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Mass Compatible Liquid Chromatography Method for Degradation Study of Aliskiren Hemifumarate and Identification of Adduct Impurity in Presence of Lacatose Excipient

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ABSTRACT

A rapid, specific and accurate stability indicating liquid chromatographic method compatible with Mass Spectrophotometer has been developed for Aliskiren Hemifumarate. The LC method is carried out on a Hypersil BDS C8 150*4.6 mm maintained at 25°C. The mobile phase consisted of 0.1 M ammonium acetate adjusted to pH 6.5±0.05 and mixed with Methanol in ratio of 375:200 v/v. A gradient programme used with acetonitrile with flow rate of 1.0 mL/min, with ʎmax 278 nm. The chromatographic separation is obtained with Aliskiren retention time at about 18 minutes and it is linear in the range 1 – 4 ppm (0.05% - 0.2 % of Test concentration). The specificity and stability-indicating capability of the method are proven through degradation studies, which also showed that, there is no interference of degradation products and peak due to excipients with main peak. Developed method was used for identification of Aliskiren Lactose adduct impurity. The method partially validated for its intended use and can be applicable for stability study.

Keywords: Aliskiren Hemifumarate, Aliskiren Lactose adduct, mass spectrophotometer.

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INTRODUCTION

Structural elucidation of degradation impurity is an essential part of drug development and pharmaceutical formulation development for a new drug entity. Information on impurity structures leads to the development of new compounds with improved stability and facilitates the design of more stable formulations. LC/UV is still the most commonly used technique for the detection and quantitative determination of degradation impurity. Most mobile phases used in the LC/UV methods require non-volatile buffers such as phosphate buffer to separate the peaks of interest from the degradation products. These mobile phases are typically not be used with a mass spectrometer for sensitivity and robustness reasons. However, when unknown degradation impurities are discovered during stability study or during stress studies, LC/UV is of little or no use for the identification step. Therefore, LC/MS is often used to determine the molecular mass and MS/MS is used to provide structural characterization[1]. In recent scenario, development of new drug uses mass detectors built around the needs of analytical scientists during chromatographic analysis is available. These are robust, reliable and requiring no sample adjustments, it integrates with current LC, UPLC and other purification systems. Aliskiren is a (ALS),(2S, 4S, 5S, 7S)- N-(2-carbamoyl-2-methylpropyl)-5-amino-4- hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifumarate (Figure. 1).The first oral direct rennin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the ranin system at its rate-limiting step by directly inhibiting the catalytic activity of ranin, thereby reducing generation of angiotensin I and angiotensin II. Millard L.R. suggested that primary and secondary amine undergoes condensation reaction with Lactose, a widely used excipients in pharmaceutical formulation as diluent. The resulting product is generally named as Glycosylamine, which results from the condensation reaction of lactose and amines with loss of one mole of water molecule. This condensation product undergoes in amadori rearrangement. Literature review suggested reaction between amines and lactose to be largely restricted to primary amines[2-5]. This article illustrate a possibility of Aliskiren hemifumarate to undergo Millard reaction with lactose to form its lactose adduct and has provided identification procedure of Aliskiren lactose adduct using developed high performance liquid chromatography, which is compatible with mass spectrophotometer. Literature survey on Degradation study and impurity characterization of Aliskiren hemifumarate, revels that, method can be used for its intended purpose for stability studies only[6,7]. HPLC method used for degradation study and impurity characterization uses non-

