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Research

Analytical method validation for the determination of Acetic acid content in Decitabine API by Gas Chromatography

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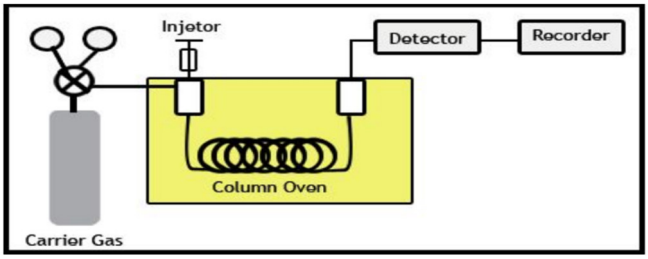
Abstract

The analysis of residual organic solvent acetic acid in Decitabine, an active pharmaceutical ingredient was investigated. Decitabine is nucleic acid synthesis inhibitor beta blocker used for the treatment of myelodysplastic syndromes, a class of conditions where certain blood cells are dysfunctional, and for acute myeloid leukemia (AML). The Auto sampler gas chromatography method described in this investigation utilized ZB-WAX Plus, 30 meter x 0.53 mm with 1 µm film thickness, Flow rate at 3.0 mL/minute, FID detector.. The injector temperature was set at 240ºC. Nitrogen was used as a carrier gas. The method was validated to be specific, linear, precise, sensitive, rugged and showed excellent recovery.

Keywords: myelodysplastic syndromes, Acute myeloid leukaemia (AML), FID detector

INTRODUCTION

Gas Chromatography is helpful for separation, identification and quantification of component compounds in a mixture. In contrast to the High-Performance Liquid Chromatography technique Gas Chromatography uses a gas as carrier mobile phase instead of a liquid. It is specially suited for compounds that are volatile, thermally stable and have low molecular weights[8].



Parts of Gas Chromatography

The carrier gas is led at a constant flow rate to the column packed with the stationary phase. Before entering the column the sample mixture (gas or liquid) is injected into the carrier gass stream. The liquid mixture is vaporized to the gaseous state by the high temperature inside the injector before being led to the column. On reaching the column the sample components retained on the basis of physico–chemical interactions between the analytes molecules and the stationary phase. The mobile gas stream moving at a steady rate elutes the mixture components in a sequence determined by the operating conditions. Different detectors are employed for detection and quantification of eluted compounds. You will now be introduced to the significant role of each part of the GC system[9].

Gases play a crucial role in a Gas Chromatography system.

• Transportation of injected sample to the column and subsequent transfer of separated components to the detector • Act as fuel to support combustion in the detector

• Support combustion process in the flame when using flame ionization detection Colour coding of gases

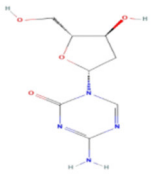
Carrier Gas: A carrier gas facilitates transport of the injected sample to the column for separation of components of the mixture. Typical purity levels should be 99.995%or higher. Desirable features of carrier gas are: Inertness towards sample and stationary phase should not have a response on the detector, commonly used gases are Nitrogen, Helium and Argon. Helium gas is preferred as carrier with TCD detector because of high explosion hazard of hydrogen. Traps are recommended in the gaslines to prevent moisture or other impurities from reaching the columns[16].

Analytical Method Validation: The following are all included in the method validation research.

System Suitability: Method development and validation are followed by the formulation of system appropriateness requirements.

Performance calculations: To obtain the total system performance, compute the subsequent values of Relative retention, Theoretical plates, Capacity factor, Resolution, Peak asymmetry, Plates per meter, The parameters that were used to determine these system performance metrics for the separation of two chromatographic components are included in the following data and the validation parameters are Linearity, Precision, Repeatability, Intermediate precision, Reproducibility, Accuracy, Specificity/selectivity, Limit of Detection (Based on Visual Evaluation, Based on Signal-to-Noise, Based on the Standard Deviation of the Response and the Slope), Limit of Quantification, Based on Visual Evaluation (Based on Signal-to-Noise Approach, Based on the Standard Deviation of the Response and the Slope), Robustness, Ruggedness.

