline hydrochloride and ipratropium bromide from nasal spray dosage form

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## Abstract

**Background:** A simple, robust, precise, and an accurate HPLC method was established for simultaneous estimation of xylometazoline hydrochloride and ipratropium bromide from a nasal spray dosage form. The effective separation was obtained by injecting 10 µl of sample and standard solutions on to an Inertsil ODS column, 250 × 4.6, mm, 5 µ at 45 °C using phosphate buffer with 1-pentane sulphonic acid sodium salt at pH 4.7 as a mobile phase A and acetonitrile as the mobile phase B. The gradient was optimized with a flow rate of 1 mL/min and a wavelength of 210.0 nm.

**Result:** The complete analytical method validation was successfully carried out as per ICH guidelines. The retrieval study was carried out at 50% to 150% level of working concentration, and results were in the range of 99 to 101% for both the analytes. The linearity was proven from 4 to 150% of working concentration with linear regression curve ($R^2$=0.999) for both the analytes. The developed method was robust for different parameters like column temperature, flow rate, mobile phase pH, composition, and gradient.

**Conclusion:** The developed HPLC method can be successfully used for the estimation of xylometazoline hydrochloride and ipratropium bromide from nasal spray dosage form as a release test in QC department of manufacturing units.

**Keywords:** RP-HPLC, Method validation, Nasal spray, Ipratropium bromide, Xylometazoline, Stability study

## Background

Nasal spray drug products contain medicinally active constituents dissolved or suspended in solutions or mixtures of excipients like preservatives, viscosity modifiers, emulsifiers, buffering agents in non-pressurized dispensers that deliver a spray containing a metered dose of the active constituent controlled by a spray pump. A nasal spray unit can be intended for unit dosing discharge up to several hundred metered sprays of formulation containing the drug ingredient. Nasal sprays are used to deliver drugs locally in the nasal cavities for local and/or systemic effects, such as allergic rhinitis and nasal congestion [1].

Ipratropium bromide (Fig. 1a) is a quaternary ammonium derivative of atropine an anticholinergic agent [2]. The IUPAC name of ipratropium bromide

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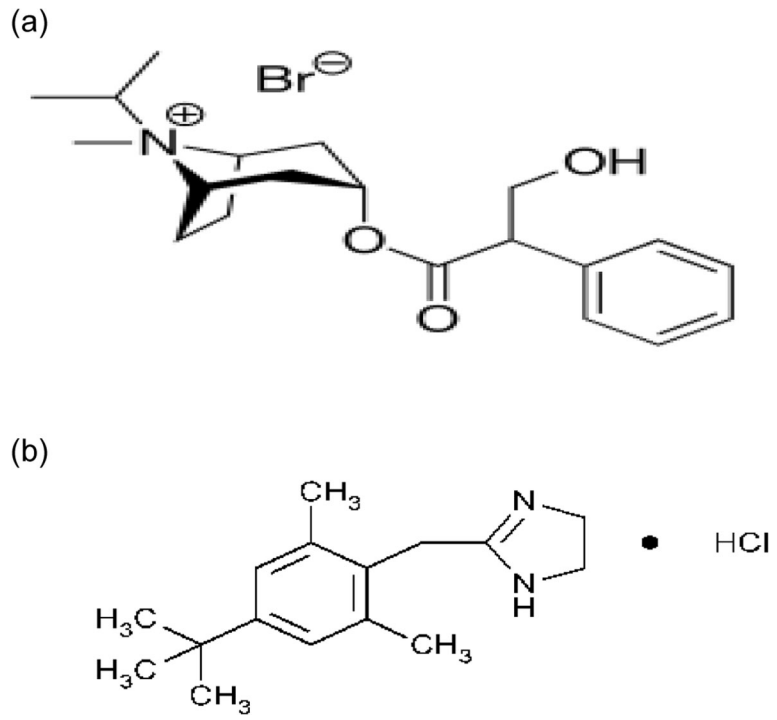



