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Original Article

LC-MS/MS STUDY OF THE TRACE LEVEL IMPURITIES OF IRBESARTAN AN ANGIOTENSIN II RECEPTOR ANTAGONIST MOLECULE TO ITS ORIGIN THROUGH nMS² TECHNIQUE

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ABSTRACT

Objective: The mass characterization of five trace level related impurities of Irbesartan was performed through the "nMS2" technique of triple quadrupole mass spectrometer and also to Correlated to the impurity origin.

Methods: A simple & effective patented process was applied to get the impurity profile, and mass characterization was performed through the "nMS2" technique of triple quadrupole mass spectrometer analyser.

Results: The simple production scan in differential collision energies is coined as "nMS2" technique of the triple quadrupole analyzer. The molecular ion fragmentation occurs with multiple collision energies and provides meaningful MSMS fragments for characterizing five trace level impurities less than 0.5% of angiotensin II receptor antagonist-Irbesartan. The origin of the impurity formation in the synthetic process was successfully related to the Spiro ketones.

Conclusion: The results obtained in this research clearly indicates the approach of "nMS²" technique was very useful in the identification and structural prediction of trace level related impurities of Irbesrtan.

Keywords: nMS²technique, Irbesartan, LCMSMS of Irbesartan, Impurity Characterization

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INTRODUCTION

Safety and efficacy of pharmaceutical substances are the two fundamental issues in drug therapy. The safety of a drug is determined by its pharmacological or toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The drug should be safe, i.e. it should have an acceptably low risk of adverse effects with doses of drug which provide the desired therapeutic effects. The impurities in drugs often possess unwanted pharmacological or toxicological effects by which any benefit from their administration may be outweighed. Thus the quality of the drug is directly related to safety. The quality and safety of a drug is generally assured by monitoring it using suitable analytical techniques and controlling the Impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important aspect in the modern pharmaceutical analysis [1-5]. The International Conference on Harmonization (ICH) guidelines defines the impurity as "Any component of the medicinal product which is not the chemical entity defined as the active substance or an excipient in the product". The impurity profiling is hence considered to be the analytical activities with the aim of detecting, identifying or elucidating the structure and quantitatively determining impurities in bulk drugs and pharmaceutical formulations [6-10].

The impurities were broadly classified as related and non-related impurities. In pharmaceuticals the manufacturing facilities follows stringent manufacturing practices, thus the possibility for the non-related unknown impurity formation will be very rare considerably difficult to identify and characterize this totally unknown impurity. Development of a cost-effective process is always desirable for sustained commercialization of any drug substances. Therefore, continuous innovations are constantly employed by process chemist to implement cost-effective synthesis scheme in scale-up operations and hence successful commercialization of the drug substances. This kind of effort requires the use of commercially available conventional raw materials along with plant-based solvents for the process, workup and purification of the final product ensuring the quality of the product. This report demonstrates how the MSMS architecture of LCMS-8040 acts as an "enabler" in identifying process-related impurities appropriately to ensure the quality of the drug substance Irbesartan. Irbesartan is the angiotensin II receptor antagonists intended for the treatment of hypertension and also for the delay progression of diabetic nephropathy. It is often formulated with a lesser dosage level of thiazide diuretic The salient features of LCMS 8040 like Synchronized Survey Scan (SSS) provides the MS and MSMS data simultaneously in a single run and paves the way for rapid identification of impurities. Further, the simultaneous MS analysis in both positive and negative modes, MSMS analysis with different collision energies and MSMS analysis with neutral loss function have enabled us to characterize the impurities of Irbesartan.

MATERIALS AND METHODS

Chemicals and reagents

The synthetic chemicals are LR grade for step 1 and step2 of synthetic reaction scheme. AR grade reagents are used for step3. Cyano methyl biphenyl and spiroketone is procured from LobaChemi along with scheme reagents. Analytical grade reagents and LCMS grade solvents is used for the LCMS and LCMSMS analysis.

Instrumentation

An UFMS consisting of the Shimadzu UHPLC NEXERA as a frontend for the Shimadzu LCMS 8040 triple quadrupole mass spectrometer. A reverse phase Shimadzu shimpak XR-ODS (50 X3.0 mm, 2.2µm) column was used for the chromatographic separation. The chromatographic data is recorded with Shimadzu Lab solution software. The LCMS and LCMSMS were performed with the FCV12 divert valve arrangement. ESI was used as the ionization source for the mass analysis in both positive and negative polarity.

