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Validated RP-HPLC method for the determination of Benzoic acid in bulk and pharmaceutical formulation

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Benzoic acid in Bulk and Pharmaceutical Formulation. Isocratic elution at a flow rate of 1.0 ml/min was employed on symmetry C18 5 µm (4.6 x 150 mm) column at ambient temperature. The mobile phase consisted of Acetonitrile: Buffer in the ratio of 45:55 v/v. The UV detection wavelength was 254nm and 20µl sample was injected. The retention time for Benzoic acid was ± 18 min. The percentage RSD for precision and accuracy of the method was calculated. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Benzoic acid in the rapid and reliable determination of Benzoic acid in Pharmaceutical formulation.

Key words: Benzoic acid, HPLC, UV detection, recovery, precision.

INTRODUCTION

The molecular formula for benzoic acid was found to be $C_7H_6O_2$. Benzoic acid is a white, crystalline powder or colourless crystals, odorless or with a very slight characteristic odour. It is slightly soluble in water, soluble in boiling water, freely soluble in alcohol and in fatty oils. Benzoic acid occurs naturally in many plants [1] and it serves as an intermediate in the biosynthesis of many secondary metabolites. Salts of benzoic acid are used as food preservatives and benzoic acid is an important precursor for the industrial synthesis of many other organic substances. Benzoic acid is a constituent of Whitfield's ointment which is used for the treatment of fungal skin diseases such as tinea, ringworm, and athlete's foot.[2][3] Benzoic acid is relatively nontoxic. It is excreted as hip uric acid.[4] Benzoic acid is metabolized by butyrate-CoA ligase into an intermediate product, benzoyl-CoA,[5] which is then metabolized by glycine N-acyltransferase into hip uric acid[6]. Cornelia Petronela ENE et. al., [7]described the determination of the antimicrobial preservative benzoic acid in different food matrix. The determination of the preservative was performed employing a HPLC system equipped with UV diode array detection. The HPLC working parameters were optimized and the method was validated by establishing the analytical criteria of performance. The results obtained for the determination of benzoic acid on real soft drink samples from different Romanian companies showed values of preservative content between 0 and 110 mg/L, meeting the national regulated limit. Jankana Burana-osot et.al.,[8]proposed a simple, sensitive and specific HPLC method was validated for the simultaneous determination of benzoic acid and sorbic acid in noodles. Sample preparation involved the extraction with methanol and water (60:40, v/v). The separation was achieved on a

Germini- C18 modified silica column (50 mm x 4.6 mm i.d., 5 µm particle diameter). The mobile phase consisted of 0.05 M ammonium acetate (pH 4.4) and methanol in the ratio of 60:40 (% v/v) at a flow rate of 1 mL/min and detection was performed at 234 nm using a diode-array detector (DAD). The method was validated with respect to specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness. The calibration curve for benzoic acid and sorbic acid was found to be linear. The values of LOD and LOQ were 0.42 and 1.14 µg/mL and 0.32 and 0.99 µg/mL respectively. The accuracy determined by spike recovery measurement was 85.61-102.04% for benzoic acid and 83.62-102.47% for sorbic acid. Sonal Shah et.al.,[9] descried the identification and quantification of *p*-hydroxy benzoic acid and agnuside in the extracts of Vitex negundo *and* Vitex trifolia. The separation was achieved using acetonitrile and O-phosphoric acid–water (0.5%, v/v) as the mobile phase in an isocratic elution mode. The developed method was validated as per the ICH guidelines for limit of detection, limit of quantification, linearity, accuracy and precision. Good linearity ($r^2 \geq 0.999$) was observed for both the compounds in wide concentration range. Relative standard deviation values for intra-day and inter-day precision studies were less than 2%. The analytical recoveries of *p*-hydroxy benzoic acid and agnuside by the developed HPLC method were 93.07% and 106.11% respectively. Chiara Guarino et.al. [10]Demonstrated a single method, based on RP-HPLC with UV detection, was developed with the aim of simultaneously quantifying four preservatives in cheeses: benzoic acid, sorbic acid, natamycin and lysozyme. The preservatives were extracted from different cheeses by using the same procedure, and separated by a single RP-HPLC gradient elution showing good resolution, in a short time. Recoveries were always higher than 91%; MDLs ranged from 0.4 to 4.0 µg g^{-1}, and MQLs were included between 1.3 and 13.3 µg g^{-1}; RDS ranged from 1% to 7%. Quantitation was performed in reference to a matrix matched calibration curve. The method was also applied to real samples for the determination of the four preservatives, with satisfying results.

