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A NOVEL ASSAY METHOD OF 1-DEOXY NOJIRIMYCINE (DNJ) BY ION CHROMATOGRAPHY

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Key words:

1-Deoxynijiromycine, Ion Chromatography, Conductivity Detector, Method development, Method validation, UPLC-TOF

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ABSTRACT: A novel Assay method for the determinationof1-Deoxy nojirimycine(DNJ) was proposed by the authors using ion chromatography. The method was found to be accurate, simple and precise. A method precision RSD of 0.5%, RSD for intermediate precision of 0.6% and correlation coefficient was found to be 0.9999. A recovery of 99.7 to 100.1% at 50%, 100% and 150% levels was found. The DNJ solutions and mobile phase were found to be stable up to 48 hrs. The robustness study was found that the method was robust at low flow, high flow, low strength and high strength. Specificity of DNJ with other cations and organic bases were not interfered. The peak of DNJ was homogeneous, this specificity of peak homogeneity study was performed with UPLC-TOF by collecting peak of DNJ from detector end of Ion chromatography. All analytical parameters were performed by using ICH guidelines.

INTRODUCTION: 1-Deoxynojirimycin (DNJ) is the natural product which is located in Mulberry (*Morus alba* L.; Moraceae) plants and which is present in Asian countries like India, China, Japan, Thailand, Korea. Which is most potent glycosidase inhibitor, the enzyme from decomposing starch and sugar and preventing the glucose absorption, resulting in decrease of blood sugar level, and the Medicine like Miglitol, Miglustat are the drugs which are function as this, these can synthesized from 1-Deoxynojirimycin (DNJ), this is acting as Key starting material for miglitol and miglustat which lack of chromophore in its molecule and therefore difficult to be detected by HPLC-UV analysis and another difficulty of DNJ analysis is the separation method as DNJ is highly polar. The interaction between the stationary phase of reversed phase columns and DNJ is so weak that DNJ does not retain in the column.

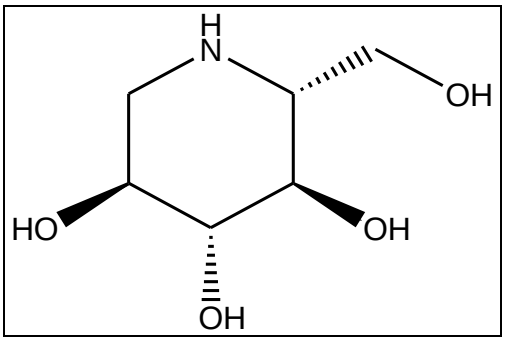

FIG. 1: CHEMICAL STRUCTURE OF 1-DEOXYNOJIRIMYCINE (DNJ)

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Other HPLC, UPLC, LC-MS and GC-MS methods helps to determine the DNJ, The content of 1-Deoxynojirimycin (DNJ) in mulberry resources was determined by HPLC with an evaporative light scattering detector (ELSD) [1]. A simple, selective, and rapid method of high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) to quantify DNJ in mulberry-based food products was developed [2]. A method of pre-column derivatization with FMOC-Cl followed by RP-HPLC with UV detection was established for the determination of 1-Deoxynojirimycin (DNJ) content in different varieties and sites of mulberry leaves [3]. A Sensitive UPLC Method Development and Validation with LC-MS Compatible for the determination of 1-Deoxynojirimycin in Mulberry Leaves using Fluorescence Detection [4]. HPLC with evaporative light scattering detector (ELSD) using external standard calibration, With the use of evaporative Light Scattering detector (ELSD), an SUPELCOSIL LC-NH2(25 cm×4.6 mm5 μm) column [5], HPLC-ELSD method for impurity test of miglitol and 1-Deoxynojirimycin (1-DNJ) [6]. HPLC-MS/MS method was developed for the quantitative determination of 1-Deoxynojirimycin (DNJ) [7]. Method development was done according to ICH Q2(R1]. The present work on assay method development and method validation on 1-Deoxynojirimycin (DNJ) by ion Chromatography, which can be used determine the assay of 1 -Deoxynojirimycin (DNJ).

