IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TARTARIC ACID FROM EFFERVESCENT GRANULES BY RP-HPLC

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Abstract: The analytical method was developed and validated for Tartaric acid Effervescent formulation by using RP- HPLC. The appropriate method optimized for the assay of Tartaric acid and validated as per ICH 2005 guidelines. For the analysis, 210 nm wavelength on UV/visible detector were selected, with the Mobile phase 0.01 M Potassium Dihydrogen Phosphate, pH 2.6. maintained with Orthophosphoric Acid. The chromatographic conditions for the best separation of Tartaric acid, RP-column C18 Shimadzu GIST Shim Pack (4.6 x 250 mm, 5 µm particle size) with a flow rate of 1.0 mL/ min, and injection volume of 20 µL. The oven is set to a temperature of around 30 °C. The method was validated for specificity, linearity, precision, accuracy, and robustness. The developed method was found to be linear and precision was evaluated by replicate analysis in which Relative Standard Deviation (RSD) values for areas were found below 2.0%. The recoveries obtained (100.83%–101.54%) ensured the accuracy of the developed method. Accordingly, the proposed developed and validated procedure was proved to be suitable for routine analysis of Tartaric acid.

Index Terms **- Organic acid, Tartaric acid, RP- HPLC, ICH guidelines, Validation.**

1. Introduction

The selected drug for this study is Organic Acid i.e., Tartaric Acid which is mainly used as Pharmaceutical Aid (Natural Acidifying[2] and Preservative). It is commonly mixed with Sodium Bicarbonate and is sold as baking powder used as a leavening agent in food preparation. It has been used in the production of effervescent salts, in combination with citric acid, to improve the taste of oral medications[1].Some studies show that it also enhances adrenergic Receptor activity[3]. Tartaric Acid in Wine may have a positive effect on cardiovascular health in post-menopausal women, underpinning its nutraceutical properties[4]. Synonyms of Tartaric acid are Cream of Tartar, Threaric acid Racemic acid[2]. IUPAC name of drug is 2, 3-Dihydroxybutanedioic[5] and structure for Tartaric acid is given in Fig no.1

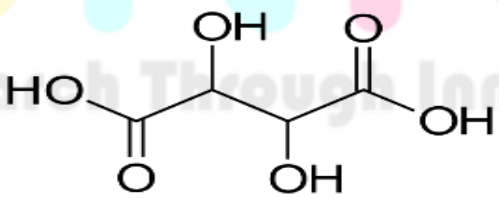

fig no. 1 structure of tartaric acid

Literature survey reveals that analytical and HPLC methods for the above stated drug have been reported in combination with other drugs in dosage forms. Studies also show that very few RP- HPLC methods for the Tartaric Acid was reported. Therefore, it was thought worthwhile to develop a novel, specific, linear, precise, accurate and robust method for the determination of Tartaric Acid by RP-HPLC using a particular mobile phase. The proposed method is optimized and validated as per the ICH guidelines.

2. Material and Methods

2.1 Material

Tartaric acid working standard from Hangzhou Bioking Biochemical Engineering and marketed formulation of SciTech specialties Pvt. Ltd. Sinnar, Nashik. Marketed formulation (Uricetra) contains 0.890 g of Tartaric acid in 4.0 g granules was used for experiment. HPLC grade water was supplied by Inhouse. Potassium Dihydrogen Phosphate AR grade was supplied by Labo-chemicals Mumbai. Orthophosphoric

Acid (HPLC grade) and Ethanol (HPLC grade) supplied by Rankem chemical industry Mumbai. Methanol and Acetonitrile supplied by Finar chemicals, Mumbai.

2.2 Instruments

HPLC of Agilent technologies (Infinity 1260) and Shimadzu (LC3050 i-series) used for the method development. Analytical Weighing Balance used was made by Shimadzu Corporation, Japan. pH Meter used to adjust pH of mobile phase was made by Lab India. Other instruments such as Ultrasonicator, and UV (Shimadzu) also used.

3. Method

3.1 Preparation of standard solution

Weighed accurately about 50 mg of Tartaric acid WS in a 100 ml volumetric flask. Add sufficient diluent (mobile phase) to dissolve completely. Make up volume to 100 ml & sonicate for 5 min (Sol A). Dilute 5 ml Sol A to 25 ml with diluent.

3.2 Preparation of sample

Weighed accurately about 1.5 g of Tartaric acid sample in a 100 ml volumetric flask. Add 50 ml diluent to dissolve the sample completely and make up the volume to 100 ml. Further dilute 2 ml to 50 ml with diluent.

3.3 Chromatographic conditions

Trials were taken by changing the mobile phase and pH of mobile phase. The optimized method was obtained when Mobile phase Potassium Dihydrogen Phosphate used at pH 2.6. Column Shimadzu C18 (4.6 mm X250 mm, 5 µm) was used for the analysis. Flow rate 1.0 ml/ min by injecting 20 µl at Oven temp 30^0C.

