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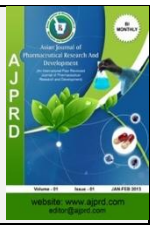

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Research Article

# Development and Validation Method for Simultaneous Investigation of Ambroxol Hydrochloride and N-Acetyl Cysteine In Bulk And Pharmaceutical Formulation By RP-HPLC.

**Lokesh Thote*, Disha Dhabarde, Jagdish Baheti, Shilpa Borkar, Swati Bhusari**

Kamla Nehru College of Pharmacy, Butibori, Nagpur 441108. (MS).

## A B S T R A C T

A simple, precise and rapid reverse phase HPLC- SPD 20-Amethod has been developed and validated for the simultaneous investigation of Ambroxol Hydrochloride (AMB) and N-Acetyl Cysteine (NAC) in tablet dosage form. The mixture of (AMB) and (NAC) was separated on $C_{18}$ 5µm.column (4.5 µm × 25 cm). All separation were monitored with a SPD-20A UV-Vis detector on 210 – 400 nm wavelength at ambient temperature and flow rate of 1 ml/min. The mobile phase was phosphate buffer: acetonitrile (75:25) and pH 2.5 adjusted with dilute ortho-phosphoric acid. The retention time of AMB and NAC were found to be 8.4024 and 3.35088 respectivelythe resolution was 9.86346. The method was mean recoveries in the range of 100.4 to 100.15 for all components. The developed method can be use for the routine analysis of AMB and NAC in tablet dosage form.

**Keywords:** Ambroxol Hydrochloride, N-Acetyl Cysteine, RP-HPLC, Validation, Simultaneous estimation.

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*Address for Correspondence:
Thote Lokesh, Kamla Nehru College of Pharmacy, Butibori, Nagpur 441108. (MS).

## INTRODUCTION:

Ambroxol hydrochloride, 4- [(2-Amino-3, 5-dibromophenyl)methylamino]cyclohexan-1-ol is an active N desmethyl metabolite of mucolite of bromohexine it may enhance the quantity and diminish the viscosity of tracheabronchial secretions. It may also act as an expectorant, rising mucolinary transport via stimulation of ciliary motility. Furthermore Ambroxol may kindle the synthesis and secretion of pulmonary surfactant the drug has been referred to as a surfactant activator[1].

N acetyl cysteine, 2-acetamido-3-sulfanyl propanoic acid is the drug of choice for the treatment of an acetomenophen overdose. It is thought to provide Cysteine for glutathione synthesis and possibly to form adduct directly with the toxic metabolite of acetaminophen[2].

There are many methods werereported for the determination of ambroxol hydrochloride alone and in combination with other drug by spectroscopic method, chromatographic method and stability indicating HPLC method. N acetyl cysteine was also investigated alone and in combination with other drug by spectroscopic method, chromatographic method and stability indicating HPLC method. To the best of my knowledge no method has been developed for the simultaneous estimation of ambroxol hydrochloride and N acetyl cysteine by simultaneous equation method. The present research work describes the rapid, accurate, sensitive and reproducible RP-HPLC method for simultaneous estimation of ambroxol hydrochloride and N acetyl Cysteine in combine dosage form.

## EXPERIMENTAL

### Instrumentation

All thechromatographic separation were carried out on Shimadzu HPLC system (Model – S0001/11118) using $C_{18}$

5µm.column (4.5 µm × 25 cm)at a flow rate of 1 ml/min, sample size was 20 µl with 214 nm detection wavelength. The system was equipped with SPD 20-A UV-Visible detector. The data was processed using LC Solution software.

### Chemical and reagents

The reference standard Ambroxol Hydrochloride (fig. 1) and NAcetyl Cysteine (fig. 2) were kindly gifted by ZIM Laboratory Pvt. Ltd. Nagpur. Marketed tablet formulation Ambronac containing 30 mg AMB and 200 mg NACwas purchased from local market. Methanol (AR grade), acetonitrile (HPLCgrade), orthophosphori acid (HPLC grade), potassium dihydrogen phthalate (HPLC grade) were purchased from Qualigens chemical ltd.