MATERIAL AND METHOD:

Chemicals & Reagents:

Analytical grade tartaric acid and 1,4-Dipicholinic acid were purchased from S.D. fine chemicals Mumbai, India. Analytical reagent grade acetonitrile was purchased from Merck Mumbai, India, High purity water was collected from a Millipore Milli-Q Water purification system (Millipore, Milford, MA, USA). Analytical grade the DNJ for research was obtained from Dr. Reddy's laboratories Ltd, Hyderabad India.

Equipments:

The ion chromatography system purchased from Metrohm AG, Switzerland used throughout this study, which is equipped with 858 Professional sampleprocessor,882 IC Pump, sampling injector with 20μL loop, 820 IC separation centre equipped with conductivity detector. Quantitation was performed from output signal, monitored and processed using the MagIC Net version 2.1 software on Compaq computer (Digital Equipment Co. Dilutions were accomplished with Hamilton precision pipettes (Bondaiz Switzerland. Peak homogeneity was done in Waters UPLC-TOF with LCT Premier XE Mass Lynx [TM] software.

RESULTS AND DISCUSSION:

Method development and optimization:

The main objective of this was to develop assay method for 1-Deoxynijorimycine in bulk levels of 1-Deoxy nojirimycine which can be used for the key starting material for Miglitol and Miglustat active pharmaceutical ingredients by using ion chromatography with conductivity detector.

During development of assay method for DNJ, the concentration of peak in ion chromatography was established, the concentration was 10 %w/w standard solution with respect to 1 mg/mL of analyte concentration, the standard solution was injected into Ion chromatography conductivity detector with column Metrocep –C2, 150mm and mobile phase was 10% Acetone with 4.0 mM Nitric acid the peak of DNJ eluted at 3.0 min retention time. Same condition with Metrocep -C4, 250 mm column then the Peak shape was the not sharp, The flow was 1.0 mL, the another condition was the 4 mM tartaric acid and 0.75mM Dipicholinic acid in Water with Metrocep -2,150mm column and the flow was 1.0 mL, peak shape was not sharp and with Metrosep -4, 250mm column and the flow was 1.0 mL, peak shape was sharp and with resolution with other cations and bases were more than 2.0. Retention time was 7.5 minutes, the optimized method condition were tabulated in **Table 1.**

Chromatographic conditions:

Analyses were performed on Metrohm AG (Switzerland), consisting of a Auto sampler as 585 Professional sample processor and Column compartment and Conductivity detector as as882 Compact IC plus. System control, data collection and data processing were accomplished using MagIC Net chromatography data software. The chromatographic condition was optimized using

Metrocep C4 250/4.0,5.0µmcolumn. 4 mM tartaric acid and 0.75mM Dipicholinic acid in Water as buffer. Filtered the solution through 0.22µ membrane filter and degas by sonication and mix with 10% acetonitrile as a mobile phase. Mobilephase was filtered through 0.22 µm nylon membrane filter and degassed under vacuum prior to use. The separation of DNJ was achieved by isocratic elution using the above mobile phase (**Table 1**). Water was used as a diluent. The finally selected and optimized conditions were as follows: injection volume 20 µL, isocratic elution (**Table 1),** at a flow rate of 1.0mL/min. Under these conditions.

Preparation of DNJ Standard Stock Solutions:
Weigh accurately and transfer about 10 mg of DNJ working standard into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well.

Sample Preparation:
Weigh and transfer about 10 mg DNJ Lab samples into a 100 mL volumetric flask. Add about 70 mL of diluent, sonicate for 60 seconds. Dilute to volume with diluent and mix well.

