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DEVELOPMENT OF BENZOIC ACID CONTNENT IN N-[(2S,3R,4R,6R)-3-METHOXY-2-METHYL-16-OXO-29-OXA-1,7,17-TRIAZAOCTACYCLO [12.12.2.12,6.07,28.08,13.015, 19.020,27.021,26] NONACOSA-8,10,12,14,19, 21,23,25,27-NONAEN-4-YL]- N-METHYL BENZAMIDE

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Abstract:

Midostaurin is a multi-targeted protein kinase inhibitor that has been used for the treatment of acute myeloid leukemia. Here, a reverse phase highperformance liquid chromatographic method has been developed for the validation of benzoic acid in midostaurin API form.[1] Chromatography was carried out on a Inertsustain C18 (150 x 4.6 mm, 5µm) column using a mixture of acetonitrile: water (90:10 v/v) as the mobile phase at a flow rate of 0.7 mL/min, the detection was carried out at 234nm and the retention time of the benzoic acid was found to be 9.89 and midostaurin was found to be 21.69. The method produce linear responses in the concentration range of 25-50 µg/mL of midostaurin. The method precision for the determination of assay was below 0.1 % RSD. The LOD and LOQ values obtained were 0.01 µg/mL and 0.03 µg/mL respectively.[2] There were no significant changes observed upon changing chromatographic conditions indicating the method to be rugged, robust, precise and linear. Therefore this validated method can be useful in the quality control of bulk of midostaurin.[3]

Keywords: Benzoic acid, Midostaurin, HPLC, LOD, LOQ and Retention time.

Introduction:

Midostaurin is a multi-targeted protein kinase inhibitor that has been used for the treatment of cancer and acute myeloid leukemia. Midostaurin is chemical name as N-((9S,10R,11R,13R)-10-methoxy-9-methyl-1-oxo-2, 3, 10, 11, 12, 13-hexahydro-9,13-epoxy-1H,9H-diindolo(1,2,3- GH:3',2',1'-lm)pyrrolo(3,4-j) (1,7) benzodiazo nin-11-yl)-nmethylbenzamide and content of benzoic acid.[4]

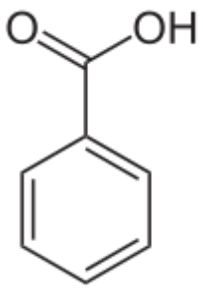


Fig-1: Benzoic acid.

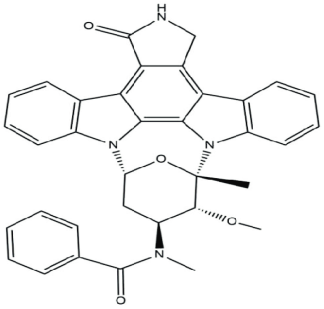


Fig-2: Midostaurin

Material and Methods:

RELATED COMPOUNDS BY HPLC:

Chemicals:

Potassium Dihydrogen orthophosphate HPLC grade or equivalent, Acetonitrile and Methanol used as: HPLC grade-Merck or equivalent, Potassiun hydroxide and water: Milli 'Q'

Chromatographic Conditions:

Column	: Inertsustain C18, 150 x 4.6 mm, 5-□m (or) equivalent
Flow rate	: 0.7 mL/minute
Detector Wavelength	: 234 nm
Injection volume	: 10 µL
Column temperature	: 40ºC
Elution mode	: Gradient
Run time	: 35 minutes
Diluent	: Acetonitrile:Water (90:10 v/v)

Preparation of buffer solution : Weigh and transfer about 1.36 g of Potassium di hydrogen phosphate into 1000 mL of Water adjust pH to 2.0± 0.05 with dilute orthophosphoric acid solution.

Preparation of Solvent-A: Buffer is used as Solvent-A

Preparation of Solvent-B: Prepare a mixture of buffer and acetonitrile in the ration of 30:70(v/v).

