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# Simultaneous validation of amoxicillin trihydrate and carbocisteine in pharmaceutical dosage by reverse phase high performance liquid chromatography

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## Abstract

*For validation of amoxicillin trihydrate and carbocisteine from the formulation i.e. capsules, a RP-HPLC method is developed. The amoxicillin trihydrate and carbocisteine were resolved on a BDS hypersil C18 (150 x 4.6 mm i.d.) 5 µ particle size. The water and methanol in the ratio of 80: 20 % (v/v) constituted as mobile phase with detection wavelength as 220 nm. The column BDS hypersil C18 was found to be most suitable for chromatographic separation. The parameters for robustness were validated. The linear ranges amoxicillin trihydrate as 125 - 375 and carbocisteine at 75 - 225 µg / ml respectively. The proposed method is much suitable to analyze capsules of marketed formulations of amoxicillin trihydrate and carbocisteine.*

**Keywords:** Amoxicillin trihydrate, carbocisteine, methanol, RP - HPLC.

## Introduction

A 6 - (D - 4 hydroxy phenyl glycyl amino) penicillinic acid trihydrate, Amoxicillin trihydrate is semi- synthetic penicillin. It is β – lactam antibiotics and it also shows antibacterial property. A (2R)-2-amino-3-[(carboxy-methyl) sulphanyl] propanoic acid, carbocisteine is a mucolytic drug. It has ability to breaks mucus inside the bodyand cleared from the body. In literature for validation of combined dosage form. HPLC[1-6] Spectro-photometric[7-12] methods were known. The previous methods in literature suggest use of buffer of some definite pH with organic solvent like acetonitrile in mobile phase. In the proposed method mobile phase was water and methanol in (80: 20% v/v) was used. The retention times were 1.74 and 3.46 minutes. Hence method required less time to complete one sample validation. The use of water (80%) in mobile phase made the method most economical. The Value of retention times as 1.74 and 3.46 min. hence the method time saving as compared literature methods. From the above discussion the both drugs were validated accurately in less time in combined formulations.

## Materials and methods

**Reagents and chemicals:** A amoxicillin trihydrate and carbocisteine, Reference standards with certificate of analysis were obtained from reputed firm. A methanol, HPLC grade from MERCK, was used for chromatographic separation. HPLC grade water, Millipore was used. For preparation of solutions, mobile phase was used.

**Chromatography apparatus and conditions:** i. Hitachi HPLC system (MERCK) with (D 7200) as sampler and (D- 7400) as ultra-violet detector. The quantification of chromatograms was done by EZChrom Elite software. ii. Analytical balance (0.01 mg) made up of SHIMADZU was used.

At ambient temperature Chromatographic separation was carried using BDS hypersil C18 column. Most suitable mobile phase was water and methanol (80: 20% v/v). Mobile phase was sonicated to degas. The flow rate of 1.0 ml /min was found to be suitable for resolution at 220 nm and the injection volume as 10 µl.

**Standard Solution:** Standard Solution of 2500 µg / ml amoxicillin trihydrate and 1500 µg / ml carbocisteine was prepared A standard solution was further diluted to give 250 µg / ml amoxicillin trihydrate and 150 µg / ml carbocisteine.

**Sample solution:** To determine average weight of content in each capsules, contents of 20 capsules were accurately weighed. From the powdered blend, 2500 µg / ml of amoxicillin trihydrate and 1500 µg / ml carbocisteine solution was prepared. Such 1 ml of such solution was further diluted to 10 ml to give working solution.

**Method Development:** The BDS hypersil C18 column offered better resolution of peaks over the other columns like ODS and others. A amoxicillin trihydrate and carbocisteine solutions were analysed separately and elution pattern as well as resolution parameters were recorded at 220 nm which was selected for analysis. The combined spectra of amoxicillin trihydrate and carbocisteine are shown in Figure-1.

Active pharmaceutical ingredient and sample chromatograms are given in Figure-2, 3.

