Evaluation of N-Nitrosodibutylamine a Genotoxic Impurity in Labetalol Drug Substance by GC-MS Technique

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Abstract—**During the analysis of the pharmaceutical drug Labetalol, an unidentified impurity was detected in the final active pharmaceutical ingredient (API). The impurity was suspected to originate from the presence of a secondary amine or from intermediates formed during different stages of the synthetic process used for Labetalol production. The unknown impurity was successfully isolated and comprehensively characterized using advanced analytical techniques, including Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), Liquid Chromatography–Mass Spectrometry (LC–MS), and Gas Chromatography (GC). Structural characterization confirmed the impurity as N-Nitrosodibutylamine (NDBA). Consequently, a sensitive, reliable, and accurate Gas Chromatography–Mass Spectrometry (GC–MS) method employing Electron Ionization (EI) and Flame Ionization Detection (FID) was developed and validated for the quantitative determination of NDBA in Labetalol bulk drug. NDBA, with a molecular mass of 159 Da, generated characteristic fragment ions at *m/z* 99, 84, and 56 during EI fragmentation. The ion at *m/z* 56 exhibited the highest response and was selected as the quantifier ion, while *m/z* 84 served as the qualifier ion. Chromatographic separation was achieved using an HI-225-MS capillary column (30 m × 0.32 mm × 3 µm) with argon as the carrier gas, an injector temperature of 220 °C, and a programmed oven temperature profile. The method demonstrated excellent resolution, LOD and LOQ values of 0.0012 and 0.0024 ppm, respectively, recoveries ranging from 93.71% to 113.72%, and was successfully validated according to ICH guidelines for specificity, precision, linearity, accuracy, ruggedness, LOD, and LOQ.**

Index Terms—**Limit of Detection (LOD), Limit of Quantitation (LOQ), Precision, Linearity, Accuracy and Ruggedness.**

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I. INTRODUCTION

Labetalol is a widely prescribed antihypertensive agent belonging to the class of α- and β-adrenergic receptor blockers. It is commonly used for the management of hypertension, including severe hypertension and hypertensive emergencies. The drug exerts its therapeutic action by blocking both α_1- and β-adrenergic receptors, resulting in vasodilation, reduced peripheral vascular resistance, and a decrease in heart rate without significantly affecting cardiac output. These pharmacological properties make Labetalol an effective medication for controlling elevated blood pressure and reducing the risk of cardiovascular complications associated with hypertension [1].

In recent years, increasing attention has been focused on the presence of nitrosamine impurities in pharmaceutical products because of their potential toxicological risks. Nitrosamines represent a class of chemical compounds that may be generated during the synthesis, purification, storage, or packaging of drug substances when secondary or tertiary amines react with nitrosating agents. Many nitrosamines have demonstrated mutagenic and carcinogenic properties in experimental animal studies and are therefore considered probable human carcinogens. Among these impurities, N-Nitrosodibutylamine (NDBA) has emerged as an impurity of regulatory concern owing to its genotoxic potential and possible adverse health effects following long-term exposure [2].

The pharmaceutical industry places significant emphasis on maintaining the quality, purity, and safety of active pharmaceutical ingredients (APIs). Even trace quantities of genotoxic impurities can

compromise product safety and may present unacceptable risks to patients receiving long-term therapy. Consequently, the identification, monitoring, and control of nitrosamine impurities, including NDBA, have become essential components of pharmaceutical quality assurance programs. Comprehensive impurity profiling not only ensures patient safety but also supports regulatory compliance and consistent product quality throughout the drug lifecycle [3].

Nitrosamines, including NDBA, are classified as genotoxic impurities because they possess the ability to interact with DNA, potentially causing mutations that may contribute to carcinogenesis. Since no completely safe exposure threshold has been established for many nitrosamines, their concentration in pharmaceutical products must be minimized to the lowest reasonably achievable level. Continuous monitoring and strict control of these impurities significantly reduce the potential health risks associated with chronic exposure and improve the overall safety profile of pharmaceutical products [3]. Regulatory authorities worldwide, including the United States Food and Drug Administration (USFDA), the European Medicines Agency (EMA), and other international organizations, have issued comprehensive guidance regarding the assessment and control of nitrosamine impurities in drug substances and drug products. These agencies require pharmaceutical manufacturers to perform scientific risk assessments, identify possible sources of nitrosamine formation, establish suitable control strategies, and demonstrate compliance with the acceptable intake limits specified for individual nitrosamines. Adherence to these regulatory requirements is essential for obtaining and maintaining product approval and ensuring patient safety [4–6].

The occurrence of NDBA in pharmaceutical products generally indicates contamination or its formation during one or more stages of the manufacturing process. Possible sources include raw materials, reagents, solvents, catalysts, processing conditions, degradation pathways, or interactions occurring during storage. Therefore, implementation of robust manufacturing practices, validated analytical procedures, and effective quality control systems is necessary to minimize the formation of nitrosamine impurities. Reliable analytical techniques capable of detecting NDBA at trace levels are indispensable for routine quality control and regulatory compliance [7]. Maintaining stringent control over nitrosamine impurities also plays an important role in preserving public confidence in pharmaceutical products. Regulatory actions involving nitrosamine contamination have highlighted the importance of implementing preventive strategies throughout pharmaceutical manufacturing. Demonstrating effective impurity control through validated analytical methods strengthens product reliability, ensures regulatory acceptance, and reinforces confidence among healthcare professionals and patients regarding the safety of marketed medicines [8].

Several preventive approaches have been recommended to control NDBA and related nitrosamines in APIs. These include systematic risk assessment of the synthetic process, selection of high-purity starting materials and reagents, implementation of Good Manufacturing Practices (GMP), optimization of manufacturing conditions, qualification of raw material suppliers, and continuous monitoring throughout the supply chain. Furthermore, sensitive and selective analytical methods should be developed and validated to accurately identify and quantify nitrosamine impurities at concentrations well below their regulatory limits. Appropriate corrective and preventive actions should also be implemented whenever elevated impurity levels are detected [9–12]. Because nitrosamines possess relatively low molecular weights, appreciable volatility, and high toxicological significance, analytical methods intended for their determination must exhibit excellent specificity, high chromatographic resolution, and superior sensitivity. Regulatory agencies therefore recommend confirmatory testing whenever process evaluation indicates a potential risk for nitrosamine formation. Gas Chromatography coupled with Mass Spectrometry (GC–MS) is considered one of the most suitable techniques for the determination of volatile nitrosamines because of its excellent selectivity, sensitivity, and structural confirmation capability [13].