Gradient Programme:

Time (Minutes)	Solvent-A(%v/v)	Solvent-B(%v/v)
0.01	65	35
5	65	35

10	30	70
20	10	90
25	65	35
35	65	35

Preparation of Standard solution: Weigh accurately and transfer about 25.0 mg of Midostaurine Standard in to a 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Reference solution: Weigh accurately and transfer about 5.0 mg of Benzoic acid and Midostaurine Standard in to a 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix. Dilute 2.5 mL of above solution into 50 mL volumetric flask dissolve and dilute with diluent.[5]

Preparation of Test solution: Weigh accurately about 25.0 mg of test sample into a 50 mL volumetric flask, dissolve and dilute to the volume with diluent. Prepare in duplicate.

Procedure: Equilibrate the column for at least 35 minutes. Inject diluent as blank solution into the system and record the chromatogram. Program the data processor to inhibit the peaks due to blank solution and perform blank correction if necessary.

Inject Reference solution twice into the system and record the chromatogram. Check for the system suitability acceptance criteria, if met the requirements, proceed further.

System suitability acceptance criteria: The tailing factor for Midostaurine peak from Standard solution should be not more than 2.0. Inject the test solution into the system and record the chromatogram. Inject the test solution into the system and record the chromatogram. Calculate the % of Benzoic acid by using the area normalization method.[6]

Sample Information:

S.No	Sample	~ RT (minutes)	~RRT
1.	Benzoic Acid	9.9	0.45
2.	Midostaurine	21.7	1.00

VALIDATION PARAMETERS & PROCEDURE:

The following parameters should be considered for the validation.

System suitability/System precision:

Preparation of Standard stock solution: Weigh accurately about each 5.0 mg of Benzoic acid standard into

50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Standard solution (1.0%): Dilute 2.50 mL of standard stock solution to 50 mL with diluent and mix.

Procedure: Inject diluent as blank solution and record the chromatogram.

Inject Standard solution into the system for six replicates and record the chromatograms.

Acceptance Criteria: The % RSD for the peak area of Benzoic acid obtained from six replicate injections of standard solution should be not more than 10.0

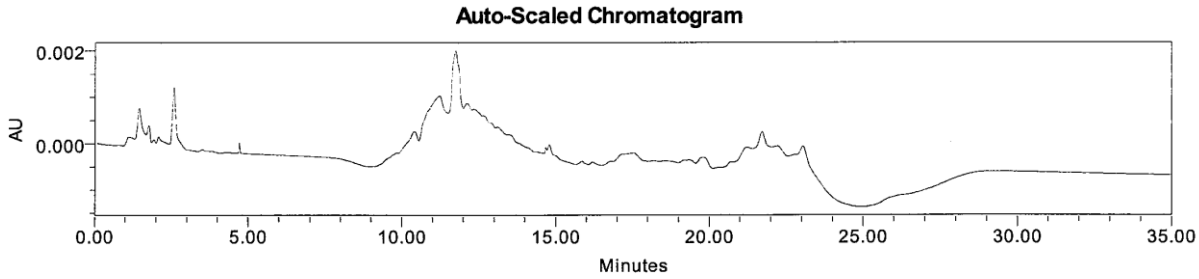


Fig-3: Blank Preparation.

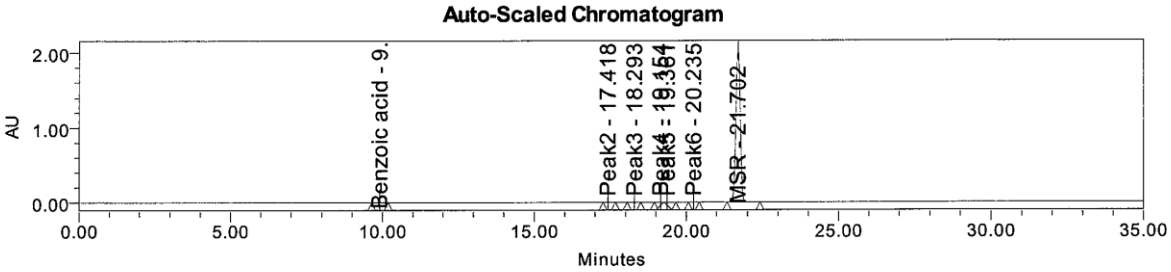


Fig-4: SST Preparation.