TABLE 1: SUMMARIZED OPTIMIZED CONDITIONS

Buffer	:4 mM Tartaric acid and 0.75mM Dipicholinic acid in Water
Mobilephase	:Buffer: ACN(90:10%v/v)
Diluent	:Water
Flow	:1.0 mL/min
Inj. Vol	:20µL
Column	: Metrocep C4 250/4.0,5.0µm
Run Time	: 25.0 min.
Detection	: Conductivity

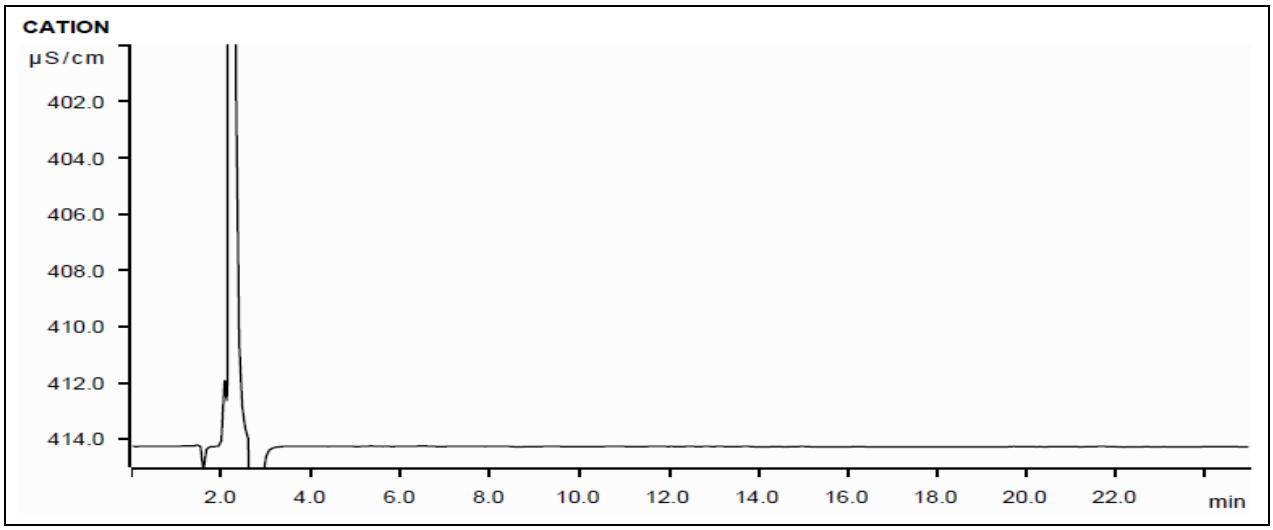


FIG.2: CHROMATOGRAM OF BLANK SOLUTION

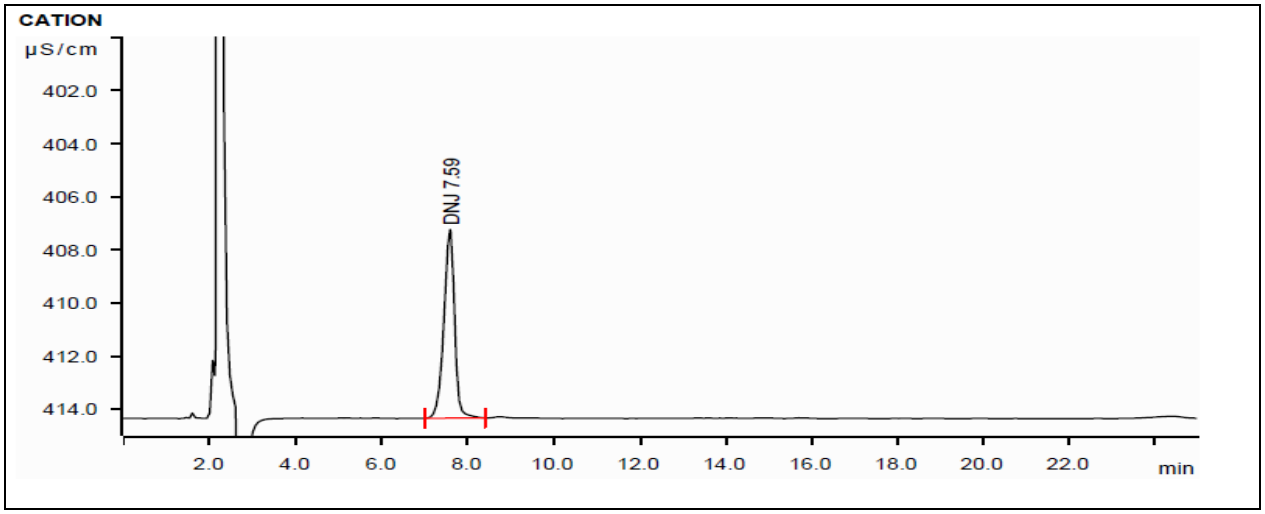


FIG.3: CHROMATOGRAM OF STANDARD SOLUTION

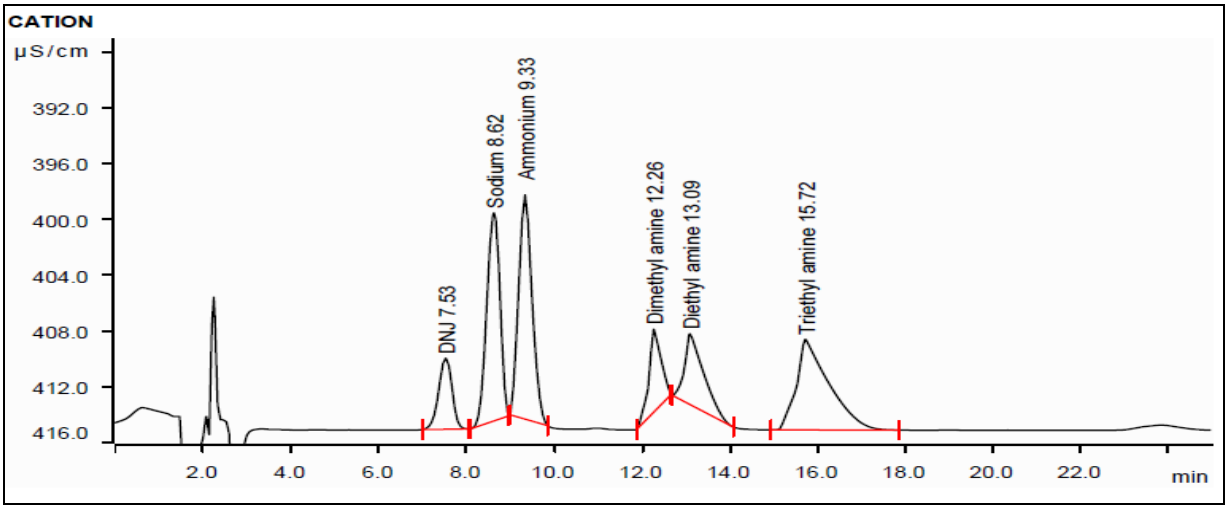


FIG.4: CHROMATOGRAM OF SPECIFICITY WITH DNJ SOLUTION

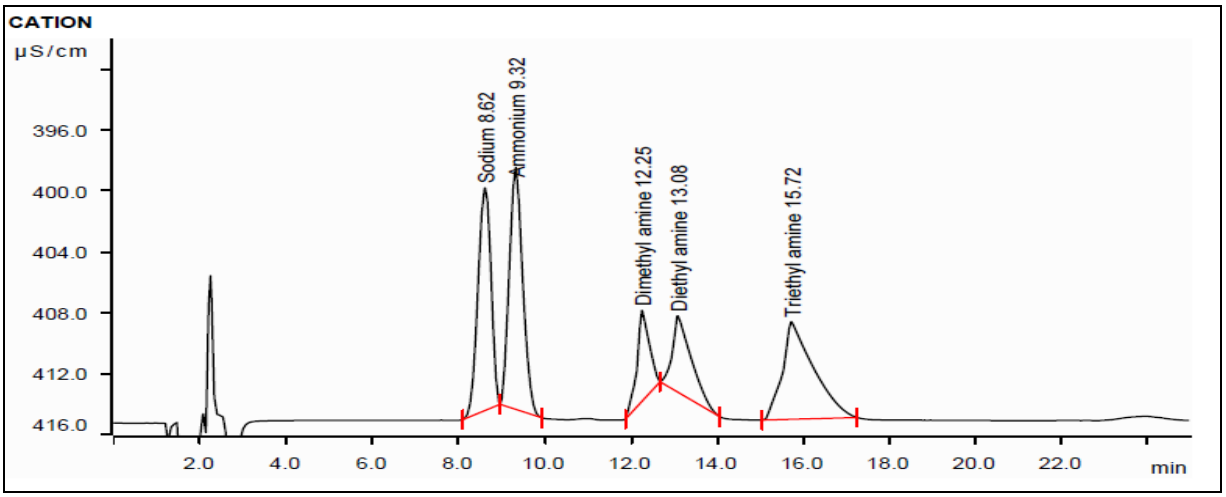


FIG.5: CHROMATOGRAM OF SPECIFICITY WITHOUT DNJ SOLUTION

Analytical parameters and validation:

After satisfactory development of method it was subjected to method validation as per ICH guideline [8]. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, accuracy, precision, linearity, robustness, ruggedness, solution stability and specificity).

Precision:

Instrument precision: (Suitability of system):

System suitability parameters were measured so as to verify the system performance. System precision was determined on five replicate injections of standard preparation. The percentage RSD of area counts of five replicate injections was 0.4 %, which indicates that the system is precise. The results obtained were shown in **Table 2**. The parameters all complied with the acceptance criteria and system suitability was established.