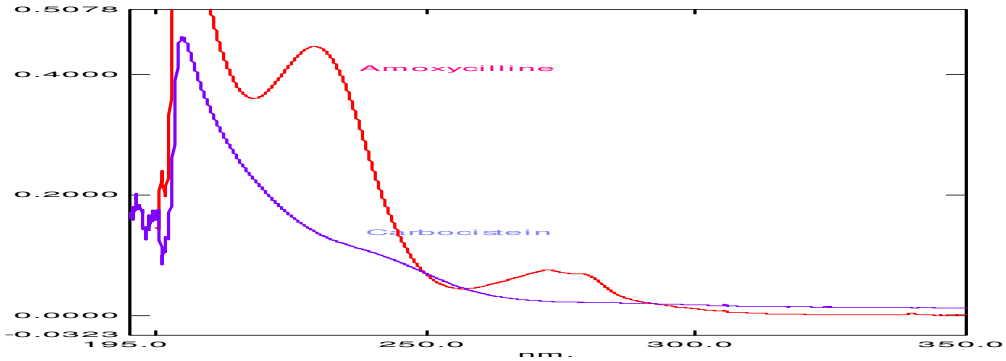


**Figure-1:** Overlain spectra of amoxicillin trihydrate and carbocisteine.

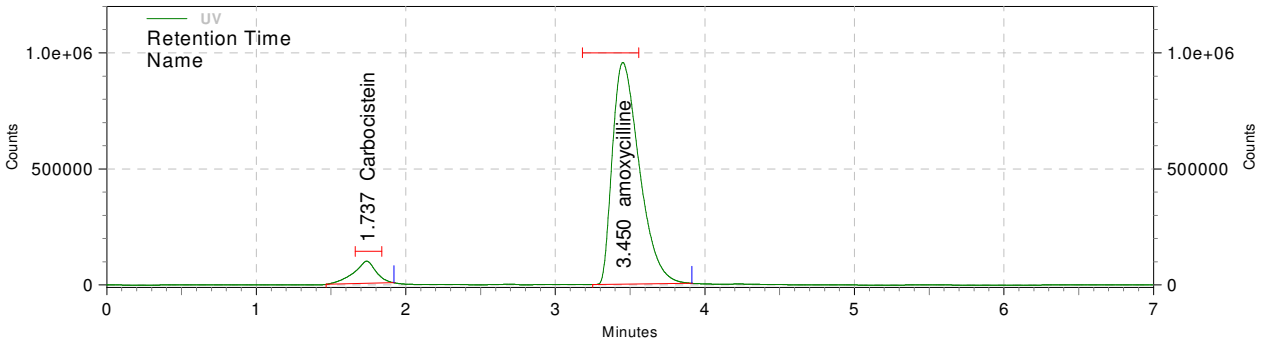


**Figure-2:** Amoxicillin trihydrate and carbocisteine chromatograms for standard solution with retention times.

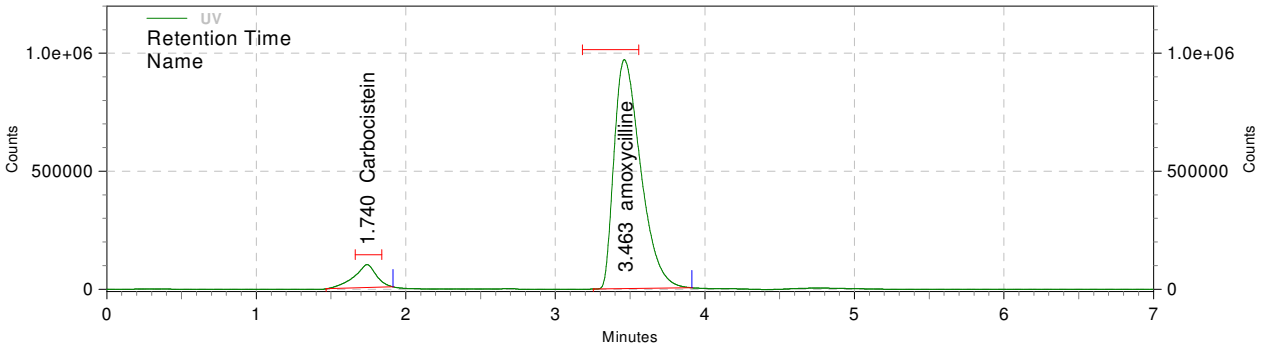


**Figure-3:** Amoxicillin trihydrate and carbocisteine sample chromatogram with retention time.

The parameters such as retention time, area, % area, no of theoretical plates and resolution were exhibited in Table-1.