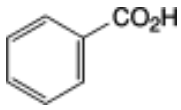

Fig: 1 Structure of Benzoic Acid

MATERIALS AND METHODS

Materials:

2.1 Instrumentation: Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a C18 5 µm (4.6 x 150 mm). Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

2.2Chemicals and Solvents: The reference sample of Benzoic acid was obtained from Jollc. The Formulation norimide was purchased from the local market. Ammonium acetate, Acetic acid, and Acetonitrile used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

2.3The mobile phase: 1 % Ammonium acetate in 2 % Acetic Acid and Acetonitrile 90:10 v/v was prepared and used as mobile phase.

2.4Sample (Capsule) solution:

Place 900 ml of dissolution medium to each of 6 the vessels of theapparatus. Assemble the apparatus and allow the temperature of the dissolution medium to equilibrate to 37 °C ± 5 °C. Place one tablet into each of the vessels, taking care to exclude air bubbles from the surface of the tablet and immediately operate the apparatus at 50 rpm. At 30 minutes, withdraw 20ml aliquot from zone midway between the surface of the dissolution medium and the top of the rotating blade, not less than 1 cm from the vessel wall. Filter the sample through a 0.45 µm filter before use, discarding the first few milli liters of the filtrate.

2.5Preparation of Standard Solution

Accurately weigh 100 mg Benzoic Acid standard into a 100 ml volumetric flask. Dissolve and dilute to volume with solvent. Dilute 10 ml of this solution to 100 ml with solvent. Filter through a 0.45 µm filter.

2.6Sample solution preparation
Accurately weigh 5.75 g of sample into a 100ml volumetric flask. Dilute to volume with solvent. Filter through a 0.45 µm filter.

3. Method Development:
Detection wavelength: The spectrum of 10ppm solution was recorded separately on UV/Vis. Spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the substance were showed maximum absorbance at 254nm.

3.1Choice of stationary phase: Preliminary trials have performed with different types, configurations and from different manufacturers. Finally the expected separation and peak shapes were obtained on C18 5 µm (4.6 x 150 mm).

3.2Selection of the mobile phase: To get low tailing factor, base line separation and sharp peak of the components, a number of trails were carried out by changing the composition of different solvents and flow rate. Indifferent combinations were tested as mobile phases on a C18 5 µm (4.6 x 150 mm).

3.3Flow rate: 0.5 – 2.0 mL/min flow rates of the mobile phase were changed for optimum separation. It was found from the results, 1.0 ml/minute flow rate was ideal for the successful elution of the analyte.

3.4Optimization of chromatographic conditions: Optimized chromatographic conditions were followed for the determination of Benzoic acid in bulk samples and in its formulations.

4. Validation of Proposed Method:
The analytical performance of the method of analysis was checked for specificity, system suitability, linearity, accuracy and method precision.