Based on the synthetic route employed for the manufacture of Labetalol and the associated process risk assessment, the formation of N-Nitrosodibutylamine (NDBA) was identified as a

potential impurity. According to the guidance published by the European Medicines Agency (EMA) regarding nitrosamines in medicinal products, the acceptable intake limit for NDBA is 26 ng/day, which corresponds to a specification limit of approximately 0.088 ppm in Labetalol based on the maximum daily dose [14]. Therefore, a highly sensitive in-house Gas Chromatography–Mass Spectrometry (GC–MS) method was developed for the determination and control of NDBA in Labetalol bulk drug. The analytical procedure was comprehensively validated according to current International Council for Harmonisation (ICH) guidelines, including evaluation of specificity, limit of detection, limit of quantification, linearity, precision, accuracy, robustness, and ruggedness, thereby demonstrating its suitability for routine quality control and regulatory applications [15–17].

II. MATERIAL AND METHODS

Chemicals and Reagents: Labetalol bulk drug samples were supplied by Supriya Life Science. HPLC-grade methanol and acetonitrile were purchased from J.T. Baker, while the N-Nitrosodibutylamine (NDBA) reference standard was obtained from Advet Biochem.

Instrumentation: Analysis was performed using a Shimadzu GC-MS-QP220 NX system equipped with a switchable Electron Ionization/Chemical Ionization (EI/CI) source, Solvent-Mediated Chemical Ionization (SMCI) unit, DI-2010 injector, HS-20 NX headspace sampler, TD-30 thermal desorption system, pyrolysis unit, and an AOC-6000 Plus multifunctional autosampler.

Chromatographic Conditions: Separation was achieved on an HI-225-MS capillary column (30 m × 0.32 mm i.d., 3 µm film thickness). The GC analysis was carried out using a programmed oven temperature profile under optimized chromatographic conditions.

Oven Program:

Rate in °c/min	Temp in °c	Hold time in min
0.0	120	2.0
10	220	3.0

GC–MS Operating Conditions: The injector temperature was maintained at 220 °C with helium as the carrier gas at a flow rate of 1.0 mL/min. Samples were injected in splitless mode with a septum purge flow of 3.0 mL/min and a split vent purge flow of 12.285 mL/min after 0.5 min. The total run time was 15 min, and N-Nitrosodibutylamine (NDBA) eluted at approximately 11.8 min. Mass spectrometric detection was performed in Electron Ionization (EI) mode with a source temperature of 250 °C, transfer line temperature of 250 °C, auxiliary temperature of 150 °C, electron energy of 40 eV, and acquisition in MRM mode (gain = 1). The collision energies were 10 and 22 eV, monitoring the transitions m/z 159→99 and 84→56 with a solvent delay of 6 min.

Preparation of Solutions: Isooctane was used as the diluent. Stock Solution A (200 ppm) was prepared by dissolving 10 mg of NDBA standard in a 50 mL volumetric flask. Stock Solution B (2 ppm) was obtained by diluting 0.5 mL of Stock Solution A to 50 mL. The working standard (0.022 ppm) was prepared by diluting 0.55 mL of Stock Solution B to 50 mL. For sample preparation, 2.5 g of Labetalol was dissolved in a 10 mL volumetric flask using isooctane, sonicated, diluted to volume (250,000 ppm), and filtered through a 0.45 µm membrane filter before analysis.

III. RESULTS AND DISCUSSION

Method Development: A mixed solution containing Labetalol (250,000 ppm) and N-Nitrosodibutylamine (NDBA, 0.088 ppm) was prepared in isooctane for method development. Various GC columns with different stationary phases were evaluated to achieve adequate separation. Initial trials using a VF-Wax column (30 m × 0.32 mm, 3 µm) did not provide satisfactory selectivity between Labetalol and NDBA. Subsequently, the HI-225-MS column (30 m × 0.32 mm, 3 µm) was selected as it produced excellent resolution and peak separation under the optimized oven temperature program.

Optimized Chromatographic Conditions: Further optimization was performed using the HI-225-MS column because of its superior chromatographic performance. The finalized method employed helium as the carrier gas with a flow rate of 3.0 mL/min, an injector temperature of 220 °C, a detector temperature of 250 °C, and a 0.5 min injection time. Under these optimized conditions, NDBA was completely resolved from Labetalol, with NDBA

eluting at approximately 11.8 min, followed by the Labetalol peak, demonstrating excellent selectivity and suitability for quantitative analysis.

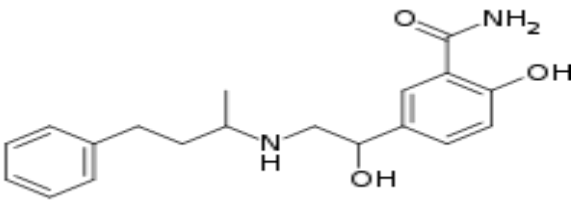

Figure-1: Structure of Labetalol

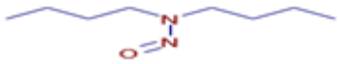

Figure-2: Structure of N-Nitroso dibutylamine

Method Validation

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix etc.

Table -1: Preparation of Specificity Solution

Preparation of Stock Solution-I					
Standard	Wt (mg)	Dilute to ml	volume taken in ml	Dilute to ml	Conc (ppm)
N-nitrosodibutylamine (NDBA)	10.78	50	---	---	215.06
Preparation of Stock Solution-II Volume of Stock Solution-I taken in ml					
Volume of Stock Solution-I taken in ml					
	0.5	50	---	---	2.36
Preparation of Standard Solution					
Volume of Stock Solution-II taken in ml					
	0.5	50	---	---	0.0237

Table- 2: Result of Retention time of Specificity Solution

Compound	Retention time	Relative Retention time
N-nitrosoDi Isopropylamine(NDBA)	11.8	0.94
Labetalol	12.5	1.00