**Fig. 1** Structure of (**a**) ipratropium bromide and (**b**) xylometazoline HCl

(IB) is (1R, 3R, 5S,8R)-3-[(3-hydroxy-2-phenyl propanoyl) oxy]-8-methyl-8-(propane-2-yl)-8-azabicyclo [3.2.1] octane-8-ium bromide. It is freely soluble in water and methanol, sparingly soluble in ethanol, and insoluble in lipophilic solvents such as ether, chloroform, and fluorocarbons. The PKa value of the ipratropium bromide is 15.15. Ipratropium bromide is a bronchodilator that works via blockade of muscarinic cholinergic receptors and is clinically used in combination with other inhalation solutions or suspensions for enhanced treatment efficacy [3]. It is used for various bronchial disorders, in rhinitis, and as an antiarrhythmic [4–6].

The IUPAC name of xylometazoline (Fig. 1b) is 2-[(4-tert.butyl-2,6-dimethyl phenyl)methyl]-4,5-dihydro-1H-imidazole hydrochloride; it is freely soluble in water, ethanol, and methanol. The pKa value is 10.29. It is used for healing nasal blocks and minor swellings due to allergies or colds.

Various analytical methods are reported for the estimation of individual xylometazoline using GC [7], HPTLC [8], HPLC [9–12], and spectrophotometry [13, 14], while for ipratropium bromide, HPLC validated method is employed [15–19]. From the literature survey, there appear no methods for simultaneous quantitation of xylometazoline hydrochloride and ipratropium bromide by RP-HPLC for any pharmaceutical dosage form. The reported methods are for the estimation of xylometazoline hydrochloride or ipratropium bromide individually or in combination with other drugs. In this paper, we have successfully developed a RP-HPLC method for the simultaneous estimation of xylometazoline hydrochloride and ipratropium bromide from a nasal spray dosage form. The developed method was successfully used for other performance tests of nasal sprays like uniformity of deliver dose and content delivered per actuation. The present work will be beneficial to scientists to save analytical method development time and cost in pharmaceutical industry since we have overcome the possible interference of placebo in estimation of these two products when estimated simultaneously by a single HPLC method. The developed method is successfully used for the quantitation of both the compounds from a marketed sample Otrivin plus nasal spray (GlaxoSmithKline).

## Methods

### Pure samples

Xylometazoline hydrochloride (99.7% purity) and ipratropium bromide (95.8% purity) working standards were used in the preparation of standard solution for quantitation of formulation products.

### Formulation

Otrivin plus (manufactured by GlaxoSmithKline Pharmaceuticals Limited) was purchased from the local market. In-house nasal spray sample was used for all the parameters of the validation study.

### Chemicals, reagents, and equipment

The liquid chromatographic system (Make-Shimadzu, Japan) LC-2010$C_{HT}$, with VU/visible detector was used for development and validation. Intermediate precision and selectivity study was performed on Waters e 2695 with PDA detector (Model-Waters 2998). The reagents and chemicals used for preparation of mobile phase such as sodium di-hydrogen phosphate dihydrate (Rankem, India), 1-pentane sulphonic acid sodium salt (Merck, USA), orthophosphoric acid (Rankem, India), acetonitrile (Finar, India) were of HPLC grade. The HPLC run of xylometazoline and ipratropium bromide was carried out by optimized chromatographic conditions on a Inertsil ODS, 250 × 4.6 mm, 5 μm column. The analytical method development and validation was carried out on a nasal spray sample prepared in-house. The placebo sample was prepared by including all the components except ipratropium bromide and xylometazoline HCL

### Preparation of mobile phase

In total, 1.56 g of sodium di-hydrogen phosphate dihydrate and 0.5 g of 1-pentane sulphonic acid sodium salt was weighed and transferred into a glass bottle containing 1 L of HPLC grade water. The solution was sonicated for 15 min and the pH adjusted to 4.7±0.1 using orthophosphoric acid. The solution was filtered through a 0.45-μm syringe filter (MDI, India) and used as mobile phase A, while acetonitrile was used as the mobile phase B. Water was used as a diluent for the preparation of standard, sample, and placebo solutions.