Peak Results

	Name	RT	Area	Height	RT Ratio	% Area	Int Type
1	Benzoic acid	9.907	72973	6996	0.457	0.35	BB
2	Peak2	17.418	5285	618	0.803	0.03	BB
3	Peak3	18.293	10072	1139	0.843	0.05	BB
4	Peak4	19.154	9623	1069	0.883	0.05	BV
5	Peak5	19.361	6785	710	0.892	0.03	VB
6	Peak6	20.235	8031	898	0.932	0.04	BB
7	MSR	21.702	20695442	2053837	1.000	99.46	BB
Sum			20808211.4				

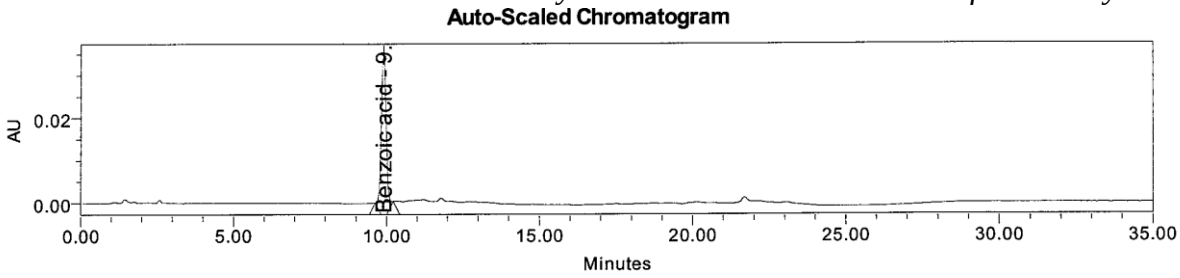


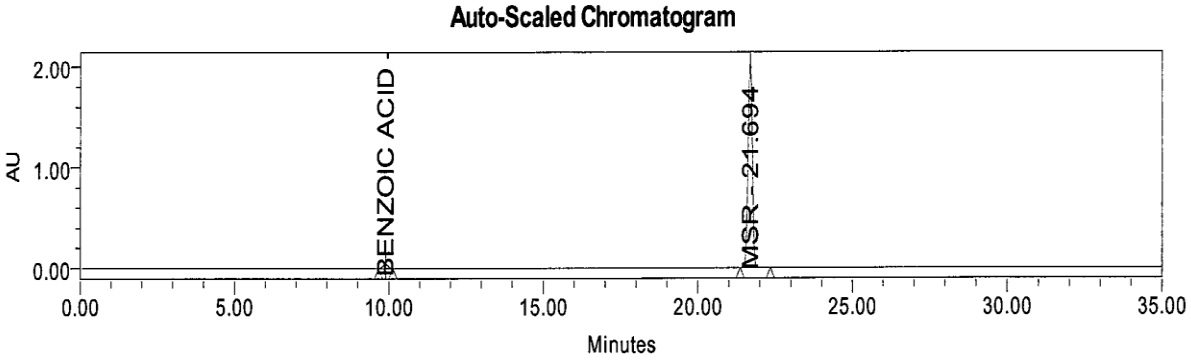
Fig-5: Standard Preparation.

Specificity:

Preparation of Benzoic acid stock solution:

Weigh accurately about 5.0 mg of Benzoic acid standard into 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Blend solution: Weigh about 25.0 mg of the test sample into 50 mL volumetric flask, add 2.50 mL of standard stock solution, dissolve and dilute to the volume with diluent and mix.



Peak Results

	Name	RT	Area	Height	RT Ratio	% Area	Int Type
1	BENZOIC ACID	9.895	442361	41665	0.456	2.15	BB
2	MSR	21.694	20090881	2044863	1.000	97.85	BB
Sum			20533242.0				

Fig-6: Blend Preparation.

Procedure:

Inject diluent as a blank and conclude the interference due to blank at the retention time of carryover impurities. Inject individual solution of Benzoic acid impurity solution and blend solution into the system and record the chromatograms. Establish retention time (RT) for Benzoic acid obtained from individual solution and blend solution.[7]

Acceptance Criteria: There should be no interference due to blank and possible known impurities at the retention time of corresponding carryover impurities. Impurity should resolve from each other and possible known impurities. The elution order and retention time (RT) of Benzoic acid obtained from individual solution and the blend solution should be comparable.