Chromatographic and mass spectrometric conditions

The UHPLC separation was carried out with Shimadzu shimpak XR-ODS (50 X3.0 mm, 2.2µm) column using 0.1% fomic acid in water at

3.4 pH and acetonitrile as mobile phase in a gradient mode. The time/%B concentration of the gradient programme is 0.01/33, 2.00/33, 10.00/60, 13.00/60,15.00/33,17.00/33. The flow rate was 0.3 ml/min, and the injection volume is 10µL. LC-MS/MS was carried out with Electrospray ionization (ESI) in positive and negative mode of detection. Nitrogen was used as a nebulizer gas. Collision-induced dissociation was achieved by argon as collision gas. The operating conditions for MS scan were: DL temperature 250 °C; nebulizer gas, 3.0L/min; dry gas, 15.0L/min; ESI voltage 4.5kV; heat block, 350 °C and the sacn range, 150 to 2000amu. The selected quazi molecular ion was subjected to the LCMSMS analysis in 11 different collision energies (CE=5v to 60v in increment of 5).

DISCUSSION

Irbesartan was in-house synthesized at Indian Institute of chromatography and Mass Spectrometry (IICMS), Chennai, India for academic research. A three-stage synthetic root is adapted as per EP1918288A1 [11]. The synthetic scheme is shown in fig. 1.

The step 1(S-1) corresponds to alkyl bromination followed by spiroketone substitution in step2 (S-2) and cyclization with a reduction to form tetrazole ring in step3(S-3). The purity of the compound was found to be 99.21% (Area normalization method) with five trace level impurities.

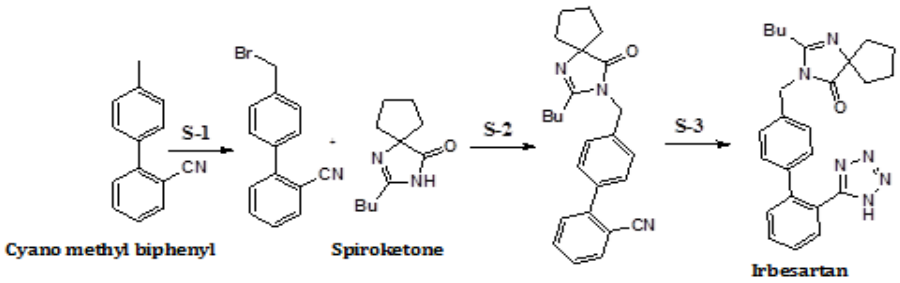


Fig. 1: Synthetic scheme for Irbesartan [11]

Though a three-step process is used to synthesize Irbesartan, the HPLC analysis shows the presence of five impurities, whose concentration varies from 0.04% to 0.32% Area (table 1). Fig. 2 shows the HPLC chromatogram of Irbesartan and its five trace level impurities. The LCMS analysis was performed for the above five impurities.