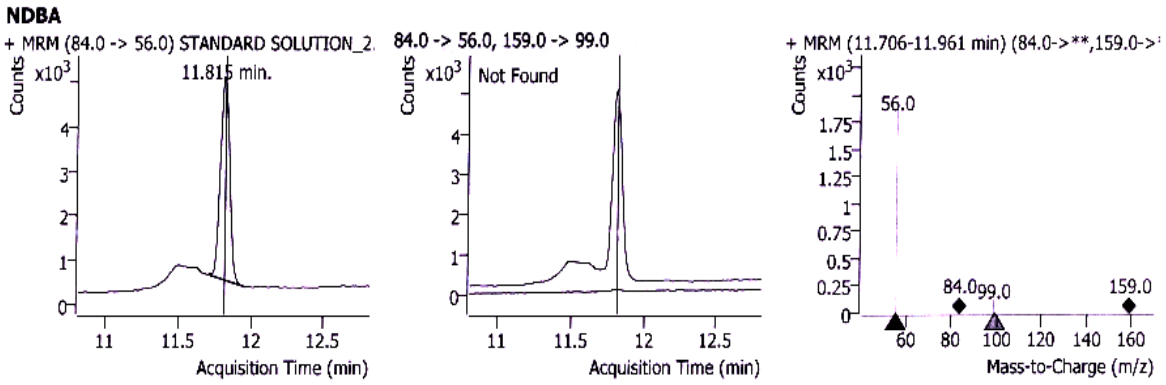

Figure -3 Specificity Solution

Precision: Precision evaluates the degree of agreement among a series of independent measurements obtained from repeated analyses of a homogeneous sample under identical experimental conditions[18]. System precision and method precision for N-Nitrosodibutylamine (NDBA) were assessed at the specification level of 0.0237 ppm against a Labetalol concentration of 250,000 ppm. The %RSD values for method repeatability and system repeatability were 3.88% and 4.79%, respectively, indicating satisfactory repeatability and demonstrating that the developed GC–MS method is precise and reliable for routine analysis.

Table- 3:System Precision

Preparation of System Precision Solution					
Standard	Wt (mg)	Dilute to ml	Volume taken in ml	Dilute to ml	Conc (ppm)
N-nitrosodibutylamine (NDBA)	10.78	50	---	---	215.06
Volume of Stock Solution-I taken in ml					
	0.5	50	---	---	2.36
Preparation of Standard Solution					
Volume of Stock Solution-II taken in ml					
	0.5	50	---	---	0.0237
Six Replicate of System Precision Standard Solution					
N-nitrosoDi Isopropylamine(NDBA)					
Injections	Area				
1	20154				
2	20684				
3	20772				
4	20685				
5	19494				
6	20908				
Mean	20450				
SD	533.85				
RSD	2.61				

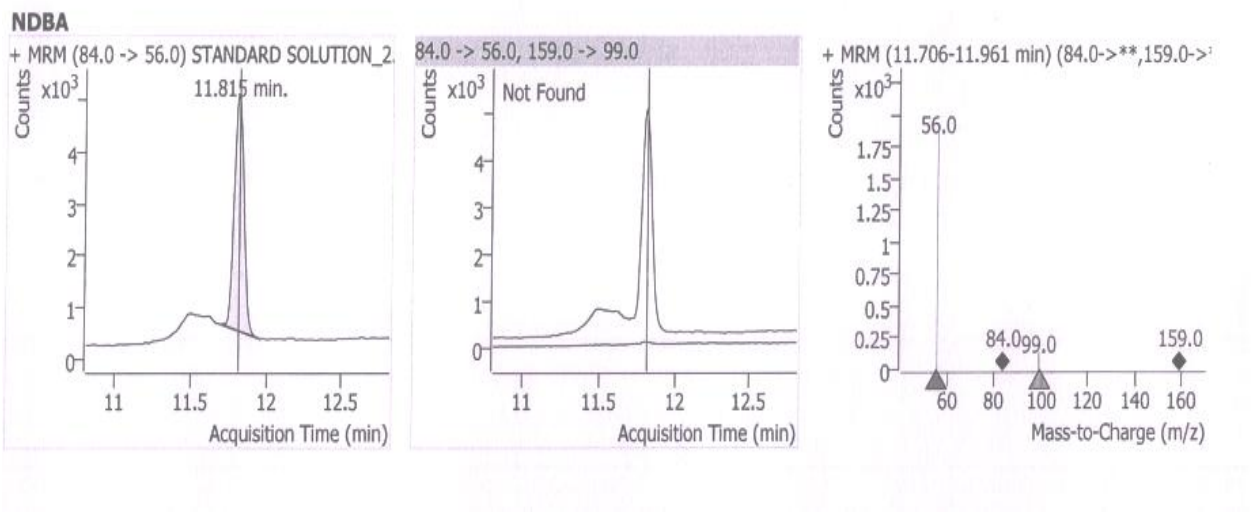

Figure -4: System Precision

Method Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Table-4: Method Precision

Preparation of Standard Solution					
Standard	Weight (mg)	Dilute to ml	Volume taken in ml	Dilute to ml	Conc (ppm)
N-Nitrosodibutylamine (NDBA)	10.78	50	---	---	215.06
Volume of Stock Solution-I taken in ml					
	0.5	50	---	---	2.36
Preparation of Standard Solution					
Volume of Stock Solution-II taken in ml					
	0.5	50	---	---	0.0237

Table- 5: Summary of Test as Such Solution

Name of Spl	Wt of Spl in mg	Diluted to ml	Area	Result of NDBA
Test 1	500.35	2	ND	ND
Test 2	500.60	2	ND	ND

Table- 6: Result Summary of Method Precision (NDBA)

Solution	Test Splike wt in mg	Amount Added in ppm	Area in test Spike	Amount found in ppm	Amount Recovered in ppm	Avg of Recovered ppm	SD	% RSD
100% Spike	500.45	0.0859	27821	0.0871	0.0871	0.0848	0.0033	3.88
	500.27	0.0860	28279	0.0886	0.0886			
	500.73	0.0859	25731	0.0805	0.0805			
	500.69	0.0859	26078	0.0816	0.0816			
	500.62	0.0859	27781	0.0870	0.0870			
	500.75	0.0859	26791	0.0839	0.0839			