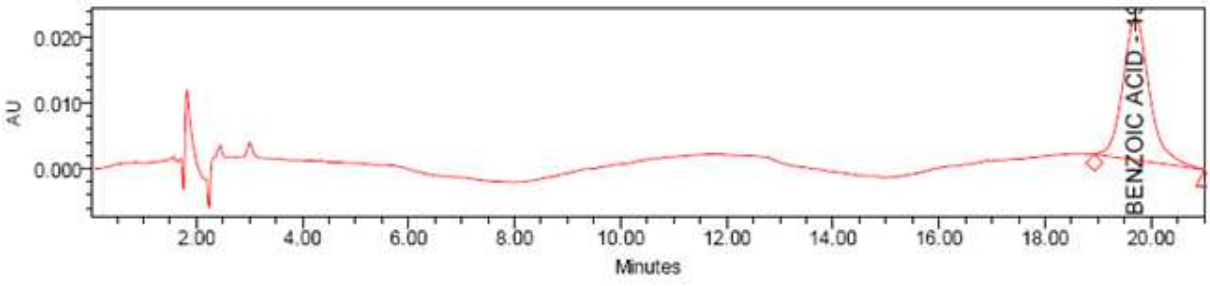

Fig: 2: Drug active – Peak due to Benzoic Acid

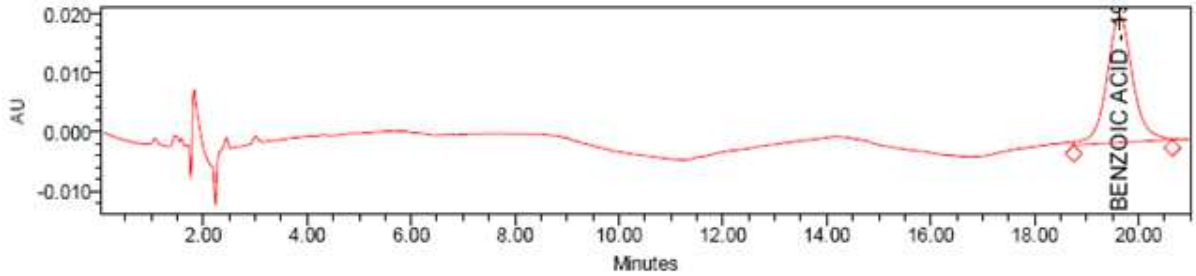

Fig: 3: Product – Peak due to Benzoic Acid

4.1Specificity
Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The solvent and placebo solutions must contain no components, which co-elute with the Benzoic Acid. The peak purity results from the photo diode-array analysis must show that the Benzoic Acid peak is pure – i.e. the purity angle (PA) must be less than the threshold angle (TH). Benzoic Acid is stable under UV light exposure. No components are seen to co-elute with the Benzoic Acid peak, and the peak purity

results indicate that the Benzoic Acid peak can therefore be considered spectrally pure. The chromatogram results are shown in the Fig: 2 to 6 and Peak purity results are shown in the Fig: 7 to 10.