TABLE 2: RESULTS OF SUITABILITY OF SYSTEM

Concentration	10.06(%w/w)
No of injections	**DNJ Area**
Injection -1	2.050
Injection -2	2.039
Injection -3	2.029
Injection -4	2.035
Injection -5	2.044
Average	2.039
STDEV	0.01
%RSD	**0.4**

Method precision: (Repeatability)

The precision of the assay method was evaluated by carrying out six independent determinations of DNJ test samples against qualified working standard. The method precision study shows the repeatability of the results obtained by the testing

method. The % RSD (n=6) was 0.5 %. The results obtained are shown in **Table 3**.

TABLE 3: RESULTS OF METHOD PRECISION

Preparation	DNJ Area	Concentrations(%w/w)
Preparation-1	2.0558	10.06
Preparation-2	2.0418	10.08
Preparation-3	2.0302	10.07
Preparation-4	2.0577	10.06
Preparation-5	2.0360	10.07
Preparation-6	2.0464	10.04
Average	2.0447	
STDEV	0.01	
%RSD	**0.5**	

Intermediate precision: (Reproducibility):

The purpose of this study is to demonstrate the reliability of the test results with variations. The reproducibility was checked by analyzing the samples by different analyst using different chromatographic system and column on different day. The analysis was conducted in the same manner as the method precision and the % RSD of all six sets of sample preparations was determined (**Table 4**). The % RSD was 0.6 % so this study proved that the method to be rugged enough for day to day use (**Table 4**).

TABLE4: RESULTS OF INTERMEDIATE PRECISION

Preparation	DNJ area	Concentrations(%w/w)
Preparation-1	1.792	10.21
Preparation-2	1.769	10.25
Preparation-3	1.771	10.24
Preparation-4	1.792	10.28
Preparation-5	1.768	10.35
Preparation-6	1.780	10.47
Average	1.779	
STDEV	0.01	
%RSD	**0.6**	

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique. The accuracy of the method was carried out by adding known amounts to three concentration levels; 50, 100, and 150% of DNJ in triplicate. The samples were given the same treatment as described in sample preparation. The percentage recoveries of DNJ at each level and each replicate were determined. The mean of percentage recoveries (n=3) and the relative standard deviation was calculated. The amount recovered was within ± 1 % of amount added, which indicates that there is no interference due to cations and organic basic impurities. It was confirmed from results that the method is highly accurate (**Table 5**).