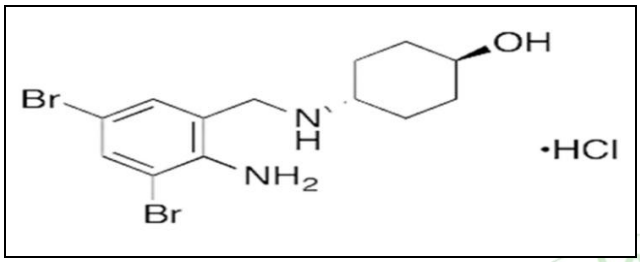


**Figure: 1** Chemical structure of Ambroxol Hydrochloride

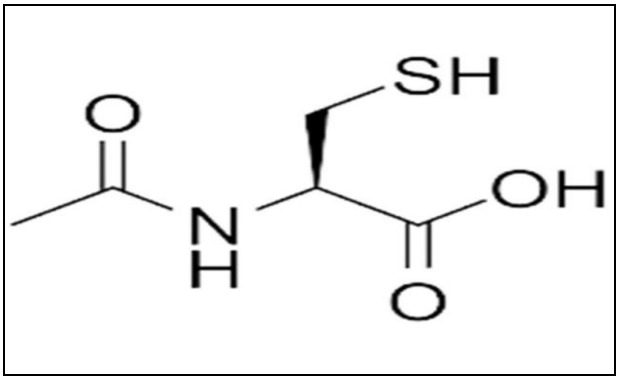


**Figure: 2** Chemical structure of N Acetyl Cystene

### Preparation of standard stock solution

The standard stock solution of AMB and NAC were prepared separately by accurately weighing 10 mg each drug into 100 ml volumetric flask. The drugs were dissolved in mobile phase {phosphate buffer: acetonitrile (75:25)} and volume were made up to mark with same mobile phase to get the final concentration of 100 µg/ml. calibration curve solution of in the range of 5 -25 µg/ml of AMB and 12-48 µg/ml of NAC were prepared from individual stock solutions. The chromatogram of standard solution of AMB (fig. 3) and NAC (fig. 4) were recorded.