### Optimization of chromatographic conditions

For better separation, and to achieve good column efficiency as per USP system suitability criteria, the method parameters were optimized using Inertsil ODS 3V, 250 × 4.6 mm, 5 μm HPLC column. The column temperature was set at 45 °C. The mobile phase flow was adjusted at 1.0 mL/min. Equal volume of (10 μl) sample, placebo, and standard samples were injected. The ipratropium bromide peak was eluted at about 5 min and xylometazoline HCL at about 12 min. The PDA detector was used during the method development for selection of wavelength and checking the peak purity of the samples subjected to force degradation. Both the compounds showed maximum absorbance at 210.0 nm.

### Gradient program

| Time (minutes) | Mobile phase A | Mobile phase B |
|---|---|---|
| 0.01 | 70 | 30 |
| 5.00 | 70 | 30 |
| 12.00 | 40 | 60 |
| 14.00 | 50 | 50 |
| 15.00 | 50 | 50 |
| 17.0 | 70 | 30 |
| 22.0 | 70 | 30 |

### Standard stock preparation

#### *Xylometazoline hydrochloride standard stock solution preparation*

Fifty milligrams of xylometazoline hydrochloride working standard was weighed and transferred into a 50-mL volumetric flask, 30 mL of diluent was added and sonicated to dissolve it completely. The volume was made up to 50 mL with the diluent and mixed well before use.

#### *Ipratropium bromide standard stock solution*

Sixty milligrams of ipratropium bromide working standard was weighed and transferred into a 50-mL volumetric flask. Nearly 30 mL of diluent was added and the mixture was sonicated to dissolve the contents completely. The volume was made up to 50 mL with the diluent, mixed well, and used for further analysis.

### Preparation of standard solution

Five milliliters each of xylometazoline hydrochloride and ipratropium bromide from standard stock solutions was measured and transferred in to a 50-mL volumetric flask. The volume was made up with diluent and mixed well.

### Sample preparation

About 2 g of the sample was weighed and transferred to a 10-mL volumetric flask, 7 mL diluent was added and sonicated for 10 min. The volume was made up to 10 mL with the diluent and mixed well. The sample was filtered through 0.45-μm PVDF filter and used for injection into HPLC system.

### System suitability

The system suitability is a significant parameter in the method development and validation study of any drug dosage form. The system suitability is checked by injecting the six replicate standard solutions into the HPLC system. The limit for relative standard deviation (%RSD) of area under the curve of six replicate standard injections was kept at not more than 2.0%. The limit for

column efficiency was kept at not less than 2000 theoretical plates. The limit for tailing factor was not more than 2.0 for both the analyte peaks.

### Calculations

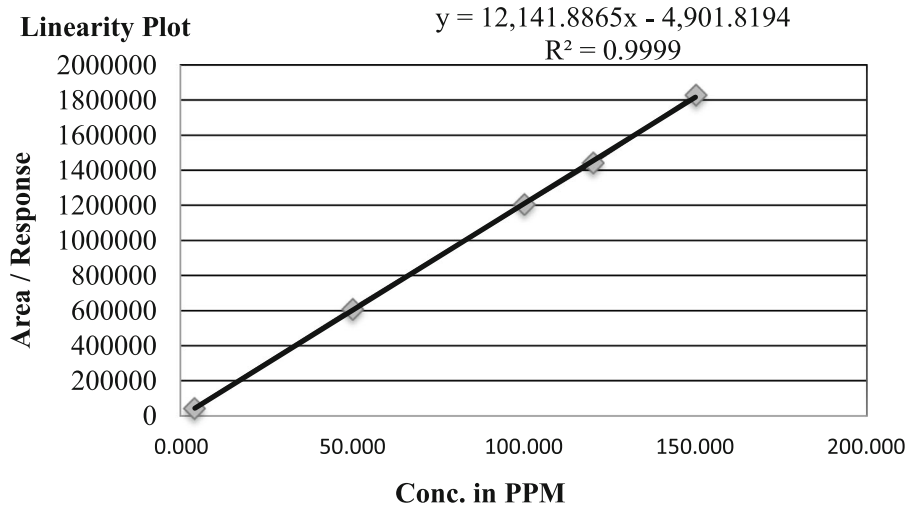
Equal volume of both the sample and standard solutions were injected into the HPLC system, and area under the curve for each analyte peak was recorded. The amount of xylometazoline hydrochloride and ipratropium bromide in % w/v was calculated.

