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# Stability Indicating RP-HPLC method Development and Validation of Salicylic Acid in Choline Magnesium Trisalicilate (Trilisate) Tablets

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**Abstract**

A stability indicating RP-HPLC method was developed and validated for the determination of Salicylic acid in Trilisate tablets using Xorabax XBD C18 column (150 × 4.6 mm, 5 µm) with mobile phase consisting of Phosphate buffer (pH 3.0), methanol (80:20 v/v) with a flow rate of 1ml/min. Detection was carried out at 230 nm. Retention time was found to be 4.6 min. Linearity was observed over the concentration range of 5-30 µg/ml ($R^2$=0.999) with regression equation y=30.55x+5.302. Salicylic acid was subjected to stress conditions including acidic, alkaline, photolysis and thermal degradation. The drug is more sensitive towards alkaline degradation. The method was validated as per ICH guidelines.

**Keywords**: Trilisate tablets; Salicylic acid; Reverse – phase HPLC; Validation; Stability indicating method.

## Introduction

TRILISATE [1] is a non-steroidal, anti-inflammatory preparation containing Choline magnesium trisalicylate. It is used to relieve the pain, tenderness, inflammation and stiffness caused by arthritis and painful shoulder. It is also indicated as anti-pyretic and analgesic.

Choline magnesium trisalicylate is a non-acetlyated salicylate. It is a combination of choline salicylate and magnesium salicylate. It acts by inhibiting prostaglandin synthesis, and also acts on the hypothalamus heat-regulating centre to reduce fever.

Choline magnesium trisalicylate has a molecular formula of $C_{26}H_{29}O_{10}NMg$, a molecular weight of 539.8, and chemical structure is given in the Figure 1.

Literature survey revealed that only few methods [2] are available for the estimation of Choline magnesium trisalicylate in pharmaceutical dosage forms. The aim of the present study was to develop a simple, economical, accurate and stable analytical method for the estimation of Salicylic acid in pharmaceutical dosage form and to validate the method according to ICH guidelines.

## Experimental Methods

### HPLC instrumentation and conditions

Chromatographic separation was achieved by using an Agilent Model 1100 series HPLC, with a 20 µl sample injection loop (automatic), and UV-Visible absorbance detector and photo-diode array detector. The output signal was monitored and integrated using Chemistation software. Xorabax XBD C18 (150 × 4.6 mm, packed with 5 µm) column was used for the separation.

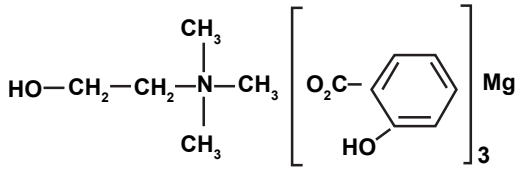


**Figure 1:** Chemical Structure of Choline Magnesium Trisalicylate.

### Chemicals and reagents

Methanol and Acetonitrile of HPLC grade were purchased from E.Merck (India) Ltd., Mumbai. Dihydrogen potassium phosphate and phosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Salicylic acid is available as Trilisate® with label claim of 500, 750 and 1000 mg of drug.

### Chromatographic conditions

The mobile phase consisting of buffer, methanol was filtered through 0.45 µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 80:20 v/v into the column (C18) at a flow rate of 1 ml/min. The detection was monitored at 230 nm and the runtime was 8 min.

### Preparation of the mobile phase

**Preparation of mobile phase:** Mobile phase was prepared by mixing the 0.05M phosphate buffer (pH 3.0) and methanol in the ratio of 80:20 v/v. The solution was filtered through a 0.45 µm membrane filter and degassed before use. Mobile phase is used as a diluent.

**Preparation of standard stock and working standard solution:** Salicylic acid Stock solution (200 µg/ml) was prepared by accurately weighing 20 mg of Salicylic acid in a 100 ml volumetric flask and making up to volume with mobile phase. Working standard solution (20 µg/ml) for HPLC was prepared by diluting 5ml of standard stock solution to 50 ml with mobile phase. Solutions were filtered through a 0.45 µm membrane filter prior to injection.