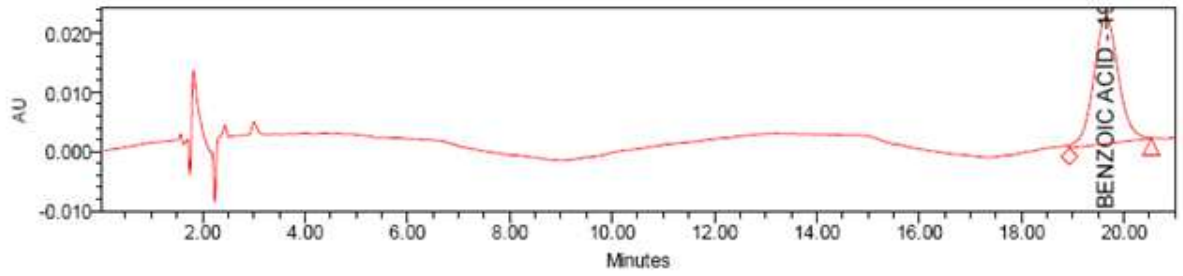

Fig: 4: Active – UV stress: Peak due to Benzoic Acid

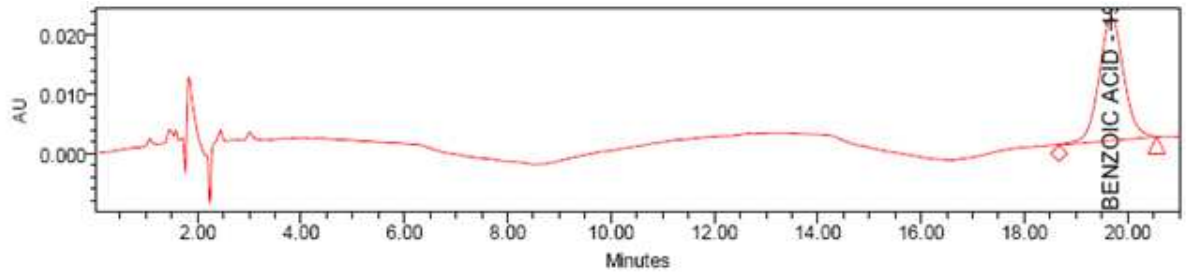

Fig: 5: Product – UV stress: Peak due to Benzoic Acid

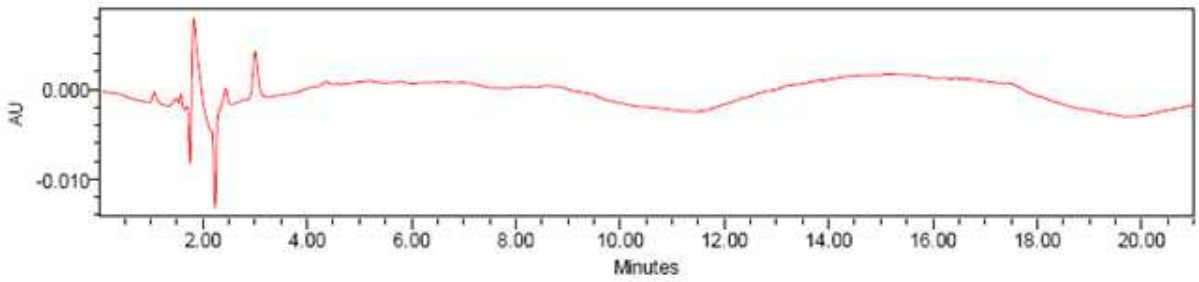

Fig: 6: Placebo – No significant peak detected.