TABLE5: RESULTS OF ACCURACY

Average area from MP	2.0447		
50% DNJ	**Area**	**Assay(%w/w)**	**Concentrations(%w/w)**
Area in Prep-1	1.0397	100.4	5.10
Area in Prep-2	1.0417	100.5	5.10
Area in Prep-3	1.0416	100.8	5.09
Average assay		100.6	
STDEV		0.2	
% RSD		0.2	
% Recovery		**100.1**	
100% DNJ	**Area**	**Assay(%w/w)**	**Concentrations(%w/w)**
Area in Prep-1	2.0558	100.4	10.07

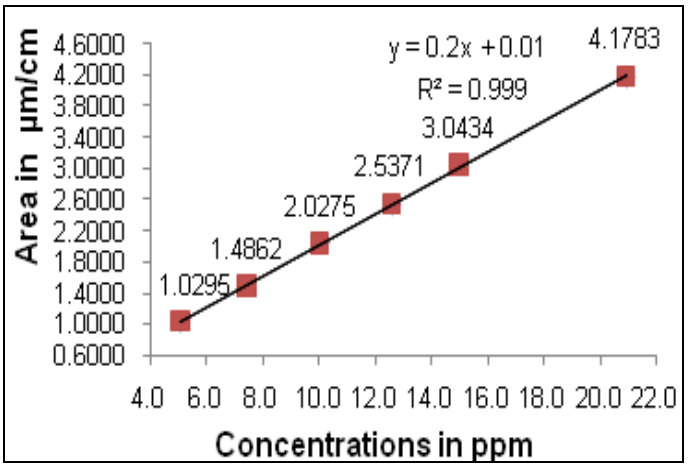
Area in Prep-2	2.0418	100.3	10.02
Area in Prep-3	2.0302	100.0	9.99
Average assay		100.2	
STDEV		0.2	
% RSD		0.2	
% Recovery		**99.7**	
150% DNJ	**Area**	**Assay(%w/w)**	**Concentrations(%w/w)**
Area in Prep-1	3.0298	100.1	14.90
Area in Prep-2	3.0351	100.2	14.91
Area in Prep-3	3.0533	100.2	15.00
Average assay		100.2	
STDEV		0.1	
% RSD		0.1	
% Recovery		**99.7**	

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. The nominal concentrations of standard solutions for DNJ at six different concentration levels ranging from 50 to200% of analyte concentration. The DNJ correlation coefficient was greater than 0.999 %intercept at 100%level was 0.7%. The regression statistics are shown in **Table 6** and **Plot 1.**

TABLE6: RESULTS OF LINEARITY

Concentrations(%w/w)	DNJ Area
5.1	1.0295
7.5	1.4862
10.1	2.0275
12.6	2.5371
15.0	3.0434
21.0	4.1783
Slope	0.2
Intercept	0.01
Correlation coefficient	**0.9999**
% Intercept at 100%	**0.7**



PLOT 1: LINEARITY PLOT OF DNJ

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The effect of change in flow rate (± 0.2mL/min) and strength of Mobile phase 10% of buffer less than normal condition and 10%more than normal condition. The robustness results were shown in **Table 7.**

TABLE 7: CONDITION.0.8mL

Concentration(%w/w)	**10.24**
Preparation	**DNJ Area**
Preparation-1	2.212
Preparation-2	2.200
Preparation-3	2.212
Preparation-4	2.172
Preparation-5	2.211
Average	2.201
STDEV	0.02
%RSD	**0.8**

Test solution			
Preparation	**Area**	**Assay**	**Weight in mg**
Test solution-1	2.219	100.7	10.254
Test solution-2	2.207	101.4	10.125
	Average Assay	101.0	

CONDITION.1.2 mL

Concentration(%w/w)	**10.24**
Preparation	**DNJ**
Preparation-1	1.473
Preparation-2	1.495
Preparation-3	1.472
Preparation-4	1.483
Preparation-5	1.459
Average	1.476
STDEV	0.01
%RSD	**0.9**