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**Preparation of the sample solution:** Accurately 20 tablets of TRISILATE were weighed and crushed into a fine powder. Powder equivalent to 100 mg Salicylic acid was accurately weighed into a 100 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution. The solution was filtered and from the filtrate 2.0 ml was taken and diluted to 100 ml with mobile phase. 20 µl of the solution was injected into the system and the peak area was recorded from the respective chromatogram.

### Assay procedure

Separately 20 µl of placebo solution, standard preparation and sample preparation were injected into the chromatographic system and chromatograms were recorded (Figures 2 and 3) and the peak areas were measured. From the peak areas, the amount of drug present in the sample is calculated by using the formula:

Mg/Tab = Spl. Area /Avg Std Area × Std Wt/Spl.Wt × Dilution factor × Avg .Wt/100 × Potency of Std %ASSAY= (Mg/Tab)/Label Amount × 100.

Where Spl=sample, Avg=Average, Std= Standard, Wt=Weight, Tab= Tablet.

## Method Validation

The developed method was validated for the following parameters: selectivity, linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), precision, accuracy and system suitability [3].

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels and 20 µl of each solution was injected into the HPLC system and the peak area of the Chromatogram obtained was noted Table 1, Graph 1.

The accuracy of the assay method was evaluated in triplicate at three concentration levels (50,100 and 150%) and the percentage recoveries were calculated Table 3.

The intra-day precision of the assay method in triplicate at a concentration of 20 µg/ml. The inter-day precision study was performed on three different days at a concentration of 20 µg/ml. The % RSD (Relative Standard Deviation) of the obtained assay values were calculated Table 2.

The LOD and LOQ were based on the standard deviation of the response and the slope of the constructed calibration curve Table 2.

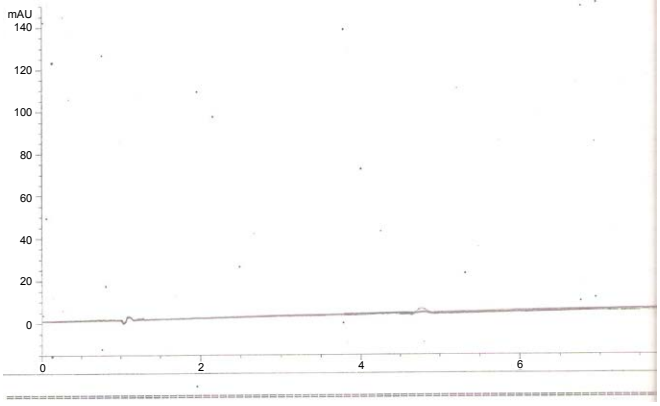



**Figure 2:** Chromatogram of Placebo Solution.

### Specificty/forced degradation studies

The study was intended to ensure the effective separation of Salicylic acid and its degradation peaks of formulation ingredients at the retention time of Salicylic acid. Forced degradation studies [4] were performed to evaluate the stability indicating properties and specificity of the method.

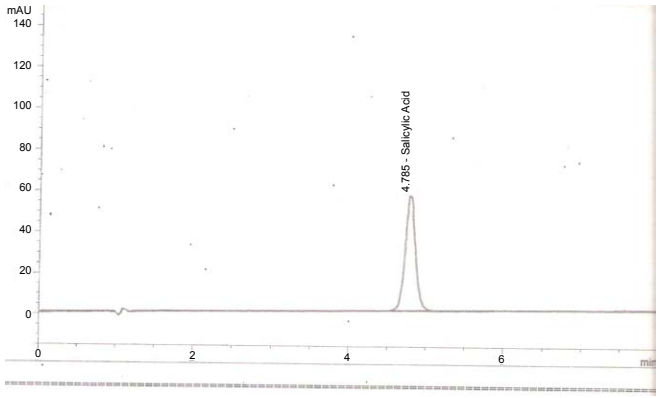
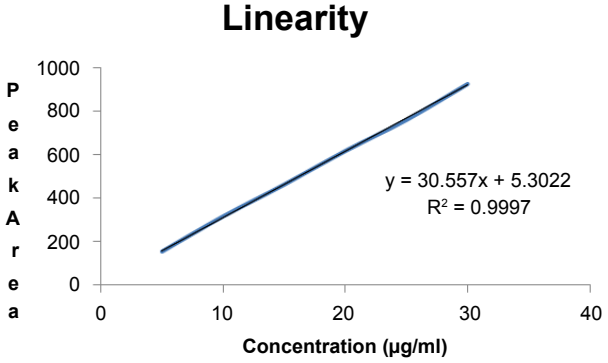