**Table-1:** The parameters for amoxicillin trihydrate and carbocisteine in replicate analysis with n = 6.

| Parameters | Carbocisteine | Amoxicillin Trihydrate |
|---|---|---|
| Retention time | 1.740 | 3.463 |
| Area | 1005664 | 11983356 |
| % Area | 7.742 | 92.258 |
| No. of theoretical plates | 2676 | 3779 |
| Resolution | 0.0000 | 5.78228 |

**Method validation: System suitability:** The six replicate analysis individually was carried for system and method precision. From these retention times, area, % area, no of theoretical plates and resolution were determined. The results indicated good performance of the system.

**Linearity:** The standard solution in the range of 0.5, 0.8, 1.0, 1.2, 1.5 times of standards solution were injected in replicates. Hence under such suitable conditions and chromatographs are recorded. From such curves areas under curves were calculated. A graphs were plotted of area Vs concentration, they are represented in Figure-4 and 5. The values of slope, intercept and correlation coefficient were tabulated in Table-2. The linearity range was observed for amoxicillin trihydrate and carbocisteine at 125 - 375 µg / ml and 75 - 225 µg / ml respectively.

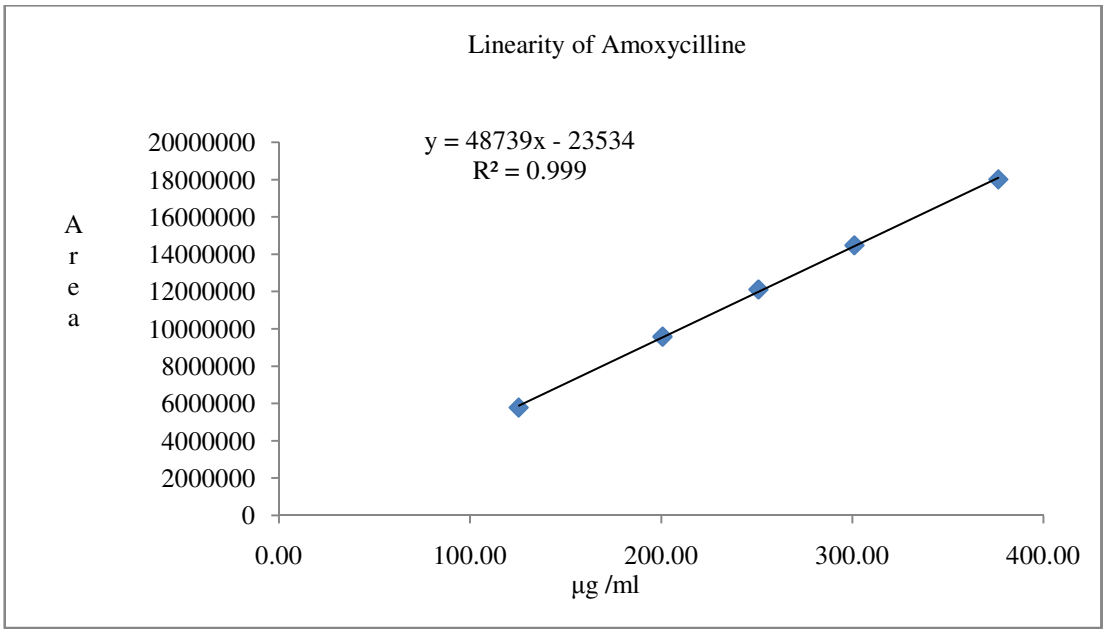


**Figure-4:** Linearity graph of Amoxicillin.

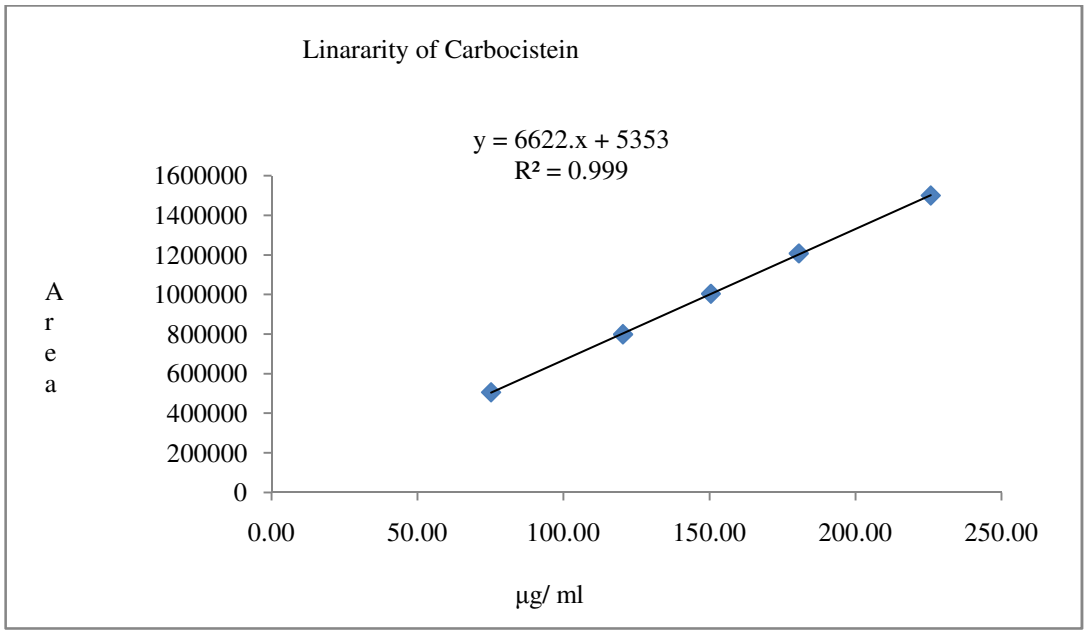


**Figure-5:** Linearity graph of carbocisteine.

**Table-2:** The analysis of regression data.

| Terms in graphs | Amoxicillin tri-hydrate | carbocisteine |
|---|---|---|
| The value of r i.e. coefficient of co-relation | 0.9999 | 0.9999 |
| Value of Intercept | - 23534 | 5353 |
| Value of Slope | 48739 | 6622 |