### Analytical method validation

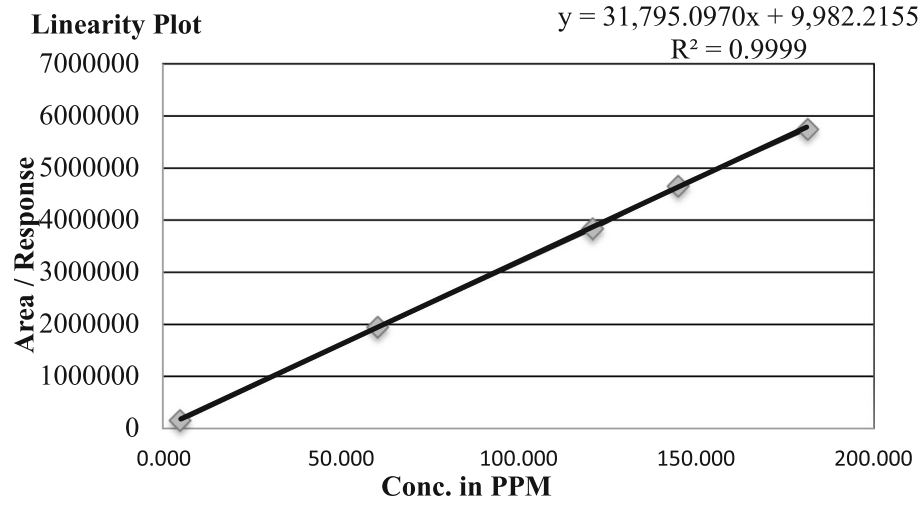
The developed method was validated in terms of, accuracy, precision specificity, linearity, range, robustness, and stability of analytical solution as per the ICH guidelines.

#### *Accuracy*

The accuracy of an analytical method reflects the closeness of agreement between the values that is acceptable either as a conventional true or an accepted reference value, and the value observed by the technique. Standard solutions were spiked in to placebo at various concentration levels, i.e., 50, 100, and 150% of working concentration and analyzed according to the described method. The mean % recovery for the analytes at each concentration level should be in the range of 98-102% and % RSD of % recovery for the analytes at every




**(a)** Linearity of Ipratropium Bromide




**(b)** Linearity of Xylometazoline HCl

**Fig. 2** **a** Linearity of Ipratropium bromide. **b** Linearity of Xylometazoline HCl

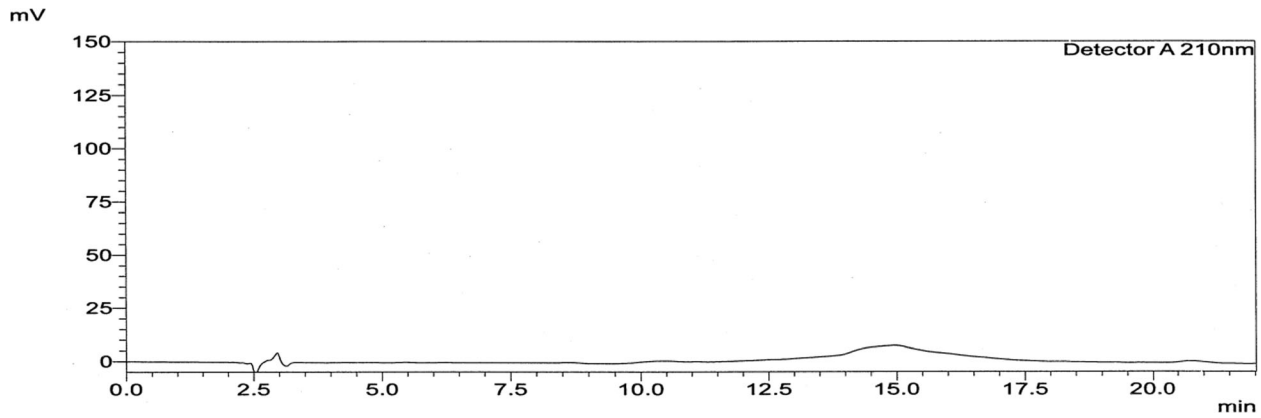



**Fig. 3** Typical chromatogram of Blank solution

level must not be greater than 2.0% as per ICH guidelines.