(± 10% Variation for retention time)

Test Solution Stability: Perform the analysis of test sample spiked with Benzoic acid at limit level and carryout solution stability upto 24 hrs

Procedure: Prepare and inject spiked test sample solution (Blend solution) containing Benzoic acid at specification level and Midostaurine at test conc. level record the chromatogram. Inject each interval stability sample solution and record the chromatograms. Compare the % variation of Benzoic acid obtained from initial sample and each time interval of solution stability sample and calculate together. Report the solution stability in hours.[8]

Note: After finding the time interval of solution at which is not stable no need to evaluate solution stability for the samples of remaining time intervals. The time intervals can be reduced and altered based on the intended application.

Acceptance criteria: The variation content (ppm) of carryover impurity obtained from solution stability study and initial result (fresh sample) should be meet ± 30.0% of the specification limit.

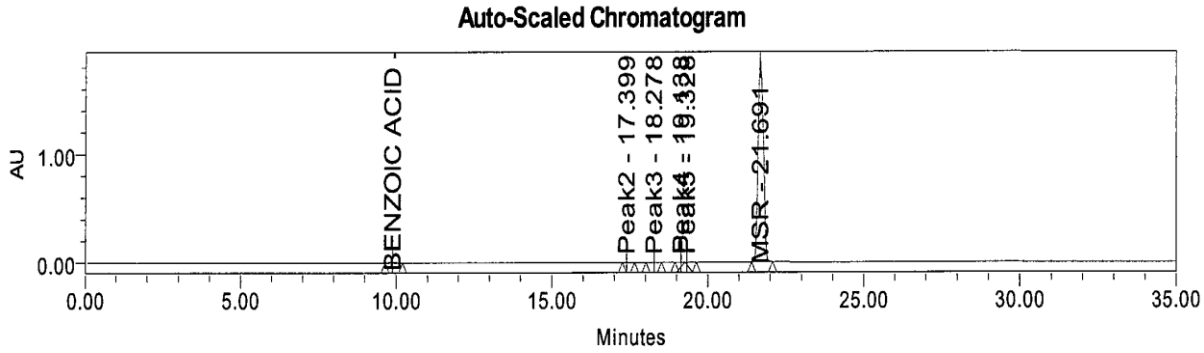


Fig-7: Solution Stability Preparation.

Detection Limit (DL): This is the measurement of lowest concentration of analyte that can be detected but not to be quantified.

Preparation of DL solution: Prepare DL solution containing carryover impurities with respect to test concentration by diluting each individual standard stock solution, so as to get S/N ratio is about 3:1 to 5:1

Procedure: Inject diluent as blank solution and record the chromatogram. Inject DL solution and record the chromatogram. Calculate the S/N ratio using the software.

Acceptance criteria: The S/N ratio should be about 3:1 to 5:1

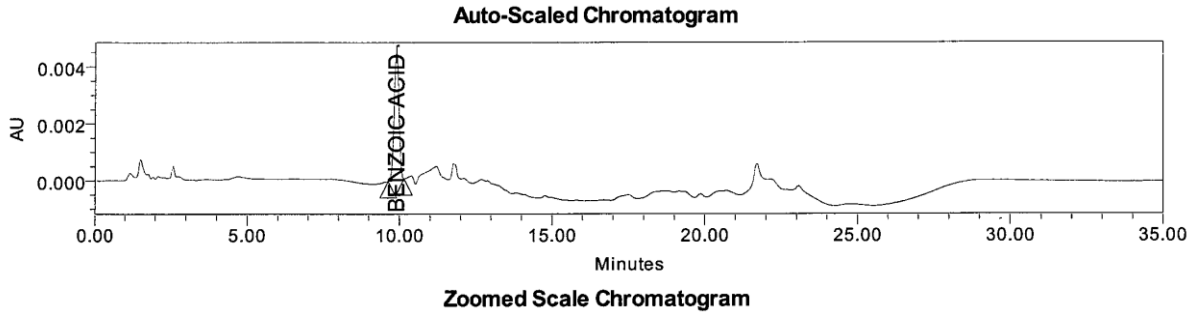


Fig-8: Detection Limit Preparation.

Quantitation Limit (QL): This is the measurement of lowest concentration of analyte that can be quantified with acceptable precision.

Preparation of QL solution: Based on the S/N ratio obtained from DL solution, derive QL concentration of Benzoic acid so as to get S/N ratio is about 10:1

Procedure: Inject QL solution and record the chromatogram.