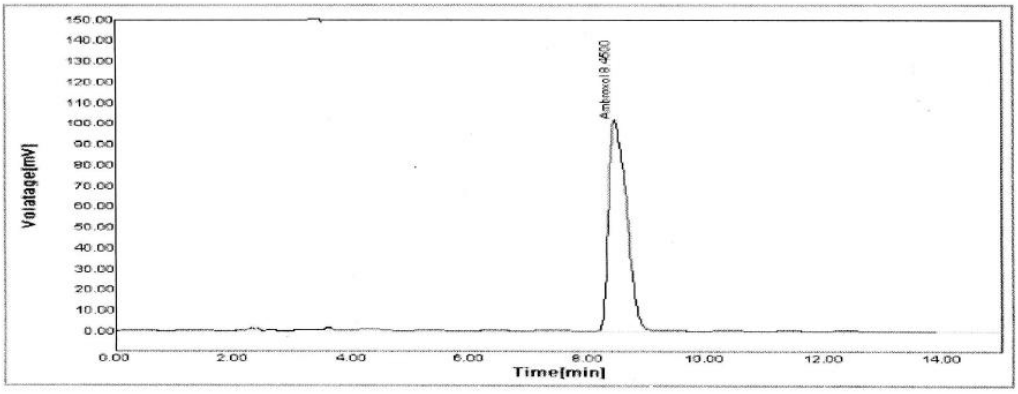


**Figure: 3.** Chromatogram of standard solution of Ambroxol hydrochloride

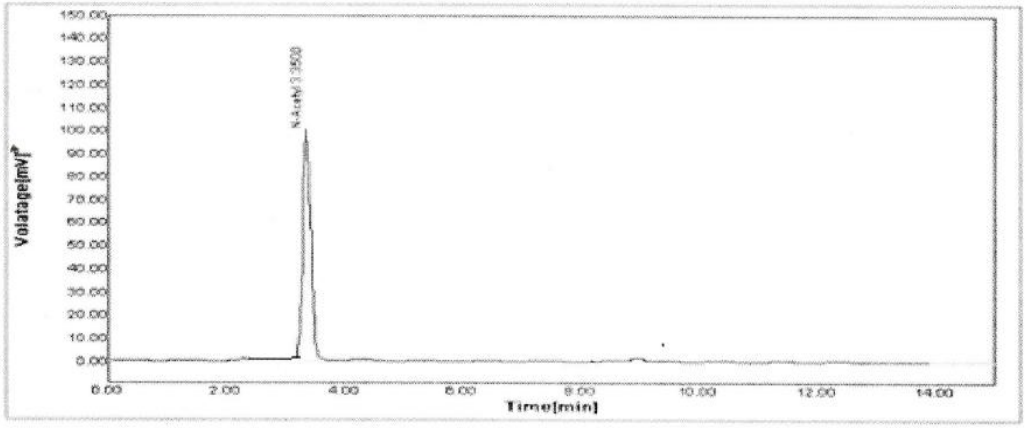


**Figure: 4.** Chromatogram of standard solution of N acetyl cysteine

### Preparation of sample solution

Twenty tablets were weighed, average weight was calculated and tablets were ground to fine powder. Tablet powder equivalent to 0.513 mg of NAC was transferred in 100 ml of volumetric flask 20 mg of pure AMB were further to it and volume was making up to the mark with mobile phase. The solution was filtered through 0.45 µ nylon membrane filter. Further dilution was done with mobile phase to get concentrations of 20 µg/ml of AMB and 120 µg/ml of NAC.

### Method Development

After several trails with various solvents, mobile phase system composed of phosphate buffer: acetonitrile (75:25) and pH 2.5 adjusted with dilute ortho-phosphoric acid was chosen for the simultaneous estimation of AMB and NAC in combined dosage form by RP-HPLC. This mobile phase composition offered maximum resolution for the drug at the detection wavelength of 214 nm. Mobile phase with the flow rate of 1 ml/min gave optimum separation with good resolution between the peaks. A reverse phase C18 column was used as stationary phase. The retention time of AMB and NAC were found to be 8.4500 and 3.3500 minutes, respectively. The percentage label claim (% Recovery) for AMB and NAC were found to be 100.2 % and 100.03 % respectively (fig. 5).

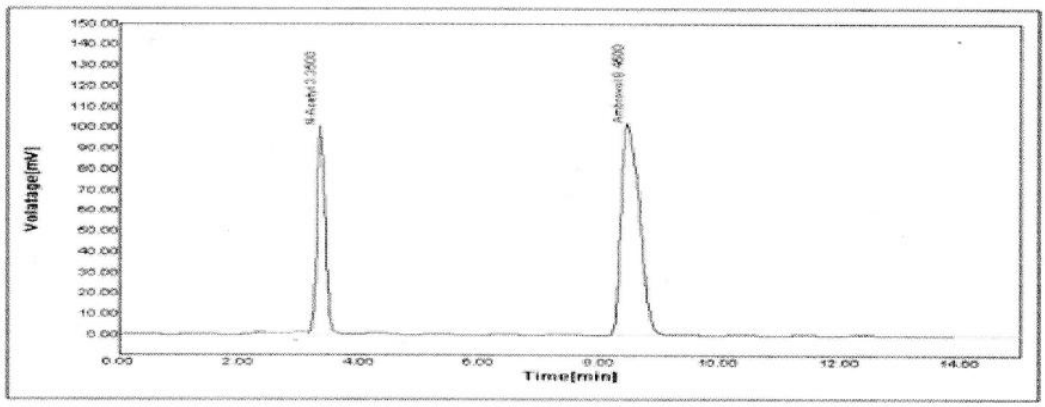


**Figure: 5** Chromatogram of sample Ambroxol hydrochloride and N acetyl cysteine

### Method Validation

From the calibration curve linear relationship was observed in the concentration range of 5 to 25 µg/ml for AMB with 0.999 as the value of correlation coefficient and for NAC the linearity in the range of 30 to 150 µg/ml with 0.999 as the value of correlation coefficient. System suitability studies were carried out in which the resolution between the peaks was found to be 9.86346. AMB was found to have a value of 3160.62 as its number of theoretical plates and for NAC it was 2481.58. The accuracy of the method was studied by performing the recovery study. The mean % recovery was found to 100.2 % and 100.03 % for AMB and NAC respectively.