### *Precision*

The precision of an analytical process defines the goodness of agreement among the series of measurements acquired from many identical samples. As per ICH validation guidelines, system precision, method precision, and intermediate precision were evaluated. The standard solution was injected in six replicates as described in analytical method and the % RSD was determined. The % RSD for peak area of both the analytes for six identical injections of standard solutions was set at not more than 2.0%.

**Repeatability** The method precision was carried out by preparing six samples of a single batch. The % assay of the six samples was calculated. The precision of the method was examined by calculating the % RSD of the results.

**Intermediate precision** Intermediate precision expresses ability of method to produce reliable results under laboratory conditions, viz., different days, analysts, system, and column. Six samples were prepared as per the test procedure by using the same batch of formulation and injected. The % assays of these samples were examined and the ruggedness of the method was estimated by calculating the % RSD of the results.

### *Specificity*

**Selectivity** Diluent, individual standard solutions for each analyte, placebo, and samples were prepared as mentioned in the analytical method section.

**Force degradation** To prove the stability-indicating nature of the developed analytical method, the forced degradation study was carried out at different stress conditions like acid, base, oxidative, and thermal degradation.

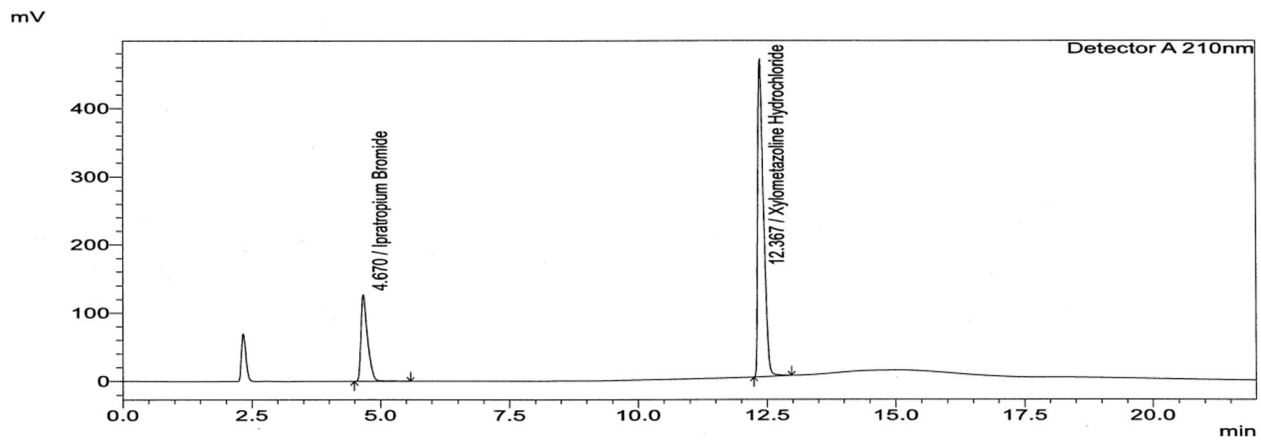



**Fig. 4** Typical chromatogram of standard solution containing Ipratropium bromide and Xylometazoline HCl

**Table 1** System precision

| Inj. no. | Xylometazoline | Ipratropium bromide |
|---|---|---|
| 1 | 3681946 | 1107548 |
| 2 | 3698831 | 1117256 |
| 3 | 3682602 | 1112935 |
| 4 | 3709109 | 1112831 |
| 5 | 3740140 | 1114566 |
| 6 | 3692709 | 1104680 |
| **Average** | **3700890** | **1111636** |
| Standard deviation | 21783.52 | 4655.80 |
| **% RSD** | **0.59** | **0.42** |

For acid degradation, 1 mL of 0.1 N hydrochloric was added in the sample, and kept at 80 °C for 8 h. The sample was neutralized with the same quantity of 0.1N NaOH solution. For degradation under basic conditions, 0.1 mL of 0.1 N NaOH solution was added in the sample, and after 5 min, the sample was neutralized with 0.1N HCl solution. During the thermal degradation study, the sample was heated at 80 °C for 48 h in an oven and the sample analyzed. For oxidative degradation, 1 mL of 30% hydrogen peroxide was added to a sample solution. The sample was kept at room temperature for 24 h and analyzed.

