# RP-HPLC method for the simultaneous analysis of ambroxol hydrochloride and nitazoxanide in API and tablet dosage form

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# RP-HPLC Method for the Simultaneous Analysis of Ambroxol Hydrochloride and Nitazoxanide in API and Tablet Dosage Form

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**Abstract:** Present work is aimed to develop a new simple, fat, rapid, accurate, efficient, and reproducible RP-HPLC method for the simultaneous analysis of Ambroxol Hydrochloride and Nitazoxanide in API& tablet dosage form. The chromatographic separation was performed using phenomenex $C_{18}$ Column having dimensions of 4.6x250mm having particle size of 5µm, with mobile phase consisting of Buffer $P^H$-3.5 and Acetonitrile (40:60% v/v), flow rate was adjusted to 1.0ml/min and detection wavelength at 235 nm. The proposed method has been validated for linearity, range, precision, accuracy and robustness were within the acceptance limit according to the ICH Q2B guidelines. The retention times of Ambroxol Hydrochloride and Nitazoxanide were 2.985 mins and 5.581 mins respectively. The linearity was performed in the concentration in the range of 7.5 µg/ml to 45µg/ml and 25 µg/ml to 150 µg/ml and with a correlation coefficient of 0.999 and 0.999 respectively. % RSD for system precision was found to be 0.212 and 0.160% RSD for repeatability 0.2 and 0.12, % RSD for intermediate precision was 0.06 and 0.06 respectively. The % percentage purity of Ambroxol Hydrochloride and Nitazoxanide was found to be 99.93% and 99.35% respectively. The method was found to be robust even by change in the mobile phase ±5% in less flow condition.

## INTRODUCTION

Ambroxol Hydrochloride is a secretolytic agent used for respiratory problems diseases with excessive mucus or viscid. It is a metabolite of bromhexine[2]. Chemically it can be represented as 4-[(2-amino-3,5-dibromophenyl)methylamino]cyclohexan-1-ol;hydrochloride with formula of $C_{13}H_{18}Br_2N_2O$, mass was 414.56 g/mol[3,4,5] (Fig 1). The physiochemical properties are white crystalline solid, odorless and is freely soluble in methanol, acetone, ethanol and tetrahydrofuran and very soluble in acetonitrile1,2 having melting point 81-82 $^0C$[6,7,15]. Nitazoxanide chemically as (-N-(5-nitro-2-thiazoyal) salicylamide acetate) [1,13,14] (Fig. 2) with molecular

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formula $C_6H_6N_6O_2$, is an anthelmenthic, antiprotazoal, giardiasis[2] cryptosporidiosis in immune-compromised patient and helmentic infection.[15]

Literature review reveals that there are few analytical methods reported for the analysis of Ambroxol Hydrochloride and Nitazoxanide.by the simultaneous estimation by using spectrophotometer, RP-HPLC are the reported analytical methods for compounds either individually or in combination with other dosage form.[8,9-14] Hence it was felt that there is a need for a new analytical method development for the simultaneous estimation of Ambroxol Hydrochloride and Nitazoxanide in pharmaceutical oral (tablet) dosage form. The developed method will be validated according to ICH Q2B guidelines.[16]