**Accuracy:** The standard addition method was used for accuracy of drugs to study of the proposed method in the 80%, 100 % and 120 % in replicate analysis. The percentage of analyte recovered was studied. The values recovery studies are formulated in Table-3, 4.

**Precision:** For the precision study six replicates were carried out. The values 0.256 % for amoxicillin trihydrate and 0.763 % carbocisteine represented as relative standard deviation. It gave idea of the sample repeatability of the method. The data is summarized in Table-5.

**Table-3:** Statistical data of accuracy study in amoxicillin trihydrate.

| % | Replicate | Amox Mg | Area | Amox Added µg /ml | Amox recovered in µg /ml | % Recovery | Mean |
|---|---|---|---|---|---|---|---|
| 80% | 1 | 25.12 | 9620783 | 200.64 | 201.13 | 100.24 | 100.02 |
| | 2 | 25.09 | 9622770 | 200.64 | 201.17 | 100.26 | |
| | 3 | 25.06 | 9554395 | 200.64 | 199.74 | 99.55 | |
| 100% | 1 | 25.14 | 12060912 | 250.8 | 252.14 | 100.53 | 100.53 |
| | 2 | 25.05 | 12069113 | 250.8 | 252.31 | 100.60 | |
| | 3 | 25.08 | 12052677 | 250.8 | 251.97 | 100.46 | |
| 120% | 1 | 25.12 | 14535049 | 300.96 | 303.86 | 100.96 | 100.89 |
| | 2 | 25.05 | 14540671 | 300.96 | 303.98 | 101.00 | |
| | 3 | 25.15 | 14498716 | 300.96 | 303.10 | 100.71 | |
| Amox: Amoxicillin | | | | | % recovery mean | | 100.48 |

**Table-4:** Statistical data of accuracy study in carbocisteine.

| % level | Replicate No. | Carbo Mg | Area | Carbo added in µg /ml | Carbo recovered in µg /ml | % Recovery | Mean |
|---|---|---|---|---|---|---|---|
| 80% | 1 | 15.03 | 808248 | 120.4 | 119.84 | 99.54 | 99.33 |
| | 2 | 15.07 | 812148 | 120.4 | 120.42 | 100.02 | |
| | 3 | 15.05 | 799248 | 120.4 | 118.51 | 98.43 | |
| 100% | 1 | 15.12 | 990895 | 150.5 | 146.92 | 97.62 | 99.27 |
| | 2 | 15.09 | 1019700 | 150.5 | 151.19 | 100.46 | |
| | 3 | 15.03 | 1012182 | 150.5 | 150.08 | 99.72 | |
| 120% | 1 | 15.10 | 1197275 | 180.6 | 177.52 | 98.30 | 98.89 |
| | 2 | 15.04 | 1212381 | 180.6 | 179.76 | 99.54 | |
| | 3 | 15.11 | 1203895 | 180.6 | 178.50 | 98.84 | |
| Carbo: carbocisteine | | | | | % recovery mean | | 99.16 |