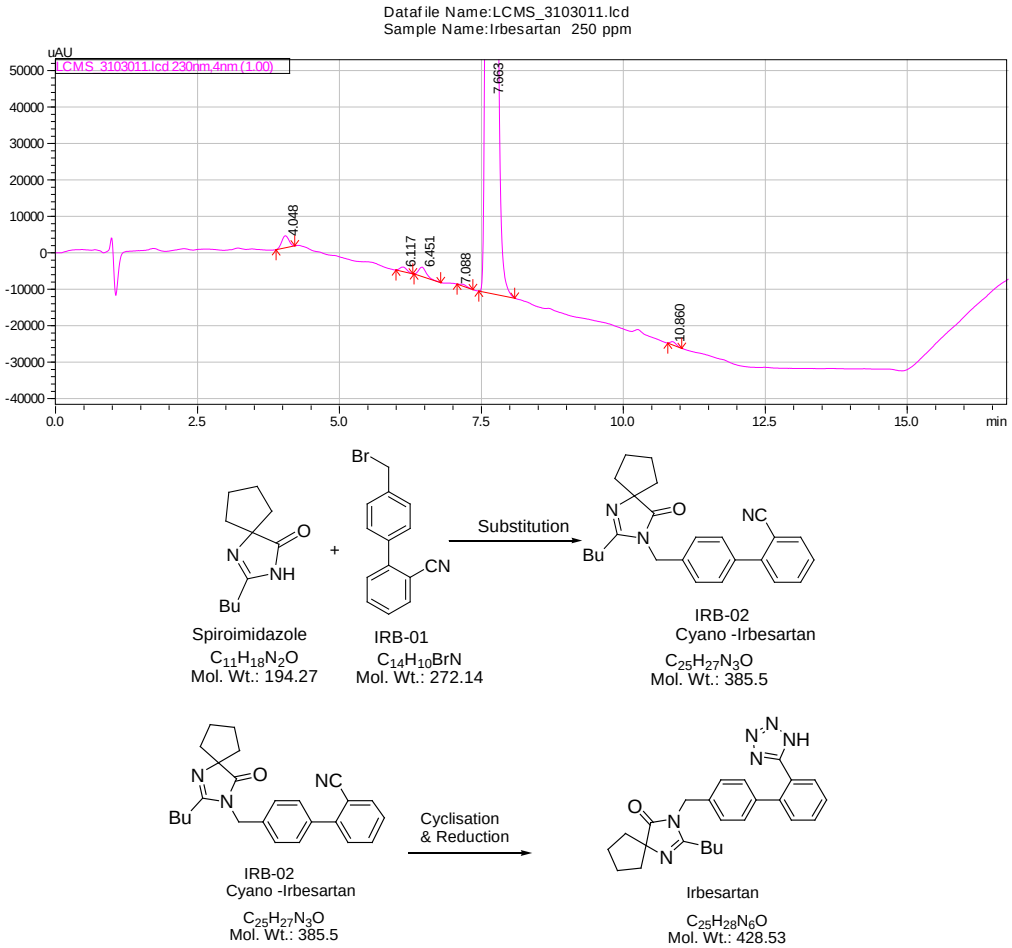


Fig. 2: HPLC chromatogram of Irbesartan and its trace level impurities

Table 1: The Retention time and area percentage of Irbesartan and impurities

Impurities	Retention time (min)	Level of impurities in UV detection (%Area)
Impurity-1	4.05	0.32
Impurity-2	6.12	0.11
Impurity-3	6.45	0.26
Impurity-4	7.09	0.04
Impurity-5	10.86	0.07

The trace level impurity area is shown in table 1

The impurity variance is between 0.04%a/a to 0.32%a/a. Thus it is less than 0.5%a/a in the analyzed chromatographic conditions [12-14]. On comparing the polarity in the chromatographic impurity elution order, it was observed that four impurities are polar with respect to irbesartan and the one with later elution is relatively nonpolar to irbesartan. This also infers that the structure of these impurities has functional group variances or aliphatic chain increments/decrements reflecting to polarity.

The mass spectrometric analysis in impurity characterization is the first approach for the possible clue for the formation of impurity in the reaction process of the intended product. The Irbesartan and its impurities were mass analyzed. The molecular ion of Irbesartan has been observed as m/z 429 [M+1] in ESI positive mode, confirmed in the simultaneous ESI negative mode analysis as m/z 427 [M-1]. Similar to irbesartan, the molecular mass of the impurities was also confirmed by the simultaneous positive [M+1] ions and negative [M-1] ions and tabulated in table 2.

Table 2: Molecular mass of Irbesartan and impurities

Name	Molecular ion		Molecular mass (Da)
	Positive	Negative	
Impurity 1	373	371	372
Impurity 2	415	413	414
Impurity 3	447	445	446
Impurity 4	427	425	426
Impurity 5	443	441	442

The impurities are correlated to Irbesartan and found all the impurities are behaving similar in mass spectrometry with ionization both in positive and negative ionization.

The LCMS/MS-"nMS²" approach

The sanctity of an LCMS analysis remains in the novel approach of its intended application. The stringent specification of international regulatory bodies has made it necessary to characterize the impurities in trace level - (0.1%). This is to minimize the adverse biological effects of the unknown impurities and also to improve the potency of the active component. Characterization of a compound and its trace level impurities requires MS and MSMS analysis. The segmental analysis was performed to obtain the above molecular masses for irbesartan. The MSMS analysis of irbesartan with nine different collision energies simultaneously in ESI positive ionization shows the characteristic fragmentation ions such as m/z 429, m/z 207, m/z 195, m/z 180 and m/z 84 of irbeartanis shown in table 3 and the proposed fragmentation pathway is shown in fig. 3.