DRUG PROFILE: Decitabine Molecular Formula is $C_8H_{12}N_4O_4$



MATERIALS & METHOD:

Gas Chromatography with Auto liquid sampler and FID detector, Analytical Balance, Glassware Class-A, **Column:** DB-WAX plus, 30 meter x 0.53 mm with 1.0 µm film thickness or equivalent. Chemicals, Standards & Sample**:** Dimethyl sulphoxide, Acetic acid, Carrier Gas-Nitrogen, Detector- FID, Diluent-Dimethyl sulphoxide

Preparation of Acetic acid Standard Stock Solution: Weigh and transfer about 1000 mg of Acetic acid into 100 mL volumetric flask containing 50 mL of diluent and make up to the mark with diluent.
Preparation of Acetic acid Standard Solution: Take 2.5 mL of above solution into 100 mL volumetric flask and make up to the mark with diluent.
Preparation of Test Solution: (Test preparation in duplicate) Weigh and transfer about 250 mg sample into 5 mL volumetric flask and make up to the mark with diluent.
Procedure
The sequence shall be in the below provisional manner:

S.No	Name of the solution	No of Injections
1	Blank	02
2	Standard solution	06
3	Test solution Preparation-1	01
4	Test solution Preparation-2	01
5	Blank	01
6	Standard solution(Bracketing)	01

- standard solution should be not more than 15.0
- The overall % RSD for peak area of Acetic acid from replicate injections of standard solution and bracketing standard solution should be not more than 15.0

S.No	Validation Parameters
1	System Suitability
2	Specificity
3	Precision i) System Precision ii) Method Precision (repeatability)
4	Limit of Detection and Limit of Quantification i) Establishment of Limit of Detection and Limit of Quantification ii) Precision at Limit of Detection and Limit of Quantification
5	Linearity
6	Accuracy

Note: More than one parameter can be performed at once with relevant sequence having common system suitability with bracketing standard preparations.

Method validation results: System suitability: Injected six replicate injections of standard solution into the chromatographic system as per the test method and evaluated the system suitability parameters.

Results: System suitability

Parameter	% RSD for the peak area of Acetic acid
% RSD for Peak Area	2.8

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0, The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0
Conclusion: The above results reveal that the system meets the required system suitability criteria.
Specificity**:** Blank interference: Injected blank solution and checked for the peak interferences found due to blank at the retention time of acetic acid peak.
System suitability:

Parameter	**% RSD for the peak area of Acetic acid**
% RSD for Peak Area	2.8
% RSD for bracketing standard-1	2.9

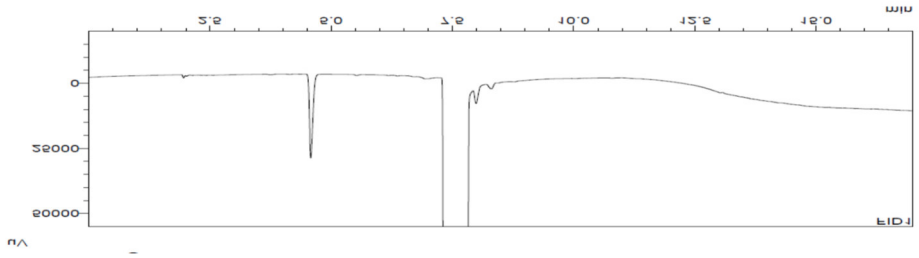
Results of Blank interference:

Sample Name	**Peak found at the RT of Acetic acid peak (Yes/NO)**
Blank Solution	No

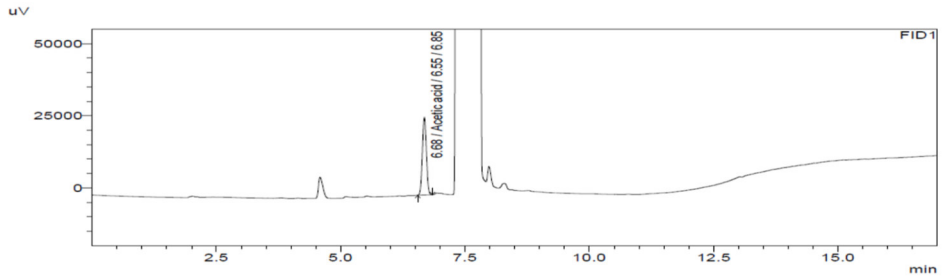
Solvents Interference: Injected individual acetic acid standard solution and spiked sample solution at specification level and evaluated the Interference of other solvents.