### *Linearity*

The linearity of the developed method was validated by analyzing the mixture of standard solution concentration in the range of 4-150% of working concentration. The solutions were injected in triplicate into the HPLC system, and the area under the curve of analyte peaks was recorded. The correlation co-efficient between concentration and peak area, slope, and intercept was evaluated.

**Table 2** Method precision

| S.N | Xylometazoline | Ipratropium bromide |
|---|---|---|
| **1** | 100.0 | 102.0 |
| 2 | 96.7 | 100.0 |
| 3 | 100.0 | 102.0 |
| 4 | 96.7 | 98.0 |
| 5 | 98.3 | 100.0 |
| 6 | 96.7 | 98.0 |
| **Average** | 97.2 | 99.3 |
| Standard deviation | 1.78 | **1.78** |
| **% RSD** | 1.83 | 1.79 |

**Table 3** Intermediate precision

| S.N | Xylometazoline | Ipratropium bromide |
|---|---|---|
| 1 | 96.7 | 100.0 |
| 2 | 95.0 | 98.0 |
| 3 | 95.0 | 96.0 |
| 4 | 98.3 | 100.0 |
| 5 | 98.3 | 98.0 |
| 6 | 95.0 | 98.7 |
| **Average** | 96.38 | 98.5 |
| Standard deviation | 1.62 | 1.50 |
| **% RSD** | 1.69 | 1.52 |

### *Range*

The developed method was checked for upper and lower amount of both the analytes in the sample for which it has been demonstrated that the method has suitable level of precision, linearity and accuracy. This study was covered under the linearity study.

### *Robustness*

Robustness of an analytical method measures the capacity to stay unaffected by minor changes in the method parameters. In this study, parameters like variation in detection wavelength, flow rate, column oven temperature, mobile phase organic composition, and mobile phase buffer pH were studied.

### *Stability of analytical solution*

The system suitability solution and sample solution was prepared on day zero of experiment and stored at room temperature. The solution was analyzed on subsequent days for 3 days. The standard solution used was prepared freshly for the investigation and the assay results were calculated for sample solution to evaluate the stability sample of solution. The solution is considered stable, if the cumulative % RSD of the stored sample and standard solution is not more than 2.0.

## Results

### Specificity

During the specificity study, no interfering peak was observed at the retention time of both the analytes in the placebo sample and the diluent indicating the peak purity of both the analyte peaks. The representative HPLC chromatogram for blank, standard and sample solution is shown in Figs. 2, 3 and 4 respectively.

### Precision

In the system precision study, the % RSD of six replicate injections was observed as 0.59 and 0.42% for xylometazoline HCl and ipratropium bromide respectively.

**Table 4** Recovery study

| | Xylometazoline | | Ipratropium bromide | |
|---|---|---|---|---|
| Recovery level in % | % Recovery | % Average recovery | % Recovery | % Average recovery |
| 50% | 99.7 | 99.4 | 98.5 | 98.3 |
| | 99.2 | | 98.3 | |
| | 99.4 | | 98.2 | |
| 100% | 101.2 | 101.1 | 101.4 | 101.3 |
| | 101.3 | | 101.4 | |
| | 100.9 | | 101.2 | |
| 150% | 101.6 | 101.6 | 101.6 | 101.5 |
| | 101.5 | | 101.5 | |
| | 101.6 | | 101.5 | |

The results are summarized in Table 1.

For the method precision and intermediate precision study, the % assay values of 12 sample solutions were found within the specifications (less than 2%). The results for both method precision and intermediate precision are represented in Tables 2 and 3 respectively.