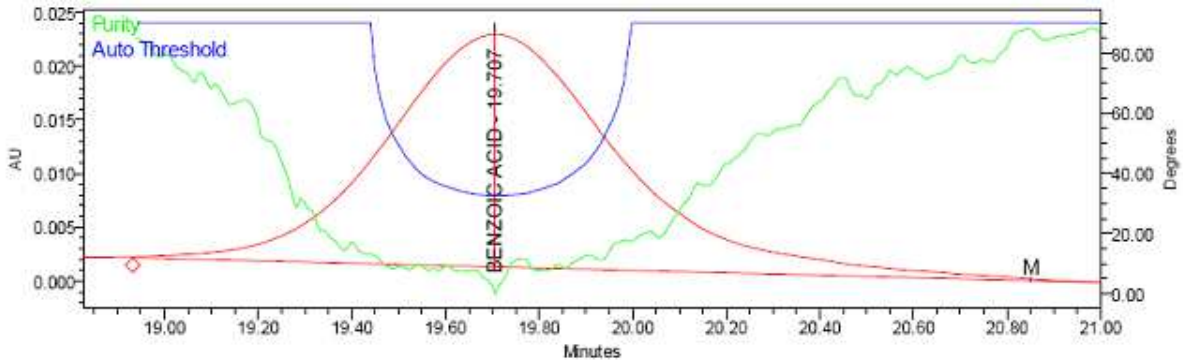

Fig: 7: Purity angle < Threshold 16.655 < 76.3

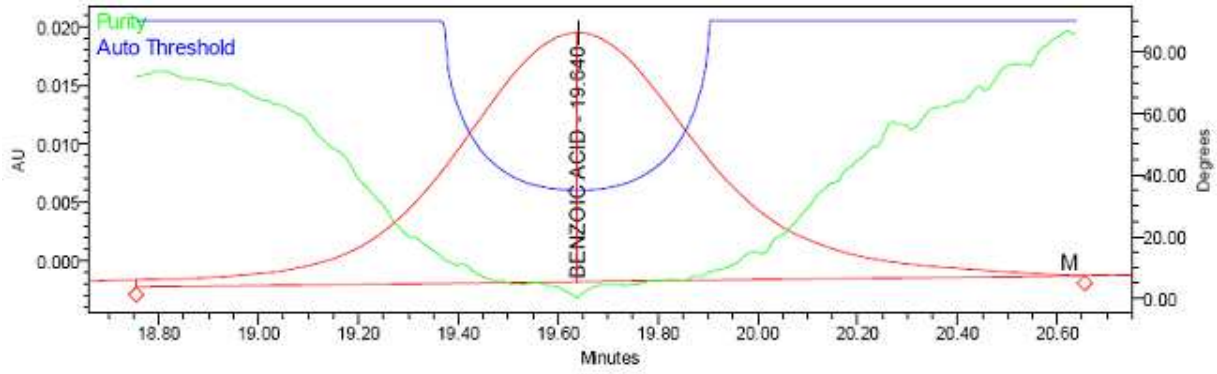

Fig: 8: Purity angle < Threshold 13.261 < 80.252

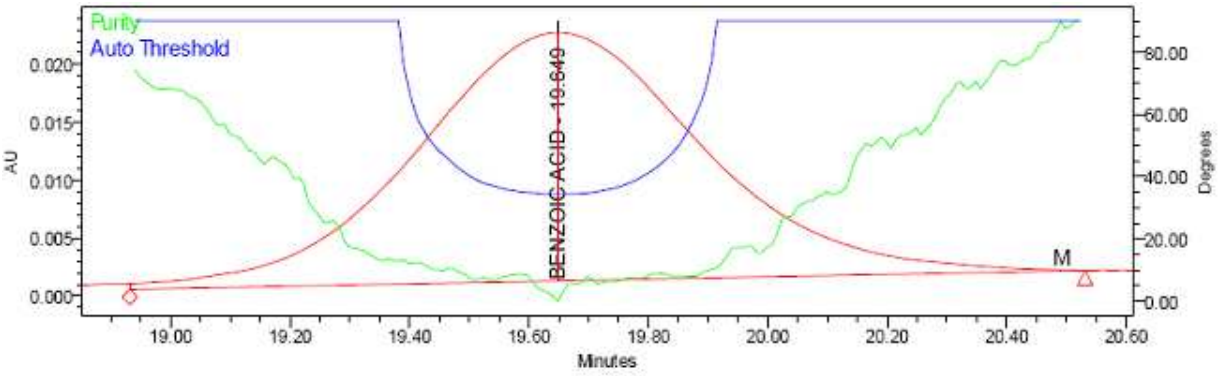

Fig: 9: Purity angle < Threshold 12.575 < 64.421

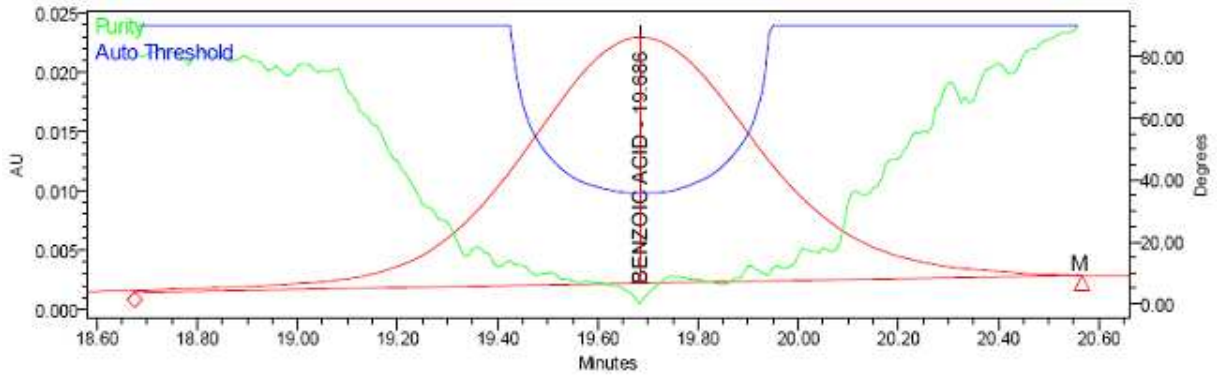

Fig: 10: Purity angle < Threshold 13.473 < 90.000

4.2System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The requirements for system suitability for this method are: The % RSD of the peak responses due to the Benzoic Acid for the six replicate injections must be less than or equal to 2.0 %. Six replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses was determined. Therefore, the analytical system complies with the requirements specified by the system suitability. The results are tabulated in the Table: 1.

Table: 1 System Suitability Results

Sample	Benzoic Acid Area
1	778267
2	780980
3	780261
4	781432
5	779235
6	781713
Mean	780314
% RSD	0.2

4.3Linearity

The linearity of an assay method is its ability to elicit test results, which are directly proportional to the concentrations of drug active in samples in a given range. Proof of linearity justifies the use of single-point calibrations. The correlation coefficient of the regression line for the Benzoic Acid should be greater than or equal to 0.99.The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when + 5 > z > - 5. Five solutions containing 50, 75, 100, 125, and 