Results of solvent Interference:

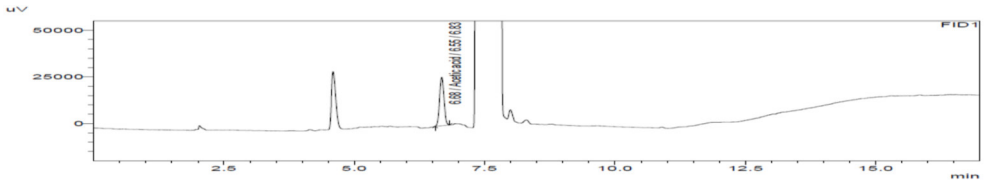
Name of the solvents	**Retention time of acetic acid peak from individual injection (In minutes)**	**Retention time from spiked sample solution (In minutes)**
Acetic acid	6.68	6.64



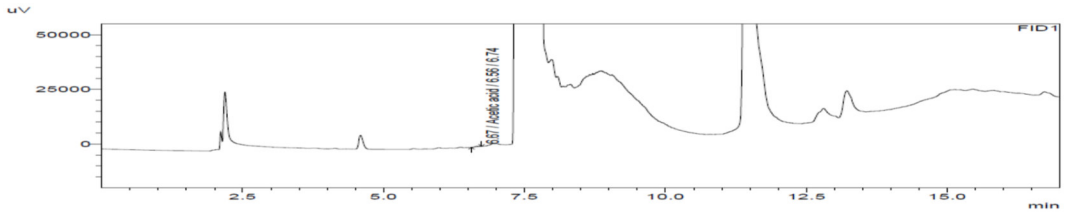
Typical chromatogram of Blank Solution



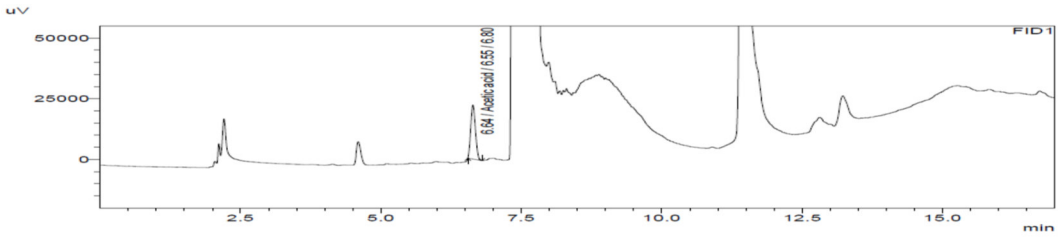
Typical chromatogram of Standard Solution



Typical chromatogram of Acetic acid



Typical chromatogram of as such sample solution



Typical chromatogram of Spiked Sample Solution

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0, The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0, The blank peak should not show any interference at the retention time of the solvent peak in the standard and sample solution.
Conclusion: No interference was observed from the blank at the retention time of Acetic acid in the standard and sample solution; hence the above results reveal that the method is specific.
System precision: Injected six replicate injections of standard solution into the chromatographic system as per the test method and evaluated the System precision parameter.

Results: System suitability

Parameter	**% RSD for the peak area of Acetic acid**
% RSD for Peak Area	2.8

System Precision results for %RSD of Acetic acid Area:

Injection No.	**Acetic acid peak area**
1	149720
2	142769
3	140608
4	141456
5	149725

6	144239
Mean	**144753**
%RSD	**2.8**

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0, The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0

Method precision: Determined the precision of the test method by injecting six samples by spiking the solvent at specification level as per the test method.

Results: System suitability

Parameter	**% RSD for the peak area of Acetic acid**
% RSD for Peak Area	2.8
% RSD for bracketing standard	2.6

Method precision Results (Acetic acid content in ppm):

S.No.	**Acetic acid content (ppm)**
Prep-1	4497.97
Prep-2	4775.95
Prep-3	4853.85
Prep-4	4904.61
Prep-5	4534.66
Prep-6	4500.72
Mean	**4677.46**
%RSD	**4.0**

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0, The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0

- The % RSD for the Acetic acid content from the six preparations of the method precision solutions should be not more than 15.0.

Conclusion: The above results reveal that the method is precise.

Limit of detection and Limit of Quantification: Injected the known concentration of all solvent and evaluated the LOD and LOQ concentration based on S/N ratio.

Results: System suitability

Parameter	**% RSD for the peak area of Acetic acid**
% RSD for Peak Area	3.0
% RSD for bracketing standard	2.8

LOD & LOQ Results:

Name of the Solvent	LOD(ppm)	S/N Ratio	LOQ(ppm)	S/N Ratio
Acetic acid	32.92	5	99.75	10

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0, The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0, The S/N ratio should be not less than 3 and not less than 10 for LOD and LOQ respectively.

Precision at Limit of Detection and Limit of Quantification Limit of Quantification
Injected six replicate injections of LOQ & LOD solutions into the chromatographic system asper the test method and evaluated the precision at LOQ & LOD levels.