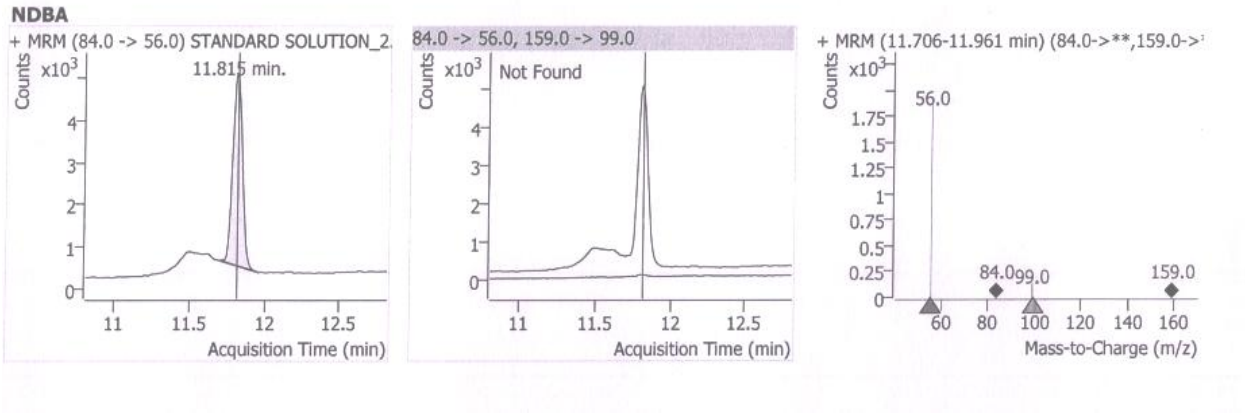

Figure -5 : Method Precision

Linearity: The Linearity of an analytical procedure is its ability (within given range),to obtain test results, which are directly proportional to concentration of analyte in sample. The Linearity of the method for NDBA was checked at six concentration levels i.e from Limit of quantitation (LOQ (10%) to 150% of NDBA at Specification level of 0.0237 ppm which is wrt to Labetalol analyte concentration. The coefficient of regression of the calibration curve was found to be 0.9983,thus confirming the excellent correlation between the peak area and concentration of NDBA.

Table 7 : Linearity

Preparation of Standard Solution					
Standard	Weight (mg)	Dilute to ml	Volume taken in ml	Dilute to ml	Conc (ppm)
N-nitrosodibutylamine (NDBA)	10.78	50	---	---	215.06
Volume of Stock Solution-I taken in ml					
	0.5	50	---	---	2.36
Preparation of Standard Solution					
Volume of Stock Solution-II taken in ml					
	0.5	50	---	---	0.0237

Table- 8 :Linearity Levels

Linearity Levels	Volume of Linearity Stock Solution taken in ml	Diluted to ml
Level-I LOQ (10%)	0.05	50
Level-II 30%	0.15	50
Level-III 50%	0.25	50
Level-IV 80%	0.4	50
Level-V 100%	0.5	50
Level-VI 120%	0.6	50
Level-VII 150%	0.75	50

Table- 9: Linearity of NDBA

Linearity Level	Conc ppm	Observed Area			Average
Level-I LOQ(10%)	0.0024	3087	3268	3109	3155
Level-II 30%	0.0071	9675	9951	10040	9889
Level-III 50%	0.0118	16909	16780	16135	16608
Level-IV 80%	0.0189	27812	28930	27962	28235
Level-V 100%	0.0236	35090	35471	37225	35929
Level-VI 120%	0.0284	47406	47225	46397	47009
Level-VII 150%	0.0355	54347	56000	55616	55321