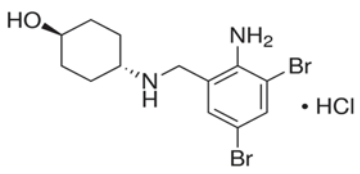



**FIGURE 1.** Structure of Ambroxol Hydrochloride

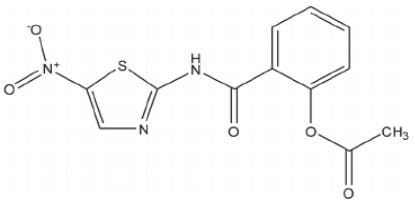



**FIGURE 2.** Structure of Nitazoxanide

## EXPERIMENTAL SECTION

Reference materials of Ambroxol Hydrochloride were supplied by Mylon, Nitazoxanide from Cipla, HPLC graded methanol, Ortho phosphoric acid and water (Merck), Acetonitrile (Thermo Fischer Scientific India), $KH_2PO_4$ and $K_2HPO_4$(Rankem) and samples of tablets were procured from local market. The mobile phase consists of phosphate buffer (pH 3.5) and HPLC graded Acetonitrile (40:60% v/v). Analysis were performed on Waters HPLC, auto sampler and UV detector. Data were together and evaluated by empower softwar and analyte elution by phenomenex $C_{18}$ Column having dimensions of 4.6x250mm having particle size of 5µm with ambient temperature programme at a detection of 235 nm. All the drugs and chemicals were weighed on Afcoset ER-200A electronic balance, pH meter (Adwa – AD 1020) and a sonicator (Frontline FS 4, Mumbai, India). The mobile phase was degassed by ultrasonic vibrations prior to use.

### Instrument and Chromatographic Separation

Chromatography was performed on a WATERS 2695 HPLC column (Waters Corporation, Milford, USA) with an autosampler with UV detector detector.Components were detected at 235 nm and data processing was achieved by Empower 2 software. The chromatographic separation was performed on phenomenex $C_{18}$ (4.6 x 250mm, 5µm) column at an ambient column temperature. The samples were eluted using phosphate buffer (pH 3.5): Acetonitrile (40:60% v/v) as the mobile Phase at a flow rate of 1 mL/min. The mobile phase was filtered through a 0.45-µm nylon filter and it was degassed before use. 10 µL of sample solutions were injected into the HPLC system.

### Standard Solution Preparation

Standard and working solutions Ambroxol hydrochloride (30mg) and Nitazoxanide (100 mg) were accurately weighed and transferred into a 100-mL clean and dry volumetric flask separately and 10 mL of the diluent was added. It was sonicated for 30 min and diluted to the final volume with the diluent. From the above stock solution, 1 mL of the solution was transferred into another 10 mL volumetric flask and then diluted to the final volume with the diluent.

### Sample Solution Preparation

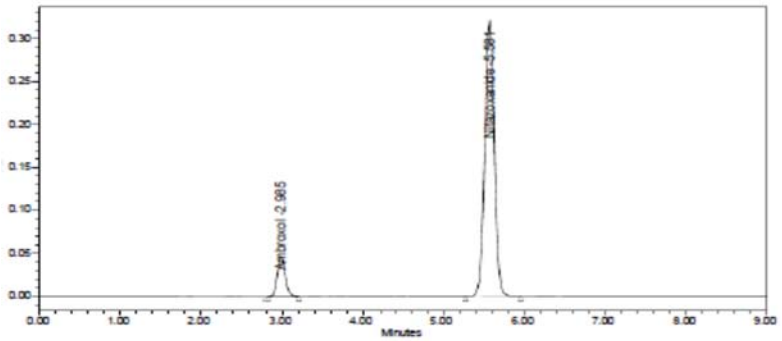
Twenty tablets were weighed and powdered finely and weight of the powder equivalent to 465 mg was transferred into a 100 mL volumetric flask and 50 mL of the diluent was added, the flask was sonicated for 30 min

and the volume was made up with the diluent and filtered. Finally 0.3 mL of the filtered solution was pipetted out, transferred into a 10 mL volumetric flask and made up the final volume with diluents.

## RESULTS AND DISCUSSION

### Method of Development and Optimization of Chromatographic Conditions

The main objective of this study was to develop and validate a assay method for simultaneous estimation of Ambroxol hydrochloride and Nitazoxanide by reverse-phase high performance liquid chromatography. Several chromatographic trials were conducted using various solvents such as methanol, ACN, water and different phosphate buffer pH levels at different ratios. During the mobile phase selection, it was found that buffer could help in separating two drugs with good resolution. The best results were achieved by using the phenomenex $C_{18}$ (4.6 x 250mm, 5µm) with mobile phase consisted of phosphate buffer (pH 3.5) and acetonitrile in the ratio of 40:60% v/v at 235 nm using a UV detector. The retention time of Ambroxol hydrochloride and Nitazoxanide was found to be 2.985 and 5.58 min, respectively. The optimized chromatograms are given in Fig 3.