Results: System suitability

Parameter	% RSD for the peak area of Acetic acid
% RSD for Peak Area	1.9
% RSD for bracketing standard	1.8
% RSD for bracketing standard	1.8

LOD & LOQ precision Results (Acetic acid content in ppm):

No. of Injections	LOD precision	LOQ precision
1	17.10	82.05
2	22.51	84.38
3	19.47	85.61
4	19.65	92.80
5	22.61	72.66
6	24.95	71.61
Mean	21.05	81.52
%RSD	13.4	10.0

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0,The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0,The % RSD for the Acetic acid content from the six injections of the LOD precision solutions should be not more than 33.0., The % RSD for the Acetic acid content from the six injections of the LOQ precision solutions should be not more than 15.0.
Conclusion: The above results reveal that the method is precise at LOD & LOQ level.

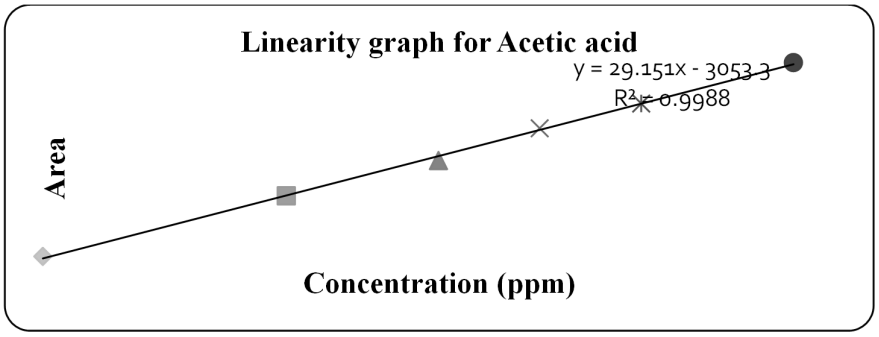
Linearity: Injected Linearity solutions from LOQ to 150% of Specification limit into the chromatographic system as per the test method and evaluated the Correlation Co-efficient.

Results: System suitability

Parameter	% RSD for the peak area of Acetic acid
% RSD for Peak Area	1.9
% RSD for bracketing standard	1.9

Linearity Solutions Results:

Linearity Levels	Acetic acid Content in ppm	Acetic acid Area
LOQ	99.75	2019
50%	2493.75	69098
80%	3989.99	108276
100%	4987.49	144008
120%	5984.99	171408
150%	7481.24	216743
Correlation Coefficient	**0.9994**	



Linearity Graph for Acetic Acid:

Acceptance Criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0, the overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0, The Correlation coefficient should be not less than 0.99.

Conclusion: The above results reveal that the method is Linear from LOQ to 150% level.
Accuracy: Prepared Recovery samples by spiking each solvent at LOQ, 50%, 100%,& 150% of Specification level on sample and injected into the chromatographic system as per the test method and calculated the %individual recovery and %mean recovery at each level.

Results: System suitability

Parameter	% RSD for the peak area of Acetic acid
% RSD for Peak Area	2.8
% RSD for bracketing standard	2.6

Table 25: Acetic acid Accuracy Results: (In ppm)

Sample No.	Spike Level	Concentration found	Concentration added	Individual % Recovery	Mean % Recovery	% RSD
1	LOQ%	74.25	99.99	74.3	80.7	8.4
2	LOQ%	87.82	99.99	87.8		
3	LOQ%	79.97	99.99	80.0		

1	50%	2361.73	2496.49	94.6	93.5	2.3
2	50%	2274.87	2496.49	91.1		
3	50%	2370.29	2496.49	94.9		
1	100%	4494.97	4992.98	90.0	94.3	4.0
2	100%	4775.95	4992.98	95.7		
3	100%	4853.85	4992.98	97.2		
1	150%	7421.21	7489.46	99.1	100.2	1.7
2	150%	7444.98	7489.46	99.4		
3	150%	7651.14	7489.46	102.2		

Acceptance criteria: The % RSD for the peak area of Acetic acid in six replicate injections of standard solution should be not more than 15.0,The overall % RSD for peak area of Acetic acid in replicate injections of standard solution and bracketing standard solution should be not more than 15.0,Individual % recovery and mean % recovery value for each level should be in between 80 to 120.,Individual % recovery and mean % recovery value at LOQ level should be in between 70 to 130., The % RSD for % recovery at each level should be not more than 15 %.

Conclusion: The present analytical method was verified as per defined protocol and it meets the specified acceptance criteria. Hence, it was concluded that the analytical method is specific, precise and accurate. Hence, the present analytical method can be used for regular analysis and its intended purpose.

CONCLUSION:

The current analytical method was validated according to the protocol, and it passes the acceptance criteria. Thus, it was determined that the analytical approach is particular, precise, linear, accurate, rugged, and robust. As a result, the current analytical approach is suitable for regular analysis and serves its intended function

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