Calculate the S/N ratio by using the software.

Acceptance Criteria: The S/N ratio should be about 10:1

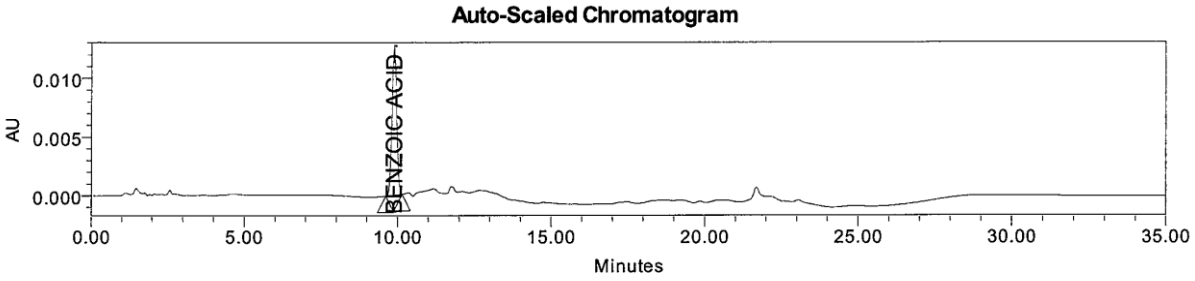


Fig-9: Quantification Limit Preparation.

Precision at QL: Inject QL solution for six replicates (six times) and record chromatograms. Calculate % RSD for the peak area of Benzoic acid obtained from six replicate injections of QL level.[9]

Acceptance criteria: The % RSD for the peak area of Benzoic acid obtained from six replicate injections of QL level should be not more than 15.0

Accuracy at QL:

Preparation of Test solution: Weigh accurately about 25.0 mg of test sample into 50 mL volumetric flask,

dissolve and dilute to the volume with diluent and mix.

Preparation of Accuracy at QL solution: Prepare the solution having 100% sample and spike the carryover impurities at QL level.

Note: Should prepare in duplicates.

Procedure: Prepare and inject test solution in single, Accuracy at QL level in duplicate and record the chromatograms. Calculate the % recovery of each impurity.

Acceptance criteria: The % recovery should be between 70 and 130 at QL level.

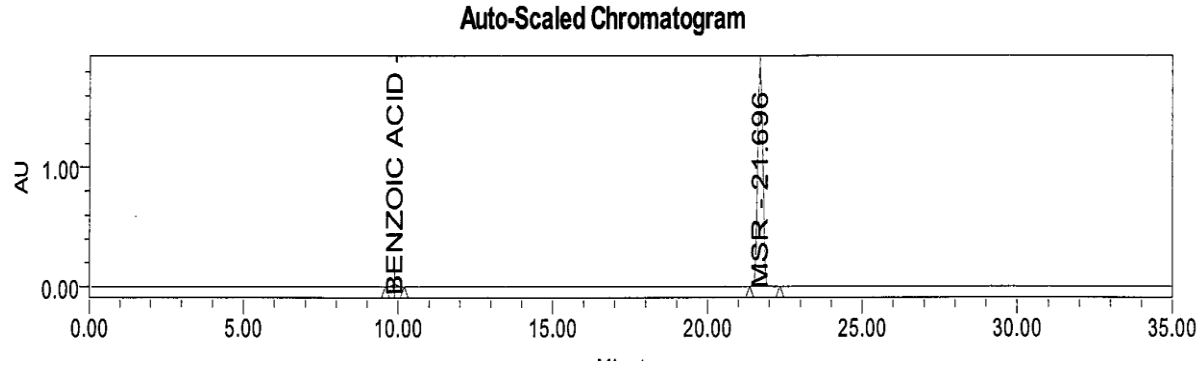


Fig-10: QL spike.

Linearity: Perform linearity with different concentrations of Benzoic acid by analyzing a minimum six concentrations i.e. QL, 50%, 75%, 100%, 125% and 150% w.r.to limit level.

Preparation of Linearity stock solution: Weigh accurately about each 5.0 mg of Benzoic acid standard into 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Linearity level -1 solution (QL): Prepare and inject QL solution in dupilcate. (or) Consider first two injections of Precision at QL.