**Table-5:** Statistical data of method precision.

| Replicate no | Amoxicillin | | | Carbocisteine | | |
|---|---|---|---|---|---|---|
| | Weight of µg /ml | Found Area | % Assay | Weight of µg /ml | Found Area | % Assay |
| 1 | 25.11 | 11975991 | 99.83 | 15.08 | 997406 | 98.07 |
| 2 | 25.07 | 11994740 | 100.14 | 15.09 | 1010004 | 99.24 |
| 3 | 25.06 | 12044359 | 100.60 | 15.17 | 1012584 | 98.97 |
| 4 | 25.12 | 12032334 | 100.26 | 15.15 | 1000626 | 97.93 |
| 5 | 25.15 | 12022561 | 100.05 | 15.09 | 1003958 | 98.65 |
| 6 | 25.08 | 12015579 | 100.28 | 15.07 | 1015795 | 99.94 |
| | Mean Assay | | 100.19 | | Mean Assay | 98.80 |
| | SD | | 0.257 | | SD | 0.754 |
| | RSD | | 0.256 | | RSD | 0.763 |

For the robustness study the variation were done as per table:

| Parameter | Variation |
|---|---|
| Flow rate | ± 0.2 ml /min |
| Wavelength | ± 5 nm |
| Mobile phase compositions | ± 2 units |
| stability | 0 hrs and 24 hrs |

The chromatograms under such variation were obtained and data was analyzed for robustness study.

**Method application:** The method was applied to simultaneous validation of both drugs in combined dosage form i.e. amoxicillin trihydrate (250 mg) and carbocisteine(150 mg). The sample solution was prepared was prepared from formulation and 10 µl was used for the study in specified conditions. The chromatograms were studied. From the chromatograms, areas amount of drug present is calculated. The data were given in Table-4.

## Results and discussion

The development and validation of drug by RP-HPLC has great importance in quality control of drugs. In new method, the retention time of amoxicillin trihydrate and carbocisteine were 1.74 and 3.46 min. The calibration range were for amoxicillin trihydrate as 125 - 375 µg / ml and carbocisteine as 75 - 225 µg / ml respectively.

The coefficient of co-relation was 0.9999 (Table-2) it indicate no interference peaks to diluent at the retention time. It suggests method specificity. The method was suitable in linear range applied. It shows that an good agreement of peak area and concentration. The value of relative standard deviation in method precision found to be less than one and mean recovery was in the range between 98.80%. to 100.18 % hence method has high accuracy. The robustness study showed that there was no effect other parameters like diluents on the drug study.

**Discussion:** The average recovery as well as % RSD of both drugs were within the acceptable limit. The method and system precision study indicate suitability of method. The robustness study of change in flow rate, mobile composition and wavelength was found to be within the acceptable limit. For all six standard replicates the relative standard deviations were less than 2% and within the acceptable limit. It represents the robustness of proposed.

The previous methods in literature suggest use of buffer of some definite pH with organic solvent like acetonitrile in mobile phase. In the proposed method mobile phase was water and methanol in (80: 20% v/v) was used. The retention time were 1.74 and 3.46 minutes. Hence method required less time to complete one sample validation. The use of water (80%) in mobile phase made the method most economical.

## Conclusion

The proposed HPLC method developed and validated for both drugs in bulk and formulation as per ICH guidelines. It was found to be accurate and precise. The method showed repeatability of results with respect to robustness and system suitability conditions. As per use of water and methanol in (80: 20 % v/v) in mobile phase and diluents, method is most economical. The Value of retention times as 1.74 and 3.46 min.

the method time saving as compared literature methods. From the above discussion the both drugs were validated accurately in less time and with more economy by using the proposed RP-HPLC method in formulation and in bulk. Hence it is strongly recommendation for the quality control to adopt such economical and time saving method for assay of drugs in combined dosage as well as individual assay of both drugs in raw material.

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