| | Peak Name | RT | Area | Height | % Area | USP Tailing | USP Resolution | USP Plate Count |
|---|---|---|---|---|---|---|---|---|
| 1 | Ambroxol | 2.985 | 300332 | 46152 | 10.345 | 1.15 | | 4822.45 |
| 2 | Nitazoxanide | 5.581 | 2600845 | 320412 | 89.655 | 1.02 | 13.45 | 10641.06 |

**FIGURE 3.** Chromatogram for Ambroxol hydrochloride and Nitazoxanide standard

### Analytical Method Validation

The proposed method was validated according to the ICH guidelines (17) for specificity, recovery, precision, linearity, system suitability, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

### Linearity, LOD and LOQ

Linearity of an analytical method was evaluated over the concentration range of standard solutions ranging between 7.5-45 µg/mL (Ambroxol hydrochloride) & 25-150 µg/mL (Nitazoxanide) were prepared for both medications and their peak areas were recorded. The linearity of the calibration curve was checked by constructing a plot of area versus concentration. The LOD and LOQ were measured from the calibration curve method. The Detection limit and quantification limit value of Ambroxol hydrochloride and Nitazoxanide was found to be 0.20, 0.63 and 0.54, 1.65. The statistical linearity data are presented in Table 1 and linearity data represented in fig. 4 and fig. 5.

**TABLE 1.** Statistical Data of Calibration Curve

| Parameter | Ambroxol hydrochloride | Nitazoxanide |
|---|---|---|
| Linearity range | 7.5-45 µg/mL | 25- 150 µg/mL |
| Regression equation | y = 10016x - 1800 | y = 25812x + 21576 |
| Limit of detection | 0.20 µg/mL | 0.54 µg/mL |
| Limit of quantification | 0.63 µg/mL | 1.65 µg/mL |