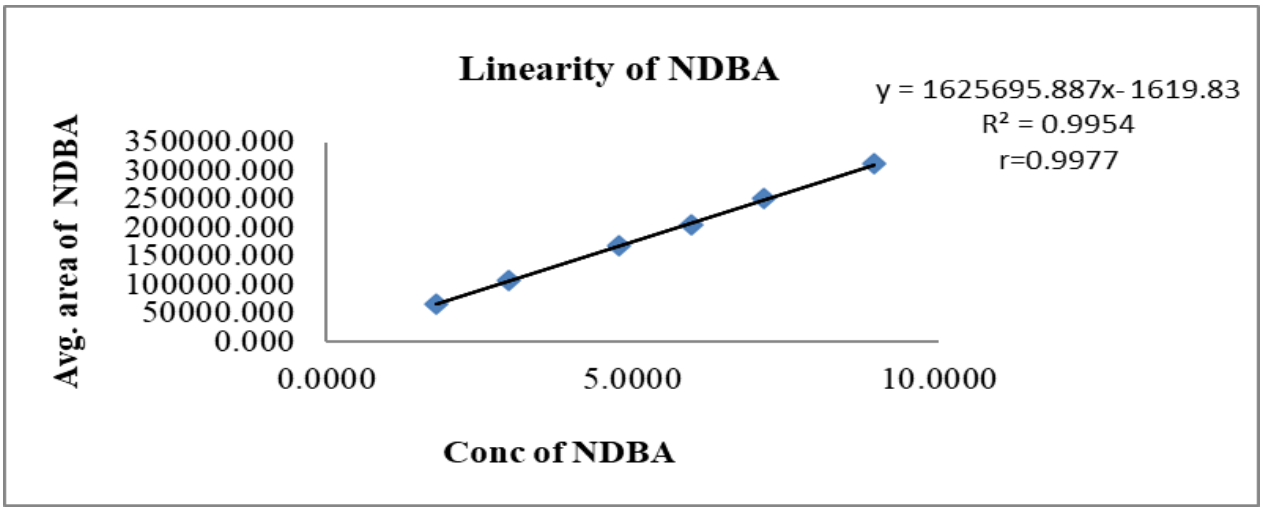

Figure-6: Linearity

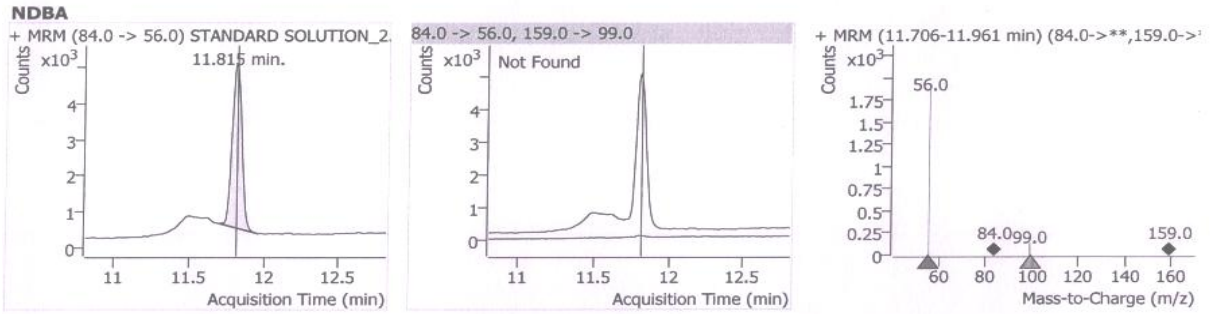

Figure-7: Linearity Level-I LOQ

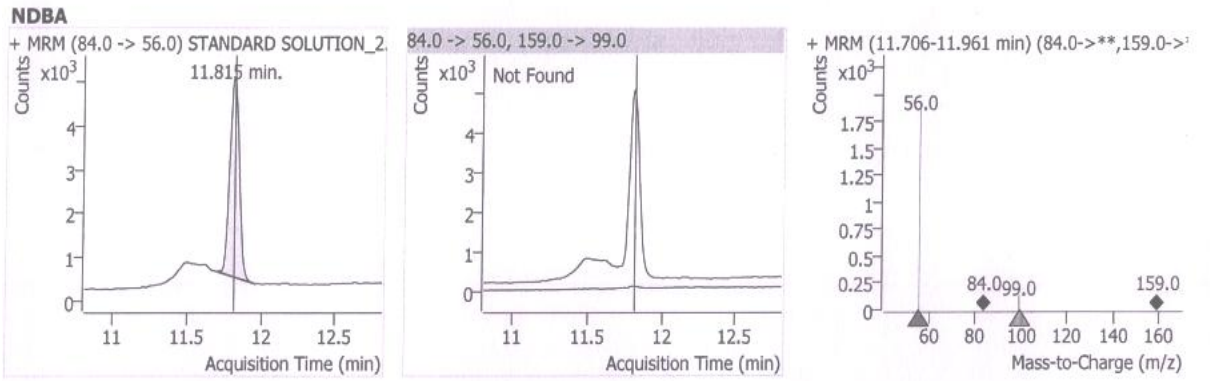

Figure- 8: Linearity Level-II (30%)

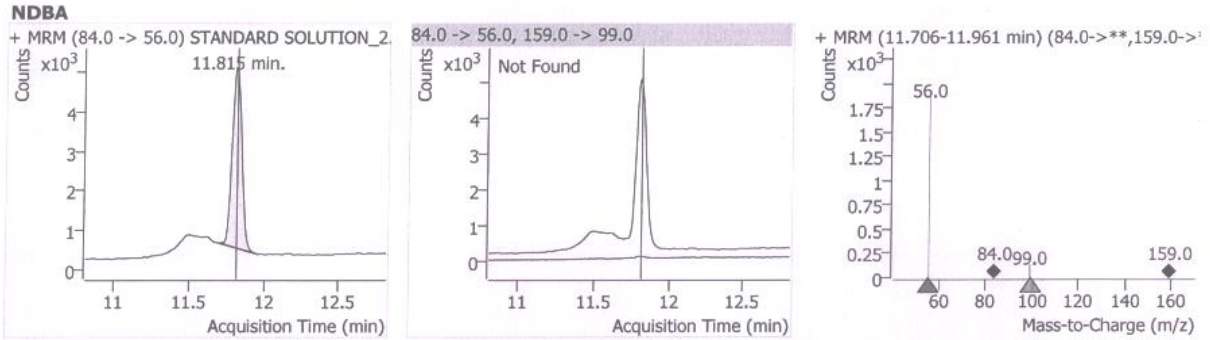

Figure- 9: Linearity Level-III (50%)

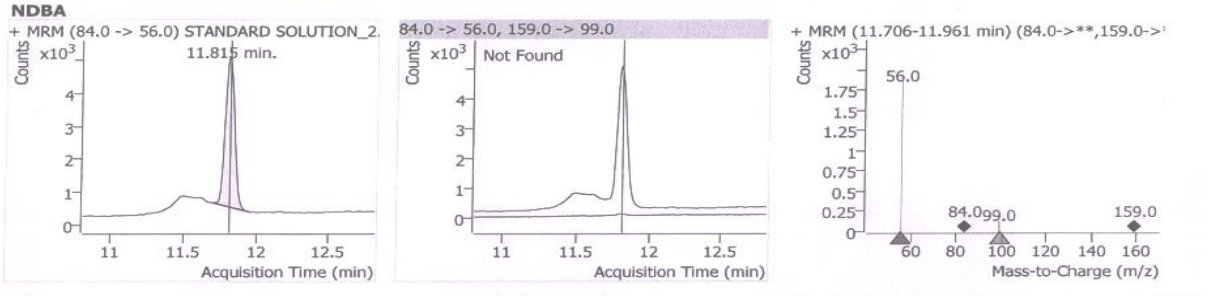

Figure- 10: Linearity Level-IV (80%)

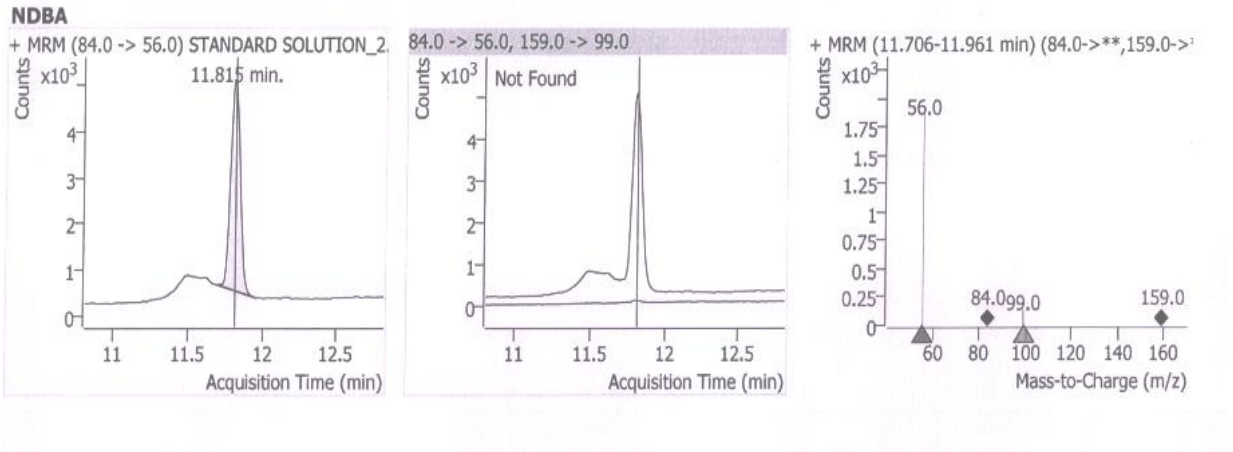

Figure -11: Linearity Level-V (100%)