### Accuracy (% recovery)

The % recovery obtained at concentration levels of 50%, 100%, and 150% was found in the range of 98-102%. The recovery of ipratropium bromide was found to be 99.4%, 101.1%, 101.6%, and for xylometazoline HCl 98.3%, 101.3%, 101.5% at 50%, 100%, and 150% respectively.

The results are reported in Table 4.

### Linearity

The linearity graph of concentration vs average peak area of analyte is reported in Figs. 5 and 2 respectively. The correlation co-efficient, peak area slope, and intercept were evaluated and found to be within the specifications. The linearity results for both analytes are summarized in Table 5.

% RSD for the area of five standard injection solutions, tailing factor, and theoretical plates generated during the robustness study are presented in Table 6. From the reported data, we can conclude that the developed method is robust.

### Force degradation study

The forced degradation study showed that the method developed is specific and there was no any interference found in the analyte peaks. All the generated impurities were resolved properly as shown in Table 7.

**Representative HPLC chromatograms**

## Discussion

From the literature reports, it is clear that currently there are no reported methods available for simultaneous estimation of xylometazoline HCL and ipratropium bromide in a combination drug dosage form, although estimation methods for the individual determinations of xylometazoline HCl or in combination with other drug

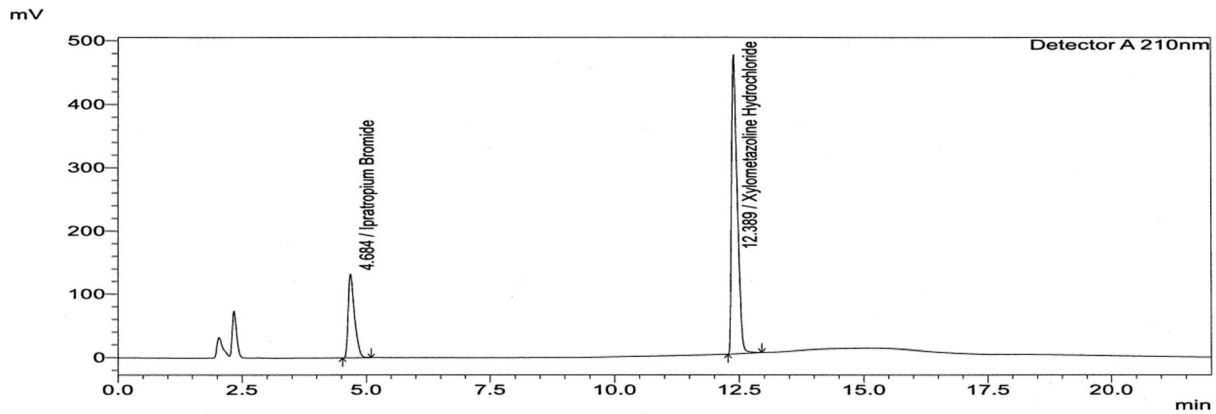



**Fig. 5** Typical chromatogram of sample solution containing Ipratropium bromide and Xylometazoline HCl

**Table 5** Linearity study of ipratropium bromide and xylometazoline HCl

| Conc in PPM | Area/response |
|---|---|
| Ipratropium bromide | |
| 4.01 | 42839 |
| 50.06 | 609125 |
| 100.12 | 1207745 |
| 120.14 | 1442510 |
| 150.18 | 1827612 |
| Slope | 12141.8865 |
| Intercept | −2623.8690 |
| Correlation coefficient [*R*] | 0.9998 |
| $R^2$ | 0.9997 |
| Xylometazoline HCl | |
| 4.83 | 152656 |
| 60.410 | 1945610 |
| 120.820 | 3830972 |
| 144.984 | 4657793 |
| 181.230 | 5750777 |
| Slope | 31795.0970 |
| Intercept | 9982.2155 |
| Correlation coefficient [*R*] | 0.9999 |
| $R^2$ | 0.9999 |

substances [7–14] and for the individual estimation of ipratropium bromide or in combination with other drug substances [15–17].