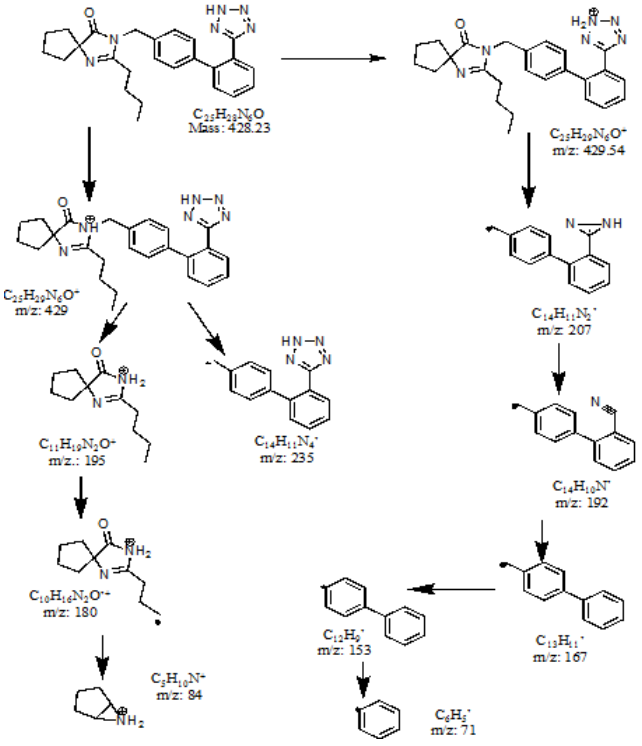
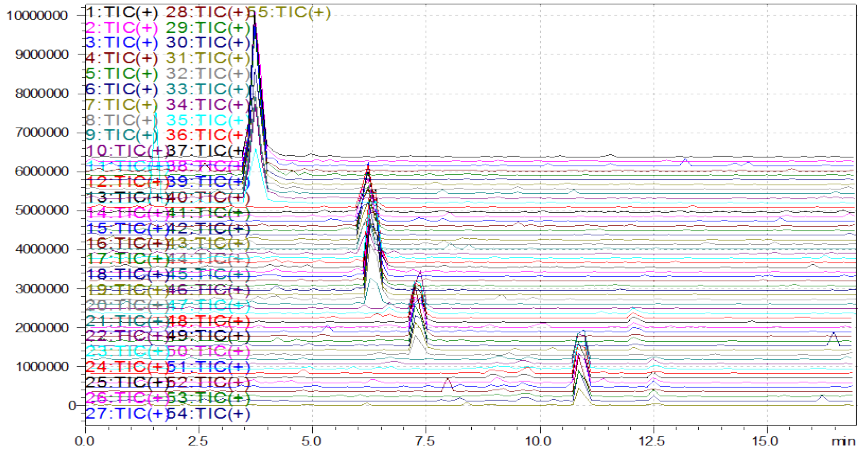


Fig. 3: The proposed fragmentation pathway of Irbesartan

Table 3: Compiled mass fragments of Irbesartan

CE	Irbesartan								
-5	429								
-10	429	207							
-15	429	207	195						
-20	429	207	195						
-25		207	195						
-30		207	195		180				
-35		207	195	190,192	180			84	
-40		207	195	190,192	180		153	84	
-45		207	195	190,192	180	165,167	153	84	
-50		207	195	190,192	180	165,167	151	84	77
-60		205		190,192	180	165,167		84	77

The MS identified impurities are subjected to the MSMS analysis simultaneously at eleven different collision energies for the five impurities in both positive and negative ESI mode. The MSMS chromatogram and compiled mass fragments in positive mode is shown in fig. 4 and the MSMS chromatogram and compiled mass fragments in negative mode is shown in fig. 5.