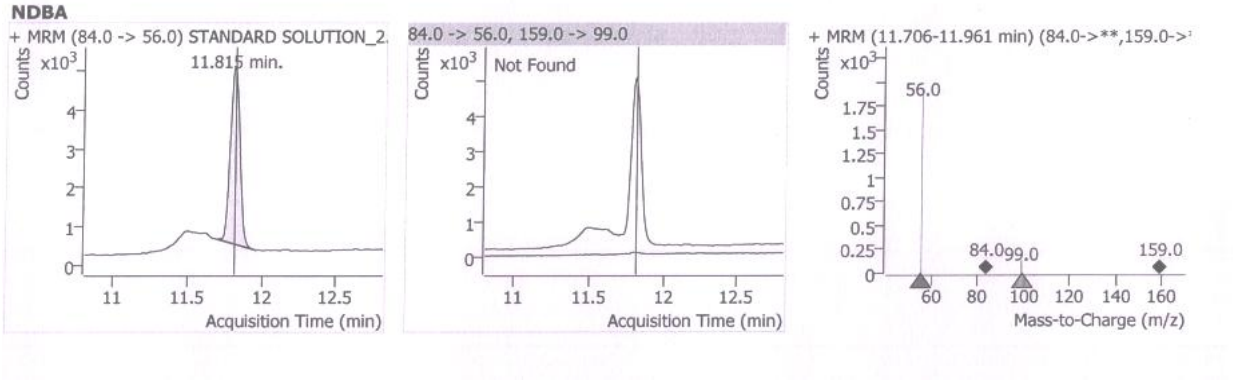

Figure- 12: Linearity Level-VI (120%)

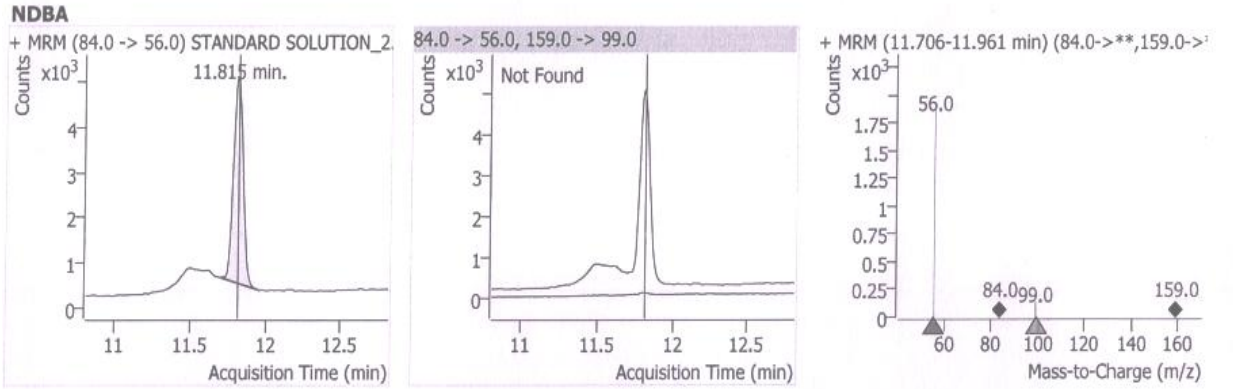

Figure- 13 :Linearity Level-VII (150%)

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD represents the minimum concentration of an analyte that can be reliably detected, whereas the LOQ is the lowest concentration that can be accurately and precisely quantified. The LOD and LOQ for N-Nitrosodibutylamine (NDBA) were established by analyzing a series of progressively diluted standard solutions. Precision at both levels was evaluated by repeated injections of the corresponding standard solutions and calculating the percentage relative standard deviation (%RSD) of the peak areas. The developed GC–MS method demonstrated excellent sensitivity, with the LOD and LOQ determined to be 0.0012 ppm and 0.0024 ppm, respectively.

Table -10: Limit of detection and Quantitation

Preparation of Standard Solution					
Standard	Weight (mg)	Dilute to ml	Volume taken in ml	Dilute to ml	Conc (ppm)
N-nitrosodibutylamine (NDBA)	10.78	50	---	---	215.06
Volume of Stock Solution-I taken in ml					
	0.5	50	---	---	2.36
Preparation of Standard Solution					
Volume of Stock Solution-II taken in ml					
	0.5	50	---	---	0.0237

Table- 11: Preparation of Limit of detection and Quantitation Solution

Standard	Volume of Stock Solution-I taken in ml	Dilute to ml	Volume taken in ml	Dilute to ml	Conc (ppm)
NDBA	0.025	50	-----	-----	0.0018
NDBA	0.05	50	-------	----------	0.0023

Table -12: Result Summary of Limit of detection and Quantitation Solution

N-nitrosodibutylamine(NDBA)		
	Area	
Injections	Limit of detection	Limit of Quantitation
1	1067	3118
2	1098	3347
3	1140	3259
4	1191	3380
5	1274	3453
6	1111	3183
Mean	1147	3290
SD	75.11	126.42
RSD	6.55	3.84