In this paper, we have successfully attempted and developed a HPLC method for efficient resolution of xylometazoline-HCl and ipratropium bromide from each other and from their degradation peaks. While ipratropium bromide eluted at about 5 min, xylometazoline HCL eluted at about 12 min under the described HPLC conditions. The developed method was validated as per ICH guideline [18, 19]. Forced degradation study was performed by applying various stress conditions to the sample to evaluate the robustness and stability-indicating nature of the developed method. During the forced degradation study, we observed that degradation of ipratropium bromide was more significant at basic and acidic conditions. Both the API's have not shown more degradation when exposed to oxidative and thermal stress conditions. During the solution stability studies, we have observed that both standard and sample solutions are stable at room temperature for 3 days. The developed method utilizes a single wavelength for analysis since both the analytes have shown optimum response at 210.0 nm. During the robustness study, the peak area, theoretical plates, and tailing factor for both API peaks was found to be within the acceptance limits indicating the robustness of the method.

## Conclusion

A simple, precise, and robust RP-HPLC method with UV detector was established for the simultaneous estimation of xylometazoline hydrochloride and ipratropium bromide for a nasal spray dosage form. The method is validated as per the Q2 (R1) ICH guidelines. This method being accurate, precise, sensitive, and time saving with minimum usage of organic solvents; it is suitable for the routine quality control

**Table 6** Robustness study

| Sr. no. | | Xylometazoline | Ipratropium bromide | Xylometazoline | Ipratropium bromide |
|---|---|---|---|---|---|
| | | **Decrease in flow rate (0.9 mL/min)** | | **Increase in flow rate (1.1 mL/min)** | |
| 1 | % RSD of six standard inj. | 0.85% | 0.47% | 0.33 | 0.36 |
| | Tailing factor | 1.5 | 1.5 | 1.6 | 1.5 |
| | Theoretical plates | 47107 | 8743 | 48581 | 8230 |
| | **Change in column oven temperature ±5 °C of 45 °C** | | | | |
| 2 | | **Decreases in column temperature (40 °C)** | | **Increases in column temperature (50 °C)** | |
| | % RSD of six standard inj. | 0.48 | 0.28 | 0.29 | 0.24 |
| | Tailing factor | 1.6 | 1.5 | 1.6 | 1.5 |
| | Theoretical plates | 50569 | 6784 | 40259 | 6510 |
| | **Change in wavelength ± 3 nm of 210.0 nm** | | | | |
| 3 | | **Decrease in wavelength (207.0 nm)** | | **Increase in wavelength (213.0 nm)** | |
| | % RSD of six standard inj. | 0.08 | 0.20 | 0.06 | 0.28 |
| | Tailing factor | 1.4 | 1.6 | 1.4 | 1.6 |
| | Theoretical plates | 50027 | 5214 | 50891 | 5323 |

**Table 7** Force degradation study results

| Stress degradation type | Conditions | Xylometazoline HCl | Ipratropium bromide |
|---|---|---|---|
| Acid degradation | 0.1N HCl | 4.0% | 13.80% |
| Base degradation | 0.1N NaOH | No degradation | 18% |
| Oxidative degradation | 30% $H_2O_2$ | 2.4% | 6.70% |
| Thermal degradation | At 80 °C for 48 h | No degradation | 6.0% |

testing of the finished nasal spray product batches. The method was developed by keeping in mind the minimum usage of the organic solvent with minimum run time to make it cost effective and ecofriendly.

### Abbreviations

IUPAC: International Union of Pure and Applied Chemistry; ICH: International Conference on Harmonization; RP-HPLC: Reverse-phase high-performance liquid chromatography; USP: United States Pharmacopeia; API: Active pharmaceutical ingredient; ODS: Octadecyl silane; SD: Standard deviation; RSD: Relative standard deviation; PPM: Parts per million

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### Authors' contributions

The authors have read and approved the manuscript. KL designed the study. BM, PS, and SL performed the experiments and analyzed the data. SK and AP reviewed the data. SB supervised the experiment. SP supported for writing the manuscript.

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### Availability of data and materials

All data is available upon request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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