CE	Irbesartan								
5	427								
10	427	399	193						
15	427	399	193	177					
20	427	399	193						
25			193						
30			193						
35			193						
40			193	164				82	
45			193	164	150		121		
50			193	165		139	121		
60			193	165		139	121	82	64

CE	Impurity I						
5	371						
10	371			137			
15	371			137			
20	371	343		137			
25	371			137			
30	371	343		137			
35	371	343	165	137			
40				137			
45				137			
50				137			
60				137	108	83	

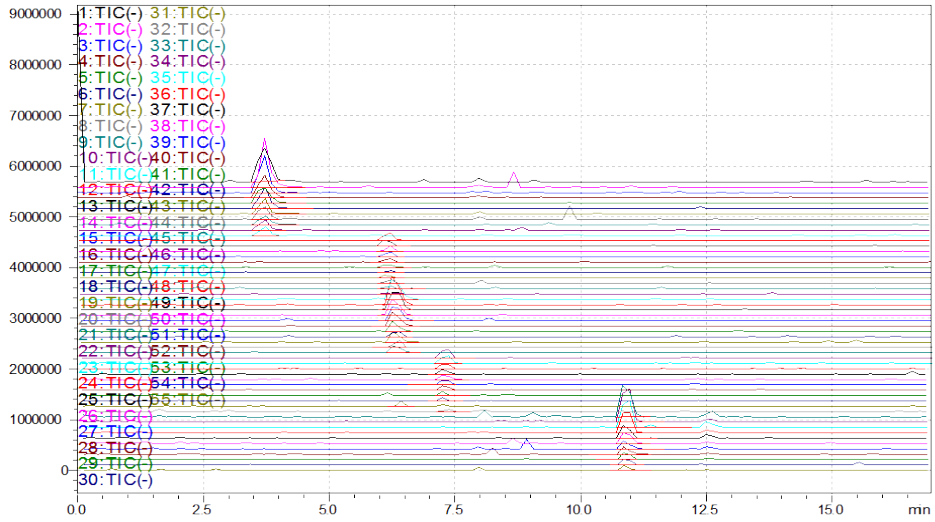
CE	Impurity II				
5	413				
10	413				
15	413				
20	413	385	179		
25	413	385	180		
30	413	385	179		
35	413		179		
40	413		179		
45			179		
50			179		
60			179	124	

CE	Impurity III						
5	445						
10	445						
15	445						
20	445	305					
25	445	305					
30	445	305	211	194	165	127	100
35	445	305	212	194	165		
40	445	305	211	194	165	127	
45	445	305	211	194	165	127	
50	445	305	211	194	165	127	
60		305	211		165	127	100

CE	Impurity IV		
5	425		
10	425		
15	425		
20	425		191
25	425		191
30	425		191
35	425		191
40	425		191
45			191
50			191
60			191

CE	Impurity V								
5	441								
10	441								
15	441								
20	441	385				207			
25	441			288		207			
30	441		315	288	219	207			
35	441		315	288	220	207			
40	441		315	288		207			
45	441		315	288		207	137		
50	441		315	288		207	137	94	
60	441			288		207		94	

Fig. 4: The overlaid MSMS chromatogram of five impurities evaluated under 11 different collisions (55 data in 15 min) and compiled mass fragments in ESI Positive mode



CE	Irbesartan								
5	427								
10	427	399	193						
15	427	399	193	177					
20	427	399	193						
25			193						
30			193						
35			193						
40			193	164				82	
45			193	164	150		121		
50			193	165		139	121		
60			193	165		139	121	82	64

CE	Impurity I						
5	371						
10	371			137			
15	371			137			
20	371	343		137			
25	371			137			
30	371	343		137			
35	371	343	165	137			
40				137			
45				137			
50				137			
60				137	108	83	

CE	Impurity II				
5	413				
10	413				
15	413				
20	413	385	179		
25	413	385	180		
30	413	385	179		
35	413		179		
40	413		179		
45			179		
50			179		
60			179	124	

CE	Impurity III						
5	445						
10	445						
15	445						
20	445	305					
25	445	305					
30	445	305	211	194	165	127	100
35	445	305	212	194	165		
40	445	305	211	194	165	127	
45	445	305	211	194	165	127	
50	445	305	211	194	165	127	
60		305	211		165	127	100

CE	Impurity IV		
5	425		
10	425		
15	425		
20	425		191
25	425		191
30	425		191
35	425		191
40	425		191
45			191
50			191
60			191

CE	Impurity V								
5	441								
10	441								
15	441								
20	441	385				207			
25	441			288		207			
30	441		315	288	219	207			
35	441		315	288	220	207			
40	441		315	288		207			
45	441		315	288		207	137		
50	441		315	288		207	137	94	
60	441			288		207		94	