Test solution Preparation	Area	Assay	Weight in mg
Test solution-1	1.446	99.1	10.125
Test solution-2	1.478	101.0	10.154
	Average Assay	100.0	
STDEV	0.00		
%RSD	**0.1**		

Test sample

Preparation	DNJ	Assay (%W/W)	Concentration (%w/w)
Area in Prep-1	2.7407	100.2	10.05
Area in Prep-2	2.7422	100.7	10.07
Average		100.5	

CONDITION HIGH STRENGTH BUFFER

Preparation	DNJ	Concentration (%w/w)
Preparation-1	2.0548	**10.03**
Average	2.0548	

Test sample

Preparation	DNJ	Assay(%W/W)	Concentration(%w/w)
Area in Prep-1	2.0647	100.5	**10.03**

CONDITION. LOW STRENGTH BUFFER

Preparation	DNJ	Concentration (%w/w)
Preparation-1	2.7280	10.02
Preparation-2	2.7243	
Average	2.7262	

Stability of sample in diluent and mobile phase:

Stability of sample solution was established by storage of sample solution at ambient temperature (25°C) for 48h and at same mobile phase,assay was determined for the DNJ, compared against fresh sample. Sample solution did not show any appreciable change in assay value when stored at ambient temperature up to 48h, the difference of fresh and old sample difference is -0.8 and -0.2 of solution stability and mobile phase stability respectively. Which were shown in **Table 8**.

TABLE 8: RESULTS OF SOLUTION STABILITY AND MOBILE PHASE STABILITY

100% Test sample of DNJ	Area	Weight in mg
Area in Prep-1	2.0558	10.011 mg
Area in Prep-2	2.0418	
Area in Prep-3	2.0302	
Average	2.0426	
STDEV	0.0	
%RSD	0.6	
Solution stability sample of after 48 hrs DNJ	**Area**	**Weight in mg**
Area in Prep-1	2.0395	10.025 mg
Assay(%W/W)	99.7	
Difference	**-0.8**	
Mobile phase stability sample of after 48 hrs DNJ	**Area**	**Weight in mg**
Area in Prep-1	2.0513	10.025 mg
Assay(%W/W)	100.3	
Difference	**-0.2**	

Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its impurities, because of the conductivity detector, DNJ shows specificity the cations and organic bases ,assay was not vary in presence of all, this is present in **Table 9. Fig. 2** and **3** are shows that there is no any interferences, and peak homogeneity was done by collecting the DNJ peak from Ion chromatography detector end and injecting into UPLC-TOL, Peak homogeneity spectrum with single 164(M+H) and Formulae of DNJ were shown in **Fig. 6** and **7**.

TABLE9:RESULTS OF SPECIFICITY

Concentration	10.06(%w/w)		
No of injections	**DNJ**		
Injection -1	1.799		
Injection -2	1.780		
Injection -3	1.778		
Injection -4	1.786		
Injection -5	1.793		
Average	1.787		
STDEV	0.01		
%RSD	**0.5**		
Test solution Preparation	**Area**	**Assay**	**Weight in mg**
Test solution-1	1.8116	101.7	10.025