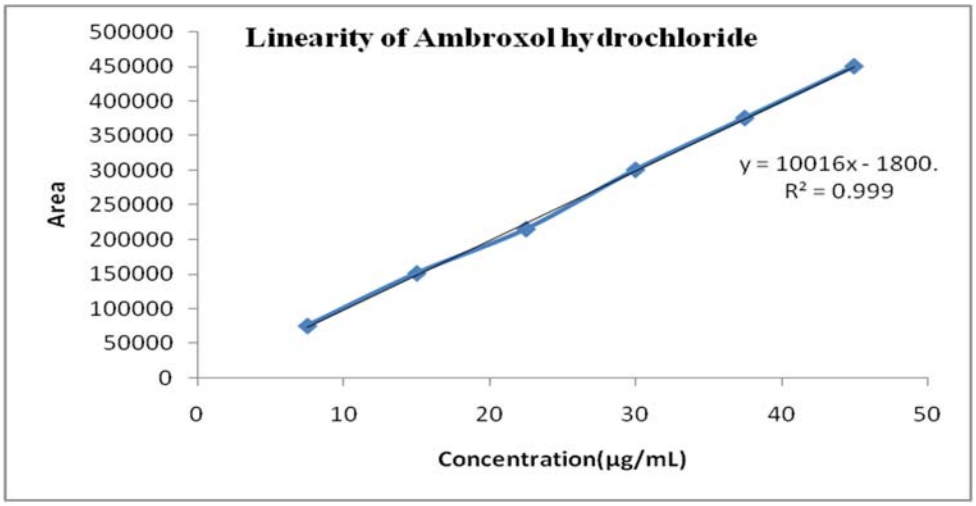



**FIGURE 4.** Linearity graph of Ambroxol hydrochloride

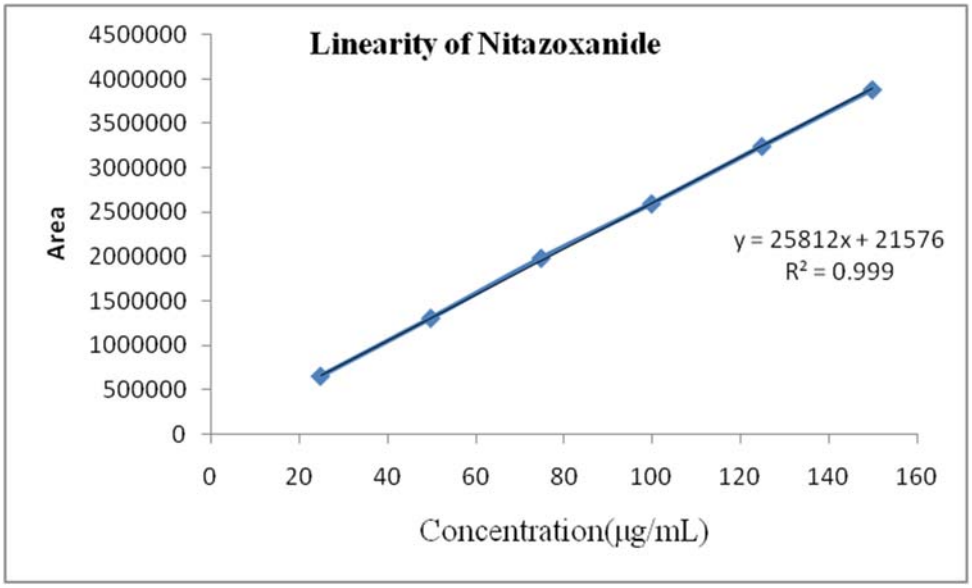



**FIGURE 4.** Linearity graph of Nitazoxanide

## Precision

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Ambroxol hydrochloride (30 µg/mL) and Nitazoxanide (100 µg/mL) have been analyzed by injecting them into a HPLC

column on the same day and on consecutive days. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in Table 2.

**TABLE 2.** Results of Intra-Day and Inter-Day Precision

| Sample | Concentration (µg/mL) | Mean area | | % RSD | |
|---|---|---|---|---|---|
| | | Intra-day | Inter-day | Intra-day | Inter-day |
| Ambroxol hydrochloride | 30 | 300740 | 2601847 | 0.212 | 0.16 |
| Nitazoxanide | 100 | 301032 | 2601496 | 0.268 | 0.12 |

## Recovery

The percentage recovery was calculated by preparing standard drug concentrations of Ambroxol hydrochloride and Nitazoxanide with concentration levels of 80%, 100% and 120%. A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration and the mean percentage recovery of Ambroxol hydrochloride and Nitazoxanide was achieved between 99.93-100.00 and 99.58-99.96%. The results are given in Table 3.

**TABLE 3.** Recovery Study of Ambroxol hydrochloride and Nitazoxanide

| Compound | Quantity (mg/ml) | | Mean % recovery |
|---|---|---|---|
| | Amount added | Amount found | |
| Ambroxol hydrochloride | 24 | 24.01 | 100.00 |
| | 30 | 29.98 | 99.93 |
| | 36 | 35.99 | 99.97 |
| Nitazoxanide | 80 | 79.677 | 99.59 |
| | 100 | 99.960 | 99.96 |
| | 120 | 119.50 | 99.58 |

## Robustness

Robustness of the proposed analytical method was a measure of its capacity to remain unaffected and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flow rate (1.0 ± 0.1 mL) and (30 ± $5^0$C) temperature. The parameters chosen for the study of robustness was the flow rate and temperature. From the results obtained, there were no significant changes observed at the end of the study.

## Analysis of Tablet Dosage Form

The developed method was applied for the estimation of drugs in the commercial tablet dosage form (Ambroxol hydrochloride 30 mg/ Nitazoxanide 100 mg tablets). The chromatograms show good separation of the samples with acceptable limits such as tailing factor and USP plate counts. The % assay was calculated and found to be satisfactory and the results are given in Table 4.

**TABLE 4.** Assay data for Ambroxol Hydrochloride & Nitazoxanide

| Entry | Name of the compound | Label claim | Amount taken | % Purity |
|---|---|---|---|---|
| 1 | Ambroxol Hydrochloride | 30 mg | 465 mg | 99.35% |
| 2 | Nitazoxanide | 100 mg | 465 mg | 99.93% |

## CONCLUSION

A rapid, specific, and reliable isocratic reversed-phase high-performance liquid chromatographic method with UV detection has been developed and validated for the determination of Ambroxol hydrochloride and Nitazoxanide in pharmaceutical formulations. The method involves use of a simple mobile phase and minimum sample preparation, encouraging its application for quality control of Ambroxol hydrochloride and Nitazoxanide in tablets.

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