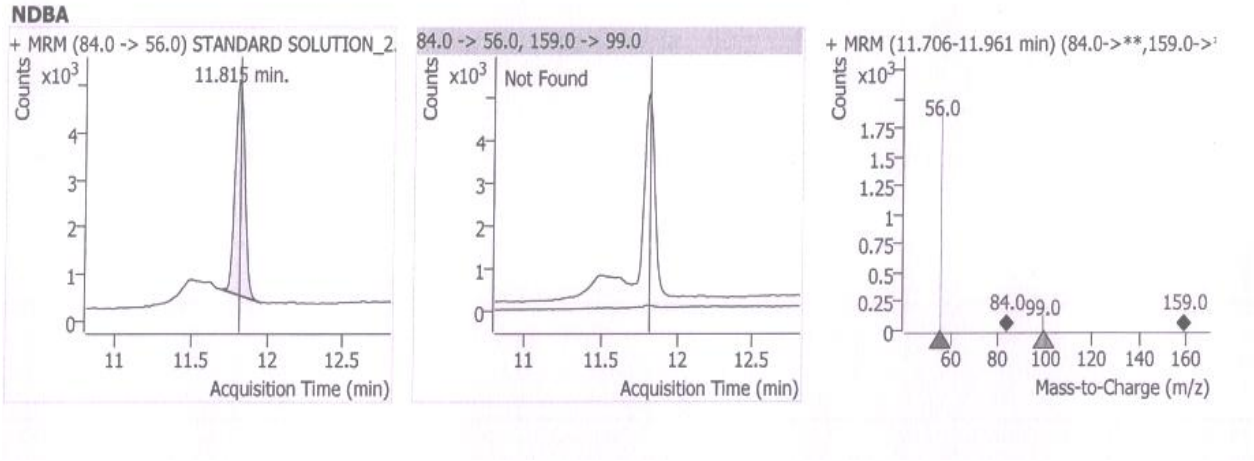

Figure-14: Limit of detection

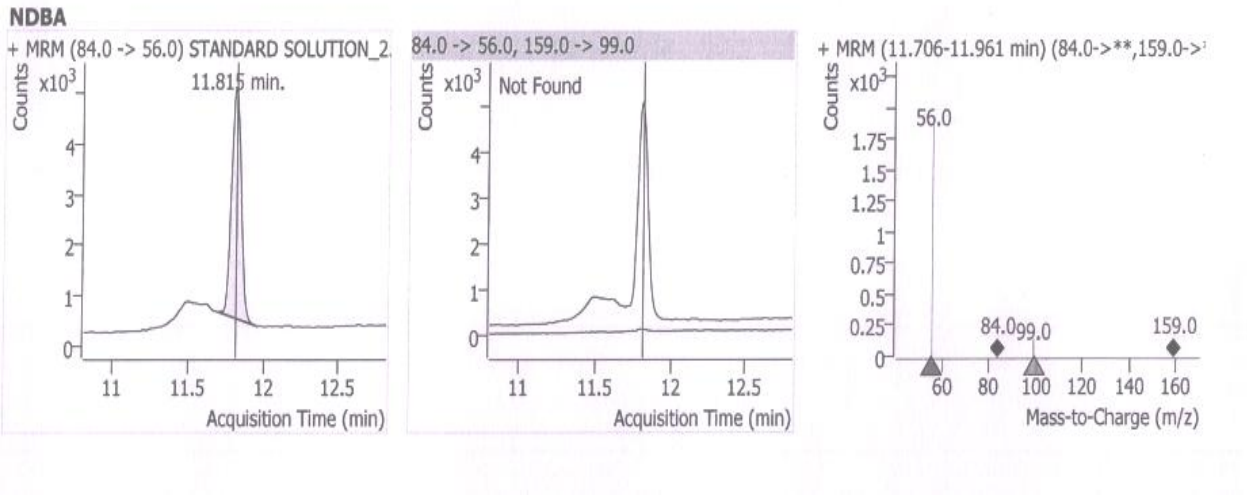

Figure- 15: Limit of Quantitation

Accuracy and Recovery: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The standard addition and recovery experiments were conducted for the NDBA in bulk samples of Labetalol in triplicate at LOQ,50%, 100%,150% with respect to test concentration. The percentage recovery ranged from 97.01% to 103.75%. Summary of Method validation.

Table- 13: Limit of detection and Quantitation

Preparation of Standard Solution					
Standard	Weight (mg)	Dilute to ml	Volume taken in ml	Dilute to ml	Conc (ppm)
N-nitrosodibutylamine (NDBA)	10.78	50	---	---	215.06
Volume of Stock Solution-I taken in ml					
	0.5	50	---	---	2.36
Preparation of Standard Solution					
Volume of Stock Solution-II taken in ml					
	0.5	50	---	---	0.0237

Table -14: Preparation of Accuracy Stock Solution

Accuracy Solution	Volume of Standard Stock-II Solution ml	Dil to ml
Accuracy Level I LOQ	0.05	50
Accuracy Level II 50%	0.25	50
Accuracy Level III 100%	0.50	50
Accuracy Level IV 150%	0.75	50

Table- 15 : Summary of Test as Such Solution

Name of Spl	Wt of Spl in mg	Diluted to ml	Area	Result of NDBA
Test 1	501.10	2	ND	ND
Test 2	500.79	2	ND	ND

Table 16 : Results of Accuracy (NDBA)

Accuracy	Test wt Spike mg	Amt added in ppm	Area in Test Spike	Amt Found in ppm	Amt Recovered in ppm	% Recovery	Mean% Recovery	SD	RSD

Level I LOQ	500.60	0.0086	3193	0.0100	0.0100	116.28	119.38	2.68	2.24
	500.90	0.0086	3326	0.0100	0.0100	120.93			
	501.21	0.0086	3320	0.0104	0.0104	120.93			
Level II 50%	500.02	0.0430	14829	0.0450	0.0450	108.14	108.92	4.46	4.09
	500.03	0.0430	15608	0.0465	0.0465	113.72			
	501.21	0.0430	14393	0.0489	0.0489	104.90			
Level III 100%	500.08	0.0859	27821	0.0871	0.0871	101.40	99.38	4.97	5.00
	500.31	0.0859	28279	0.0886	0.0886	103.02			
	500.21	0.0859	25731	0.0805	0.0805	93.71			
Level IV 150%	500.03	0.1289	47497	0.1487	0.1487	115.36	111.53	3.60	3.23
	500.12	0.1289	44571	0.1396	0.1396	108.22			
	500.13	0.1289	45703	0.1431	0.1431	111.02			