**Figure 3:** Chromatogram of Salicylic Acid Sample Solution.

| Concentration(µg/ml) | Peak Area |
|---|---|
| 5 | 154.703 |
| 10 | 316.660 |
| 15 | 463.258 |
| 20 | 617.956 |
| 25 | 761.230 |
| 30 | 926.529 |

**Table 1:** Linearity.




**Graph 1:** Calibration curve of Salicylic Acid.

| S.No | Parameter | Results |
|---|---|---|
| 1. | Intra-day precision | % RSD*=0.64 |
| 2. | Inter-day precision | % RSD*=0.60 |
| 3. | Linearity | $R^2$ = 0.9997 |
| 4. | Regression Equation | Y=30.557X+5.3022 |
| 5. | Theoretical Plates | 5111 |
| 6. | Tailing factor | 0.991 |
| 7. | LOD | 0.0104 µg/ml |
| 8. | LOQ | 0.0317 µg/ml |
| * RSD Six replicate injections mean Relative standard deviation | | |

**Table 2:** Method Validation Parameters.

| Sample No | Spiked Concentration (µg/ml) | % Recovery | %RSD |
|---|---|---|---|
| **1** | 15(50%) | 97.38 | 0.50 |
| **2** | 20(100%) | 97.4 | 0.63 |
| **3** | 35(150%) | 98.7 | 0.54 |

**Table 3:** Accuracy-Recovery study.

| Brand name | Label Claim | Amount found | % Assay |
|---|---|---|---|
| Trilisate | 750 mg | 742 mg | 98.93 |
| | **1000 mg** | **975 mg** | **97.50** |

**Table 4:** Amount of Salicylic acid present in the formulations.

| Degradation Type | %Drug Recovered | %Drug Decomposed |
|---|---|---|
| Acid | 96.19 | 2.92 |
| Alkali | 89.64 | 9.47 |
| Thermal | 97.96 | 1.15 |
| Photolytic | 96.80 | 2.31 |
| Oxidative | 96.27 | 2.84 |

**Table 5:** Forced Degradation Studies/Specificity.

All solutions for use in stress studies were prepared at an initial concentration of 200 µg/ml of salicylic acid. All samples were then diluted in mobile phase to give a final concentration of 20 µg/ml and filtered before injection.

Acid decomposition was carried out in 0.1 N HCl and alkaline degradation was conducted using 0.1 N NaOH and refluxed for 30 min at 80°C.After cooling the solutions were neutralized and diluted with mobile phase and analyzed Table 4.

Solutions for oxidative stress were prepared using 3% Hydrogen Peroxide at a concentration of 1 mg/ml of Salicylic acid and refluxation for 30 min at 80°C. The sample solution was cooled and diluted accordingly with the mobile phase.

For thermal stress testing, the drug solution (1 mg/ml) was heated in thermostat at 80°C for 30 min,cooled and used. The drug solution (1 mg/ml) for photostability testing was exposed to UV light at 254 nm for 24 h and analysed Table 5.

## Discussion

The % RSD was found to be less than 2, which indicates that the proposed method has good reproducibility. The drugs obeys linearity within the concentration range of 5-30 µg/ml .The percentage recovery found to be in between 97-99%, which indicates that the method is accurate and also reveals that the commonly used excipients and additives present in the formulation were not interfering the proposed method.

## Conclusion

The proposed method was found to be simple, precise, accurate and rapid for the determination of Salicylic acid in Choline Magnesium Trisalicylate tablets (Trilisate).

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