## RESULTS AND DISCUSSION

### System suitability

System suitability was studied under each validation parameters by injecting five replicates of the standard solution. The results obtained were within acceptable limits (Tailing factor < 2 and Theoretical plate's were found to be 3160.62 and 2481.58 for AMB and NAC respectively. (Table 1).

**Table1:** System Suitability Parameters for Ambroxol and N acetyl Cysteine

| Parameter | AMB | NAC |
|---|---|---|
| % RSD of retention time | 0.5974 | 0.557176 |
| % RSD of peak area | 1.079 | 1.2107 |
| Number of theoretical plate | 3160.62 | 2481.58 |
| Tailing factor | 1.11618 | 1.036 |

### Specificity

Specificity was measured as ability of the proposed method to obtain well separated peak for AMB and NAC without any interference from component of matrix. Mean retention time for AMB and NAC is 8.4500 min and 3.3500 min. value obtained were very close to that in laboratory mixture indicate no interference from the component of matrix.

### Precision

Replicate estimation of tablet analyzed by the proposed method was yield consistent result indicating repeatability of method study shows relative deviation < 2. (Table 2)

**Table 2:** Precision study of Ambroxol and N acetyl Cysteine

| S. No. | RT | | AUC | |
|---|---|---|---|---|
| | AMB | NAC | AMB | NAC |
| 1. | 8.3167 | 3.3500 | 2096.31 | 1023.39 |
| 2. | 8.412 | 3.321 | 2156.1 | 1046.36 |
| 3. | 8.4500 | 3.3500 | 2140.65 | 1013.58 |
| 4. | 8.4167 | 3.3667 | 2115.6 | 1028.47 |
| 5. | 8.4167 | 3.3667 | 2129.56 | 1036.25 |
| Mean | 8.4024 | 3.3508 | 2127.64 | 1029.61 |
| SD | 0.0502 | 0.01867 | 229677 | 124659 |
| % RSD | 0.5974 | 0.557176 | 1.079 | 1.2107 |

## Linearity and Range

A linear relationship was evaluated by plotting a graph of concentration Vs area under curve in the range 5 to 25µg/ml for AMB (fig. 6) and 30 to 150 µg/ml for NAC (fig. 7). Linear relationships were observed among the variables. The standard deviation of the slope and intercepts were low. (Table 3, Table 4).