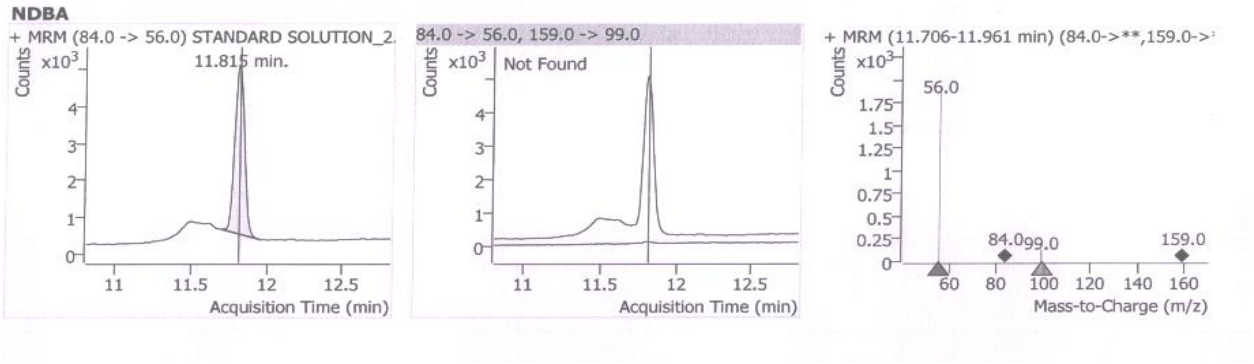

Figure-16: Accuracy LOQ Level

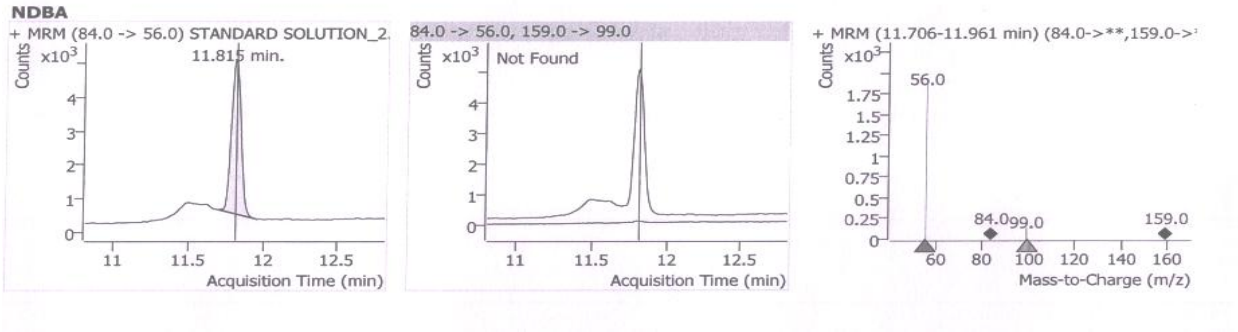

Figure – 17: Accuracy 50% Level

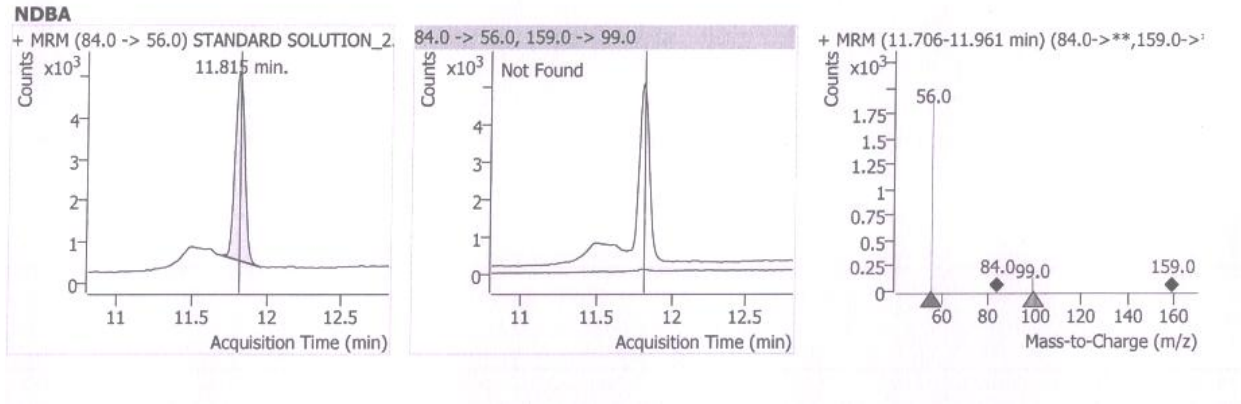

Figure- 18: Accuracy 100% Level

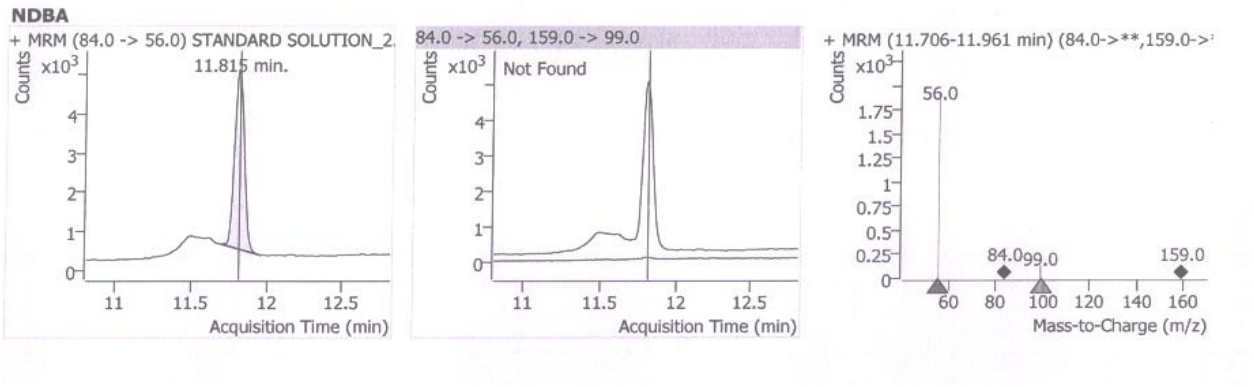

Figure-19: Accuracy 150% Level

IV. CONCLUSION

An unidentified impurity detected during the analysis of Labetalol was successfully isolated and characterized using complementary analytical techniques, including FTIR, NMR, GC, and Mass Spectrometry. Structural investigation confirmed the impurity as N-Nitrosodibutylamine (NDBA), a nitrosamine of regulatory concern. To ensure reliable monitoring of this impurity, a simple, rapid, selective, and highly sensitive Gas Chromatography–Mass Spectrometry (GC–MS) method was developed for its determination in Labetalol bulk drug. Method optimization was carried out using an HI-225-MS capillary column (30 m × 0.32 mm, 3 µm), which provided superior chromatographic separation and enhanced mass spectral fragmentation for accurate identification and quantification. The analytical procedure was comprehensively validated according to ICH guidelines. Validation results demonstrated excellent specificity, precision, accuracy, sensitivity, and reproducibility, confirming the robustness of the proposed method. Owing to its low detection limits and reliable analytical performance, the validated GC–MS method is well suited for routine quality control, impurity profiling, and regulatory compliance in the quantitative determination of trace levels of N-Nitrosodibutylamine (NDBA) in Labetalol active pharmaceutical ingredient (API).

CONFLICT OF INTEREST

The authors declare that they have no financial interests or personal relationships that could have influenced the work presented in this paper.

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