3.4 Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of 10µg/ml of each of Tartaric acid were prepared in water. After observing UV spectra of the drug, wavelength of 210 nm was selected for further study. The spectrum obtained is presented in fig. 2

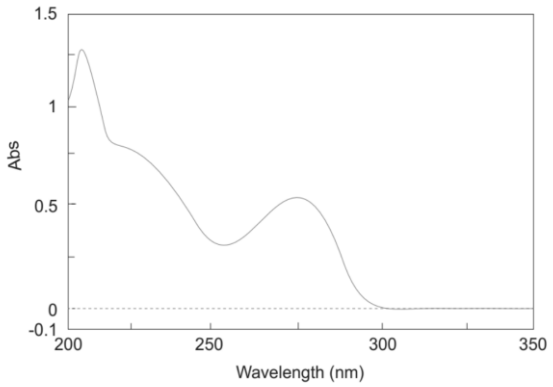

fig no. 2 absorbance maxima spectra of tartaric acid

4. Results & Discussion

4.1 Method optimization

The selection of HPLC method depends upon the nature of the sample, its molecular weight and solubility. RP-HPLC method was selected for the initial separations because of its simplicity and suitability. The chromatographic variables such as mobile phase ratio and flow rate were studied. The condition that gave the best resolution, symmetry and selectivity was selected. Optimizations in HPLC is the process of finding a set of conditions that sufficiently enable the quantification of the analyte with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed. Optimized method retention time was 3.38 min. Chromatogram obtained for Blank, Standard, Sample by optimized method presented in Fig no. 3, 4 and 5.

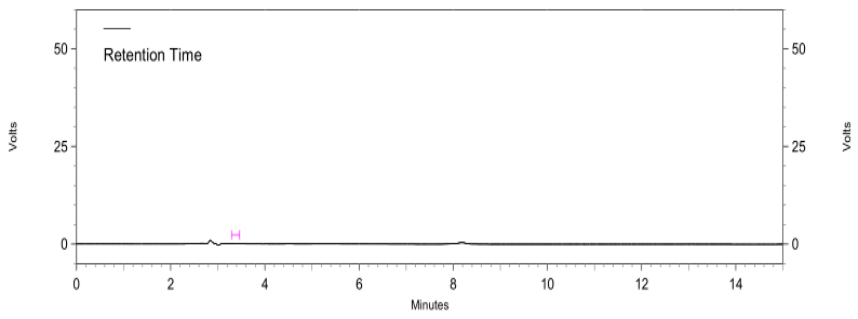

fig no. 3 typical chromatogram of blank

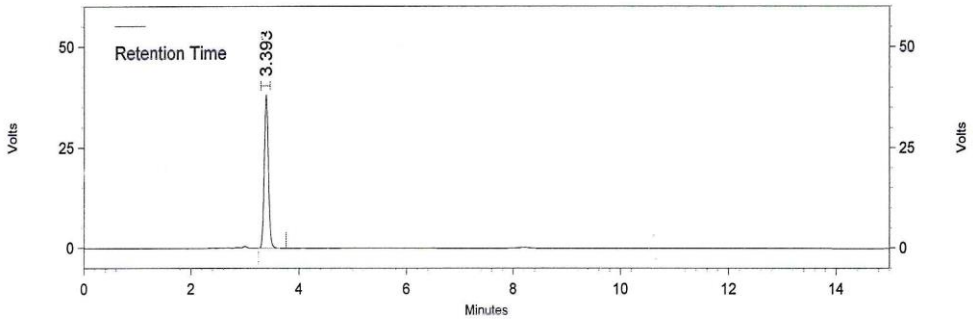

fig no. 4 typical chromatogram of standard

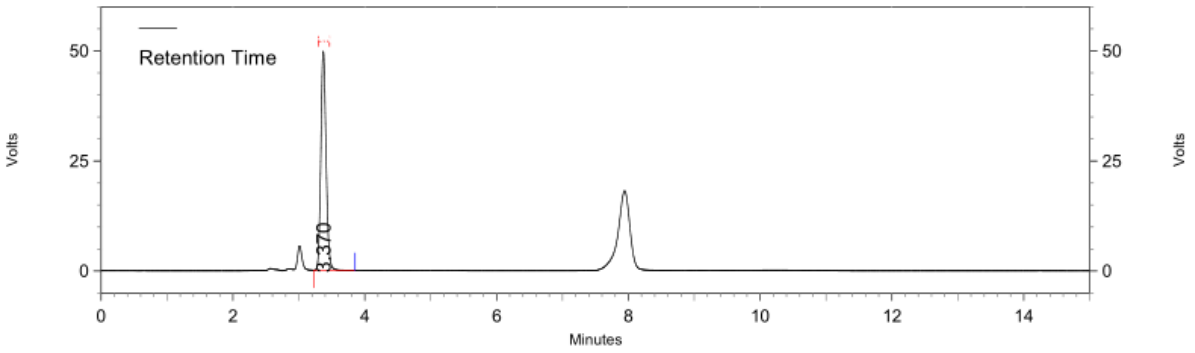

fig no. 5 chromatogram of sample

4.1.2 System suitability

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Tartaric acid was injected into the system for five times and system suitability parameters were checked. The % RSD of areas were found 0.309. Obtain results are presented in Table no. 1

table no. 1 system suitability results

Sr. No	Standard Solution	Area
1	Replicate 1	189070
2	Replicate 2	190303
3	Replicate 3	190614
4	Replicate 4	189866
5	Replicate 5	190174
	RSD	0.309

4.2 Method validation

4.2.1 Linearity

The linearity of analytical procedures is its ability to obtain test results which are directly proportional to the concentration of analyte in sample. Five samples were prepared by using Tartaric acid WS to have final Tartaric acid concentration of 80 to 120 PPM to that of the decided optimum concentration of the standard solution which is prepared for assay Procedure. Linearity results are presented in Table no. 2 and standard calibration curve represented in Fig no. 6.