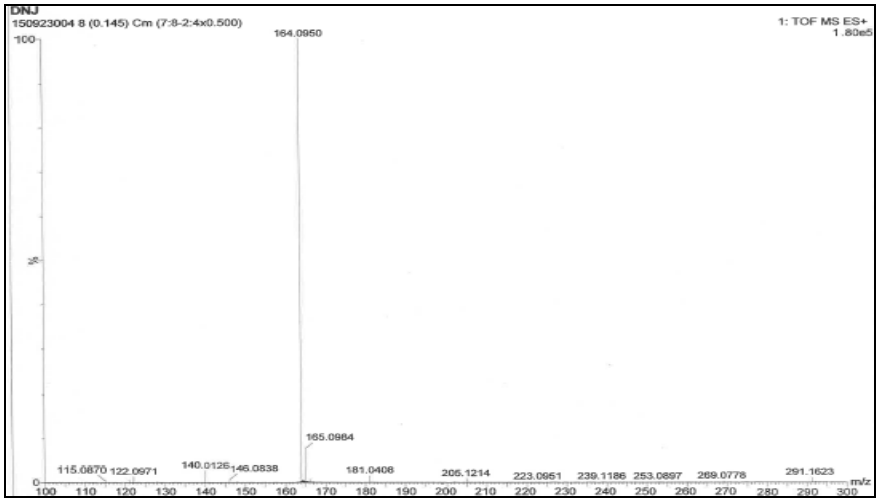


FIG.6: PEAK HOMOGENEITY SPECTRUM OF DNJ

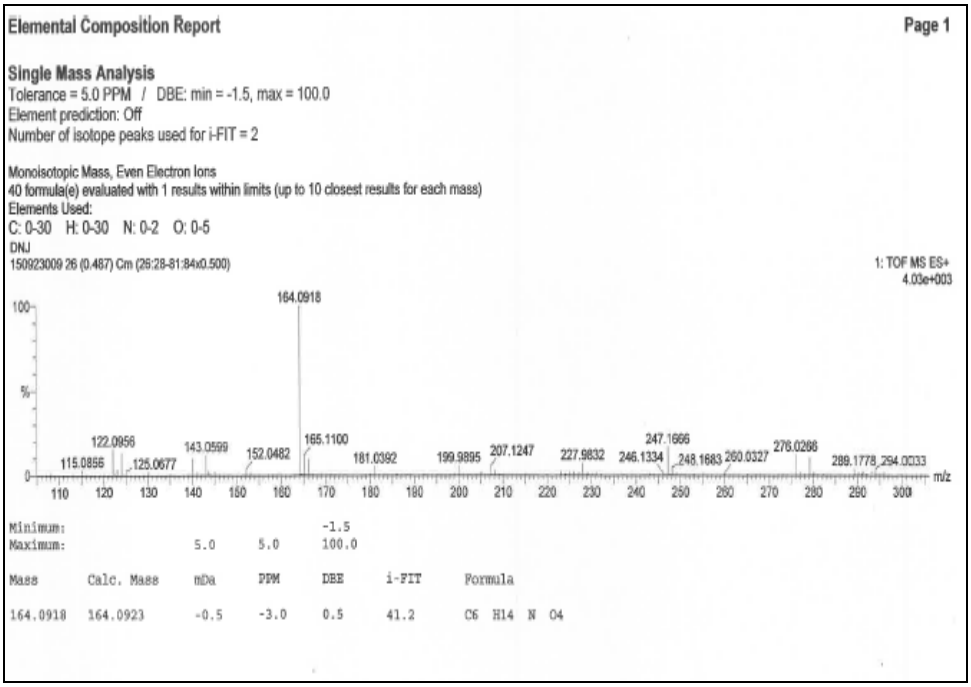


FIG.7: FORMULAE OF DNJ HR-MS

Batch Analysis:

The lab batches assay was determined by using the present validated method against to reference standard this was shown in **Table 10.**

TABLE 10: RESULTS OF BATCH ANALYSIS

Batch Name	Area	Assay	weight in mg
DNJ A	1.790	100.4	10.211
DNJ B	1.792	100.7	10.201
DNJ C	1.794	100.4	10.241
DNJ D	1.781	99.9	10.210
DNJ E	1.788	100.4	10.205

CONCLUSION: Assay method of 1-Deoxy nojirimycine (DNJ) by Ion Chromatograpy method was successfully developed for the assay determination of DNJ in bulk quantities and lab synthesized batches. The developed method is selective, precise, accurate, linear, and robust. The method is specific for the analytes and free from the interference of all cations and other organic bases. The run time (25.0) enables for rapid determination of DNJ and Peak Homogeneity by mass spectrometry confirms that no synthetic impurities co-eluting with DNJ. This is first method to determine the DNJ without derivatization. Moreover, it may be applied for content of DNJ in Miglitol and Miglustat APIs.

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