150 % of the Benzoic Acid relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R^2) and assessment values calculated. The correlation coefficient (R^2) for Benzoic Acid is 0.99.The plot is a straight line and the assessment value (z) is 3 for Benzoic Acid. Results are tabulated in the Table: 2. Linearity curve is shown in the Fig: 11.

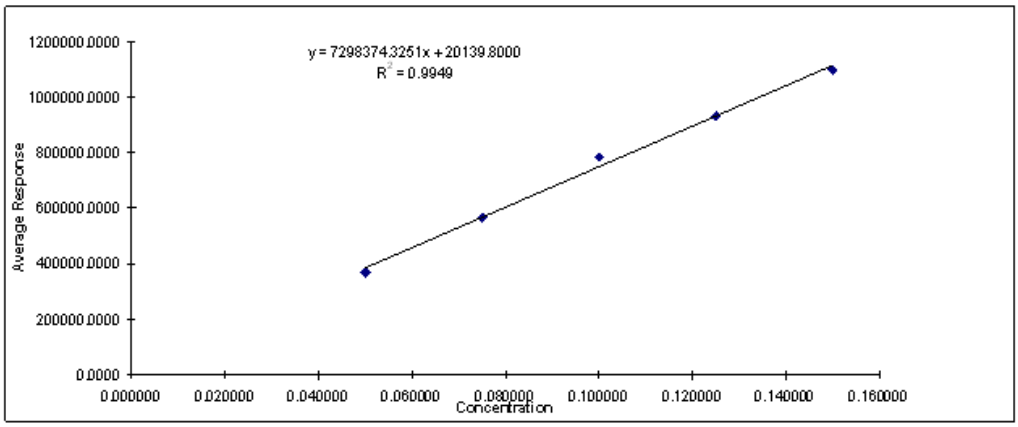

Fig: 11 Linearity Curve

Table: 2 Linearity results

Sample Number	Concentration	Response 1	Response 2	Average Response
50%	0.050010	369643.0000	368593.0000	369118.0000
75%	0.075015	565442	566998.0000	566220.0000
100%	0.100020	782676.0000	786715.0000	784695.0000
125%	0.125025	928916.0000	934585.0000	931750.0000
150%	0.150030	1098566.0000	1099098.0000	1098832.0000

4.4Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value. The percentage recovery of the active compounds, for each solution prepared, must be within 95.0 % – 105.0 % of the actual amount. Benzoic Acid samples were weighed into different 100ml volumetric flasks containing known concentrations of Benzoic Acid representing respectively 50, 75, 100, 125 and 150 % of Benzoic Acid relative to the working concentration. The above samples were injected in duplicate according to the method of analysis. From the accuracy results above, the percentage recovery values for the Benzoic Acid satisfy the acceptance criteria for accuracy across the range of 50 % - 150 %. The results are tabulated in the Table:3.