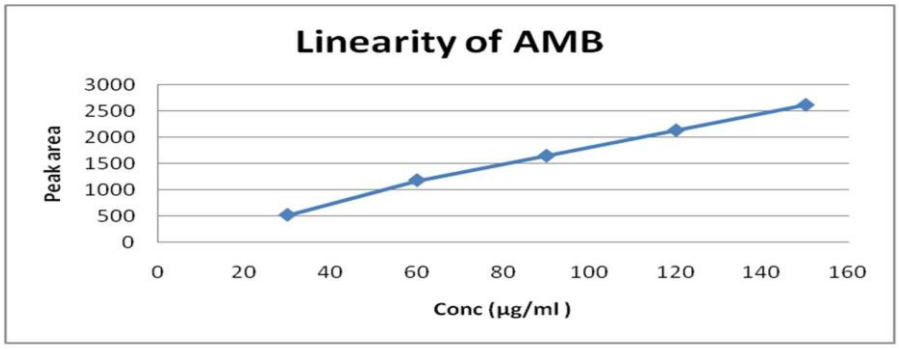


**Figure 6:** Linearity range of Ambroxol

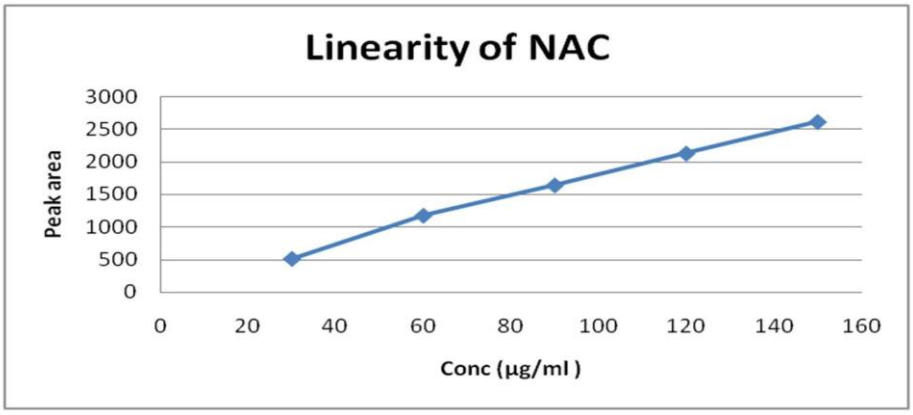


**Figure 7:** Linearity range of N acetyl cysteine

**Table 3:** Linearity data for Ambroxol

| Concentration (µg/ml) | Peak area |
|---|---|
| 5 | 2096.31 |
| 10 | 2156.1 |
| 15 | 2140.65 |
| 20 | 2115.6 |
| 25 | 2129.56 |
| Linearity dynamic range | 5-25 µg/ml |
| Correlation coefficient (r) | 0.999 |
| Slope(m) | 50.9223 |
| Intercept(c) | 13.8965 |

**Table 4:** Linearity data for N acetyl Cysteine.

| Concentration (µg/ml) | Peak area |
|---|---|
| 20 | 516.50 |
| 60 | 1179.82 |
| 90 | 1645.455 |
| 120 | 2133.52 |
| 150 | 2618.67 |
| Linearity dynamic range | 30-150 µg/ml |
| Correlation coefficient (r) | 0.998 |
| Slope(m) | 17.193 |
| Intercept(c) | 71.381 |

## Accuracy

Accuracy was ascertained on the basis of recovery studies performed by standard addition method (Average of three determinations). Satisfactory recoveries were obtained by the proposed method. This indicates that the proposed method was accurate (Table 5).

**Table 5:** Accuracy data for Ambroxol and N acetyl Cysteine

| Sr. No. | Wt. of tablet powder (g) | Amount of pure drug added (µg/ml) | | Peak area of standard | | Peak area of sample | | % recovery | |
|---|---|---|---|---|---|---|---|---|---|
| | | AMB | NAC | AMB | NAC | AMB | NAC | AMB | NAC |
| | 0.513 | 20 | 120 | 2135.46 | 1031.47 | 2136.40 | 1037.47 | 100.06 | 99.90 |
| | 0.512 | 20 | 120 | | | 2135.23 | 1035.25 | 100.0 | 100.2 |
| | 0.512 | 20 | 120 | | | 2145.32 | 1031.18 | 100.4 | 99.80 |
| | | | | | | | Mean | 100.2 | 100.03 |
| | | | | | | | SD | 0.5116 | 0.3093 |
| | | | | | | | RSD | 0.00512 | 0.00309 |
| | | | | | | | % RSD | 0.5124 | 0.3092 |

## Ruggedness

Ruggedness studies were carried out for different parameters like different time, different day and different analyst. Results of estimation by proposed method are very much similar under variety of conditions. This study signifies the ruggedness of the method under varying conditions of its performance (Table 6).