Fig.5: The overlaid MSMS chromatogram of five impurities evaluated under 11 different collisions (55 data in 15 min) in ESI Negative mode

The positive mode nMS^2 compiled fragments have given the similarity ion of m/z207 ascertainging the presence of tetrazole ring in all the five impurities. The negative mode nMS^2 compiled fragments have given the comparative differentiation 15 units leaves the prediction to the possibility of methyl/alkyl group variance in the irbesartan impurities. Thus the data analysis has enabled us to observe the presence or absence of a methyl group leading to the formation of impurity-2 and impurity-5 respectively. All the impurities except impurity-3 have their origin from the raw material spiroketones used in the synthesis of irbesartan. Impurity-3 is already reported as USP impurity-A [7].

To match the origin of the impurity, the synthetic root is screened carefully giving raise to the origin of the impurities are from the step II. Based on the MSMS spectral analysis both in positive and negative mode, the structure of the process related impurities with the molecular weight was shown in the schematic representation in fig. 6.

Thus the MSMS spectral analysis both in positive and negative mode has ascertained the possible correlations and necessitated variances with respect to irbesartan. This "nMS^2" approach and the data correlation is highly important for the structural identification of any related impurities. The MSMS correlations provide the better understanding of the functional behavior.

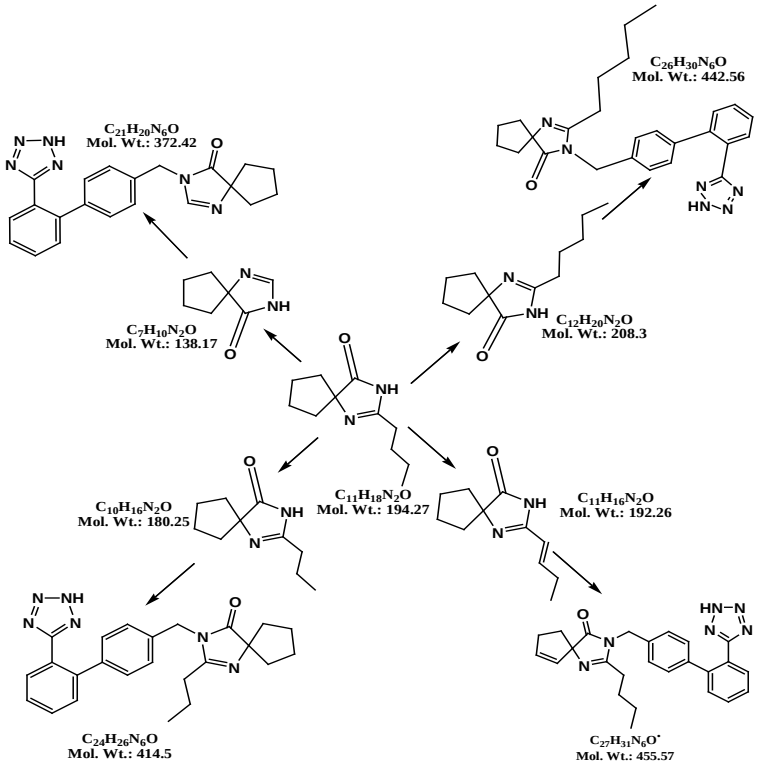


Fig. 6: The Schematic representation of Impurity origin in irbesartan

CONCLUSION

The MSMS approach of the "nMS²" technique was evaluated in detail for the structural identification the trace level impurities of Irbesarton. On the successful evaluation of the impurities, it was observed that the impurities are a methylated, demethylated and alkenylated analogue of irbesartan. This approach is very fast and efficient as the mass identification is done after chromatographic resolution. The product ion formed was predominately from the parental ion thus the ion reliability is always higher and more meaningful data for interpretation. The mass segmental analysis with "nMS²" approach is a technical advantage for the possible structural predictions of relative impurities in single analysis.

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AUTHORS CONTRIBUTIONS

Mr. Saravanan Subramaniyan has made contributions to design the experiment and performed the same. Dr. Thaminum Ansari Abubacker has made a substantial contribution in drafting and reviewing the article content critically.

CONFLICT OF INTERESTS

All authors declare that there is no conflict of interest associated with this article

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