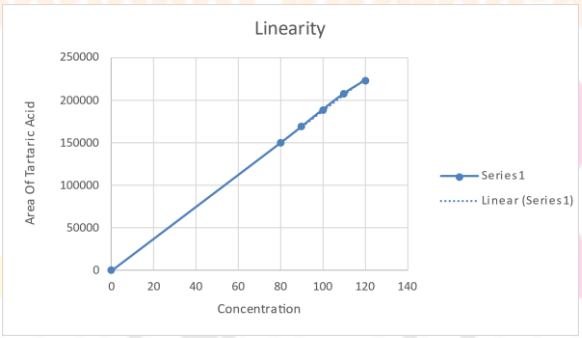

fig no. 6 calibration plot

table no. 2 linearity results

Sr. No	Concentration	Area Of Tartaric Acid
1	0	0
2	80	149919
3	90	169287
4	100	189139
5	110	207991
6	120	223481
	Slope	1877.35
	Intercept	190.66
	Co-relation coefficient	0.9999

4.2.2 Precision

Precision of the analytical method is the closeness of agreement between the series of measurements from multiple sampling of the same homogeneous sample under prescribed condition. Precision was studied by repeatability and intermediate precision. Intermediate precision expresses within laboratories variations like different analysts, different instruments etc., prepared and analyzed the three set of samples and results are presented in Table no. 3.

table no. 3 precision results

Precision parameter	% RSD	Acceptance criteria
Repeatability	0.18 %	2.0 %
Different analyst	0.67 %	2.0 %
Different instruments	0.49 %	2.0 %

4.2.3 Accuracy

Accuracy should be expressed on samples spiked with known amounts of Tartaric acid working standard and results obtain presented in Table no. 4.

table no. 4 Percentage Recovery data of Tartaric Acid

Sample	Average % Recovery	% RSD
Sample without spiking	100.71	1.22
Sample with 10% WS	100.69	0.06
Sample with 20% WS	99.53	1.28
Sample with 30% WS	100.74	0.26
Sample with 40% WS	100.71	0.20
Sample with 50% WS	100.82	0.11

4.3.3 Specificity

Specificity is the ability to asses unequivocally the analyte in the presence of components which may be expected to be present. Different set of samples were prepared of same selected batch with spiking different concentration of placebo. Results are presented in Table no. 5.

table no. 5 specificity results

Sample	Average % Assay	% RSD
Sample without placebo spiking	102.25	0.38
Sample with 10% placebo spiking	98.90	0.04
Sample with 20% placebo spiking	100.33	0.09
Sample with 30% placebo spiking	102.48	0.07
Sample with 40% placebo spiking	99.21	0.09
Sample with 50% placebo spiking	101.99	0.33

4.3.4 Robustness

Robustness determines the reliability of an analytical method with deliberate variations in the method parameters like change in pH of the mobile phase, change in flow rate and change in temperature of column oven, stability of analytical solutions etc. Robustness studied by change in flow rate, change in column oven temperature. Results are represented in Table no. 6 and 7.

4.3.5 Solution stability

Three samples from homogeneous blend of same samples were prepared and analyze the same sample on different time interval as, fresh solution, after 2 hours, after 4 hours, and after 6 hours. Results are represented in Table no. 8.

table no. 6 change in flow rate

Change in flow rate	Average % Assay	% RSD
0.8 ml/ min	100.54	0.53
1.0 ml/ min	100.45	0.66
1.2 ml/ min	100.72	0.59

table no 7 changes in temp. results

Change in temp.	Average % Assay	% RSD
27.0° C	100.16	0.03
30.0° C	99.98	0.05
33.0° C	100.36	0.08

table no. 8 stability of solution results

Sample	Average % Assay	% RSD
Fresh solution	101.69	0.10
After 2 Hrs.	101.74	0.08
After 4 2 Hrs.	11.85	0.21
After 6 2 Hrs.	101.78	0.11

5. Conclusion

During analytical method development and validation study of assay of tartaric acid from effervescent granules formulation, it is observed that developed method complies with all acceptance criteria under different validation parameter such as linearity, specificity, accuracy, robustness and precision.

Developed HPLC method with UV/Visible detector is fast, reliable, sensitive and cost-effective for the determination of tartaric acid in effervescent formulation. Because of the aqueous mobile phase, the method can be considered green and suitable for routine analysis of formulation.

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