**Table 6:** Summary of results of ruggedness studies by RP-HPLC method

| Parameter | Statistical data | Simultaneous method | |
|---|---|---|---|
| | | AMB | NAC |
| Inter-day | Mean | 99.91 | 99.71 |
| | SD | 0.03690 | 1.3672 |
| | RSD | 0.00639 | 0.01371 |
| | % RSD | 0.6320 | 1.3711 |
| Intra day | Mean | 99.82 | 100.46 |
| | SD | 0.2294 | 0.9928 |
| | RSD | 0.002298 | 0.009928 |
| | % RSD | 0.2298 | 0.9928 |
| Different analyst | Mean | 99.44 | 99.43 |
| | SD | 0.5284 | 0.7581 |
| | RSD | 0.005314 | 0.007561 |
| | % RSD | 0.53146 | 0.75612 |

## CONCLUSION

The developed method for assay of Ambroxol hydrochloride and N acetyl cysteine in tablet dosage form is very simple, economic, accurate, precise andreproducible and can be adopted to routine quality control analysis of these two drugs in pharmaceutical combine dosage form. No interference of additives, matrix etc. is encountered in this method.

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## CONFLICTS OF INTREST

No any conflicts of interest

## REFERENCES

1. Ahmad K, Ludger K, Dorotheea C AS. 4. Ahmad K, Ludger K, Dorotheea C, Annette S. An overview of efficacy and safety of ambroxol for the treatment of acute and chronic respiratory diseases with a special regard to children. Journal of multidisciplinary Respiratory Medicine, 2020; 15 (1): 51.
2. Bernhard H. Lauterburg C GB. 6. Bernhard H. Lauterburg C, George B. Corcoran M. Mechanism of Action of N-Acetylcysteine in the protection against the hepatotoxicity of Acetaminophen in rats In vivo. Journal of Clinical Investigation, (1983); 71(4): 980–991.
3. Skoog DA, Holler FJ, Crouch SR. 2009. Instrumental Analysis. New Delhi, India: Cengage Learning India Private Limited.
4. Kasture AV, Wadodkar SG, Mahadik KR, More HM. 2006. A textbook of Pharmaceutical Analysis.14th ed, Vol II. 1-2.
5. Beckett AH, Stenlake JB. 2002. Practical Pharmaceutical Chemistry. New Delhi India: CBS Publishers and Distributors. 4th ed, Vol II. 278-281.
6. Katsuyuki T, Nobuaki M SM. 5. Katsuyuki T, Nobuaki M, Shigeki M. Immunomodulatory effects of Ambroxol on airway hyper responsiveness and inflammation. Journal of Immune Network, 2016: 16 (3): 165 -175.
7. Alison L. 7. Alison L. Mechanism of action and value of N-Acetylcysteine in the treatment of early and late acetaminophen poisoning: A Critical Review. Journal of Toxicology. 1998; 36 (4): 277-285.
8. Mankaran S, Dinesh K, Gurmeet S. Simultaneous Estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in Pharmaceutical Tablet Dosage Form by Simultaneous Equation Spectrophotometric Method: A Quality Control Tool for Dissolution Studies. International Scholarly Research Notices. 2014:6.
9. Selvadurai M, Jaya K, Sokkalingam A. Development and validation of an high-performance liquid chromatographic, and a ultraviolet spectrophotometric method for determination of Ambroxol hydrochloride in pharmaceutical preparations. Journal of Pharmaceutical Technology and Research. 2013; 4(1): 65 -68.
10. Elkady Y, El-Adl SM, Baraka M, Sebaiy M. HPLC Method for Simultaneous Determination of Ambroxol, Salbutamol and Fexofenadine in Their Bulk and Dosage Forms. Journal of Pharmaceutical Sciences and Biomedical Analysis. 2020; 3 (1): 121.
11. Sreenivasa C, Sridhar C, Chandrasekhar K. Quantitative determination of N-Acetyl cysteine by RP-HPLC method in bulk and parenteral injection. Asian Journal of Pharmacy and Pharmacology. 2018; 4(5): 702-705.
12. Lea K, Njegomir R. Spectrophotometric Determination of N-Acetyl-L-Cysteine and N-(2-Mercaptopropionyl)-Glycine in Pharmaceutical Preparations. International Journal of Analytical Chemistry. 2011: 140 -146.