Preparation of Linearity level -2 solution: Take 1.25 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -3 solution: Take 1.875 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -4 solution: Take 2.5 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -5 solution: Take 3.125 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -6 solution: Take 3.75 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Procedure:

Inject QL level to level-6 solution in duplicate and record the chromatograms. Plot linearity graph between average peak area of duplicate injections against concentration of Benzoic acid determine the correlation coefficient value for carryover impurities.

Acceptance Criteria: Correlation coefficient value should be not less than 0.99

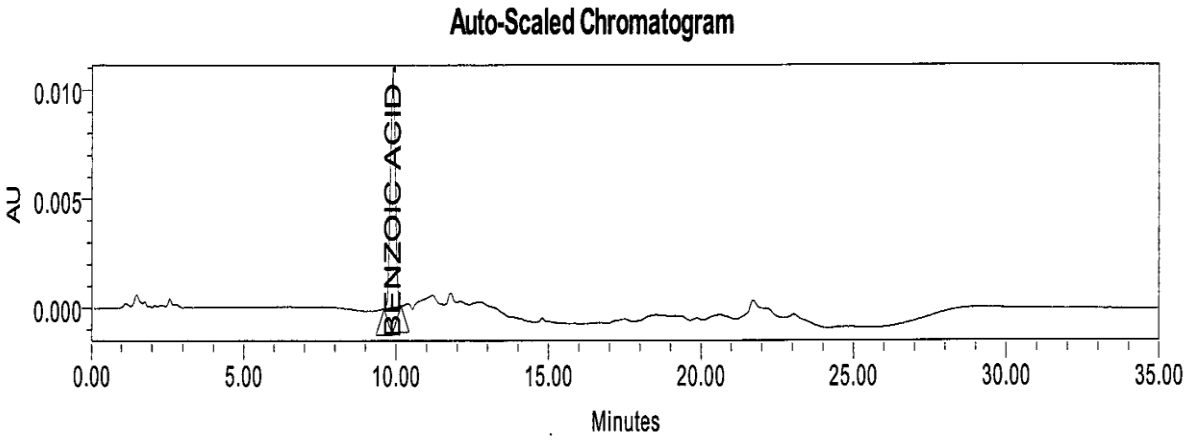


Fig-11: Linearity Preparation.

Accuracy:

Preparation of Accuracy stock solution: Weigh accurately about each 5.0 mg of Benzoic acid standard into 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Test solution: Weigh accurately about 25.0 mg of test sample into 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Accuracy at 50% level solution: Weigh accurately about 25.0 mg of test sample into 50 mL volumetric flask, add 1.25 mL of accuracy stock solution, dissolve and dilute to the volume with diluent.[10]

(This solution contains 0.50% Benzoic acid with respect to test solution concentration which covers the 50% of specification level)[15, 16]

Preparation of Accuracy at 100% level solution: Weigh accurately about 25.0 mg of test sample into 50 mL volumetric flask, add 2.50 mL of accuracy stock solution, dissolve and dilute to the volume with diluent.

(This solution contains 1.0% of Benzoic acid with respect to test solution concentration which covers the 100% of specification level)

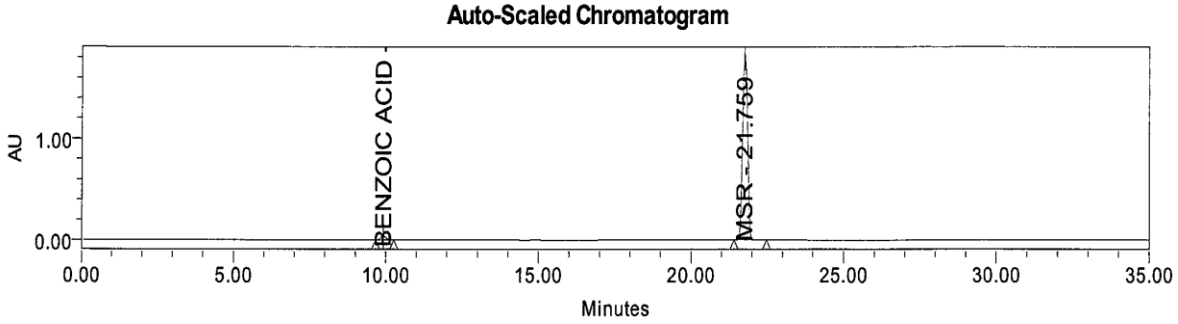


Fig-12: 100% spike recovery solution.