Table: 3 Average % recovery

Sample %	Sample weighed (g)	Theoretical % Active	Actual % Active	% Recovery	Average % Recovery
50	2.875	0.0910	0.088	96.7	96.7
			0.088	96.7	
75	4.3125	0.1365	0.131	96.0	96.0
			0.131	96.0	
100	5.75	0.1820	0.182	100.0	100.0
			0.182	100.0	
125	7.1875	0.2275	0.220	96.7	96.7
			0.220	96.7	
150	8.625	0.2730	0.264	96.7	96.7
			0.263	96.7	

4.5 Method precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

4.51Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to the Benzoic Acid concentration for the six samples must be less than or equal to 2.0 %. Six separate sample preparations were analysed according to the method of analysis. The % RSD due to the Benzoic Acid concentration for the assay meets the requirements for repeatability at 0.2 %. Results are tabulated in the Table: 4.

Table: 4 Repeatability results

Sample number	Results (% m/v)
	Benzoic Acid
1	0.20
	0.20
2	0.20
	0.20
3	0.20
	0.20
4	0.20
	0.20
5	0.20
	0.20
6	0.20
	0.20
Mean	0.20
% RSD	0.2

Table: 5 % RSD Results

Sample	Results (% m/v)
	Benzoic Acid
1	0.20
	0.20
2	0.20
	0.20
3	0.19
	0.19
4	0.20
	0.20
5	0.20
	0.20
6	0.20
	0.20
Mean	0.20
% RSD	0.7

4.52 Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed: by a different analyst, on a different day, and using different reagents, mobile phases and solvents. The % RSD due to the Benzoic Acid concentration for the six samples must be less than or equal to 2.0 %. The mean results obtained in the repeatability and the intermediate precision must not differ by more than 3.0 %. Six separate sample preparations of batch 254259 were assayed according to the method of analysis. The % RSD for intermediate precision is 0.7 %. The intermediate precision and repeatability comply as they differ by 0.0 %. Results are tabulated in the table :5 and 6 respectively.

Table: 6 Repeatability and Intermediate precision

Sample	Mean Results (%m/v)
	Benzoic Acid
Repeatability	0.20
Intermediate Precision	0.20
Mean	0.20
% RSD	0.0

4.6. Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Based on the accuracy results, the range for the assay of Benzoic Acid is 0.1 – 0.3 % m/v of Benzoic Acid, which represents 50 % to 150 % of the working concentration.

RESULTS AND DISCUSSION

No components are seen to co-elute with the Benzoic Acid peak, and the peak purity results indicate that the Benzoic Acid peak can therefore be considered spectrally pure. The % RSD of the peak responses due to the Benzoic Acid for the six replicate injections must be less than or equal to 2.0 %. Six replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses was determined. The correlation coefficient of the regression line for the Benzoic Acid should be greater than or equal to 0.99.The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when + 5 > z > - 5. Five solutions containing 50, 75, 100, 125, and 150 % of the Benzoic Acid relative to the working concentrations, were prepared and injected according to the method of analysis. The percentage recovery of the active compounds, for each solution prepared, must be within 95.0 % – 105.0 % of the actual amount. The % RSD due to the Benzoic Acid concentration for the assay meets the requirements for repeatability at 0.2 %. The mean results obtained in the repeatability and the intermediate precision must not differ by more than 3.0 %. Six separate sample preparations of batch 254259 were assayed according to the method of analysis. The % RSD for intermediate precision is 0.7 %. Based on the accuracy results, the range for the assay of Benzoic Acid is 0.1 – 0.3 % m/v of Benzoic Acid, which represents 50 % to 150 % of the working concentration.

CONCLUSION

The method for the assay of Benzoic Acid in Koastatex Suspension complies with the requirements for linearity, specificity, system suitability, method precision and accuracy across the range of 50 % to 150 % of the working concentration. The method is therefore acceptable as valid and stability indicating.

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