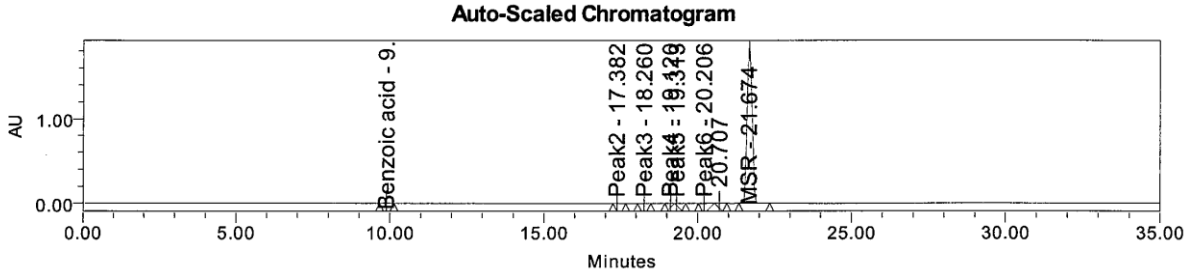


Fig-13: Batch Preparation.

Preparation of Accuracy at 150% level solution: Weigh accurately about 25.0 mg of test sample into 50 mL volumetric flask, add 3.75 mL of accuracy stock solution, dissolve and dilute to the volume with diluent.

(This solution contains 1.5% of Benzoic acid with respect to test solution concentration which covers the 150% of specification level)

Note: *Should prepare Accuracy at 50% and 150% levels in triplicate preparations and Accuracy at 100% level in six preparations. Use first three preparations of Accuracy at 100% level for accuracy study and total six preparations for method precision study.*[11, 12, 13, 14]

Procedure: Inject test solution in single, each accuracy at 50% and 150% levels in triplicate preparations and Accuracy at 100% level in six preparations. Calculate the % recovery of carryover impurity.

Acceptance criteria: The % recovery should be between 80 and 120 for 50%, 100% & 150% level.

Calculation for % Recovery:

$$\textbf{Benzoic acid (\%)} = \frac{AU}{AS} \times \frac{CS}{CU} \times P$$

% Recovery =

$$\frac{\text{Content in spiked sample (\%)} - \text{Content in test sample (\%)}}{\text{Concentration added (\%)}} \times 100$$

Where,

AU = Peak area of Benzoic acid in spiked test solution

AS = Average area of Benzoic acid in standard solution

CU = Concentration of spiked test solution (mg/mL)

CS = Concentration of Benzoic acid in standard solution (mg/mL)

P = Purity / Potency of Benzoic acid standard (%)

Method Precision: Perform the analysis by spiking the test sample with carryover impurities at specification level in six different preparations and determine the method precision.

Note: *The data from the Accuracy at 100% level six preparations can be used for method precision.*

Procedure: Prepare and inject spiked test sample in six different preparations into the system and record the chromatograms. (Consider the Accuracy at 100% level in six preparations) Calculate the ppm of Benzoic acid in each preparation as per the method.

Calculate the % RSD for the results obtained from the method precision study.

Acceptance criteria: The % RSD for the results obtained from method precision study should be not more than 10.0

Intermediate Precision: Carry out the precision study on a different day, with different instrument, different analyst and different column using with fresh preparations.

Procedure:

Establish the system suitability as per the method. Prepare and inject standard solution in six replicates and record the chromatograms. Prepare and inject spiked test sample solution in six preparations (which is analyzed under method precision study) and record the chromatograms. Calculate the ppm of Benzoic acid in each preparation as per the method. Calculate the % RSD for the results obtained from the intermediate precision study. Calculate the cumulative % RSD for the results obtained from method precision study and intermediate precision study.[17]

Acceptance criteria: System suitability should comply as per the method. The % RSD for the results obtained from intermediate precision study should be not more than 10.0. The cumulative % RSD for the results obtained from method precision study and intermediate precision study should be not more than 15.0

Conclusion:

Conclude from the above studies whether the method is valid and suitable for the content of Benzoic acid in Midostaurine by HPLC.

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