volatile buffer solution of potassium dihydrogen phosphate with 1-octane sulphonic acid salt as ion pair reagent. Multiple authors [8-11] have developed and published methods for assay content of Aliskiren alone or with combinations of angiotensin II receptor blocker, ACE inhibitor and Diuretics in past and used as reference during method development activity. Multiple validated methods[12, 13] for clinical and pre-clinical studies have been developed using phosphoric acid, ammonium acetate salts, trifloro acetic acid. F. Belal and co-authors[14] have suggested a validated automated reversed-phase liquid chromatography (RP-LC) method determination of Aliskiren in human plasma through derivatization with 1-naphthyl isocyanate. An HPLC method compatible with Mass spectroscopy was developed, which helps for identification of impurity of Aliskiren hemifumarate during product development activity and in broad view during stability study. The study also investigates the chromatographic behavior of Aliskiren hemifumarate in degradation condition of tablets formulation and a special study for degradation of drug with lactose has been carried out. An identification of Aliskiren adduct impurity in presence of Lactose has been carried out in this paper and detailed work such as Isolation, by preparative chromatography or flash chromatography and Characterization using NMR techniques are left in this paper. The method is partially validated using validation parameter like Specificity study and LOQ determination for intended purpose of proposed method during early stage development activity, like Pre-formulation study, identification of impurity and impurity profiling and tracking. The separation was performed on a Hypersil BDS C8 150*4.6 mm, 5µ column and Agilent 1200 infinity LC equipped with UV-Visible with EZChrom Elite compact version 3.3.2 SP2 software. As volatile mobile phase, buffer solution of Ammonium acetate with methanol and acetonitrile used by applying gradient programme. The analysis was completed within 30 min. The method is compatible with Mass spectroscopy and was executed as an LC-MS method, for identification of adduct impurity in presence of lactose.

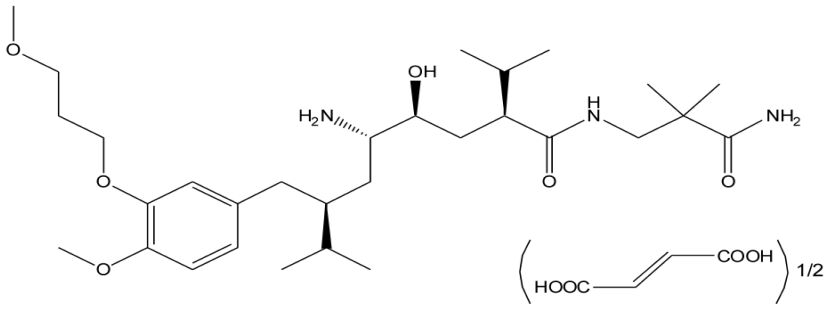


Figure-1: Aliskiren Hemifumarate

MATERIALS AND METHOD

Chemical and reagents

All chemicals used were of analytical or HPLC grade with High purity. Authentic sample of Aliskiren hemifumrate was kindly provided by Morepen Laboratories Ltd., Delhi, India and tablet formulation of Aliskiren hemifumarate (Rasilez) was obtained from local market. Methanol & acetonitrile (HPLC grade) was purchased from Spectrochem (Mumbai, India) and ammonium acetate (AR grade) was purchased from S.D. fine chem. Ltd. Nylon syringe filters (0.45 µm) were purchased from MillexHN, Millipore (Mumbai, India).

Instrumentation

Agilent 1200 infinity LC equipped with UV-Visible with EZChrom Elite compact version 3.3.2 SP2 software was used for practical work.

Methodology

High performance liquid chromatograms have been run on a Agilent 1200 infinity LC equipped with EZChrom Elite software using Hypersil BDS C8 150mm x 4.6 mm, 5µ column. 0.1M solution of Ammonium acetate is used for chromatographic separation using methanol and Acetonitrile with gradient programme of 30 minutes at 278 nm.

Mobile phase preparation:

*Buffer preparation: 3.85 gm of Ammonium acetate in 500 ml of purified water. Sonicated for 15 minutes.

Mobile phase (A): Prepare a mixture of 375 ml of *buffer preparation and 200 ml of Methanol.

Mobile phase (B): Acetonitrile (100%)

Run time: 30 minutes using Gradient programme**

Injection volume: 10 µl

Column temperature: At Room temperature 25°C

Sample temperature: At Room temperature 25°C

Flow rate: 1.0 ml

Diluent: 50 % methanol in water

**Gradient programme:

Time (minutes)	MP(A)	MP(B)
0.01	100	0
10.0	90	10
20.0	50	50
25.0	100	0
30.0	100	0

Blank preparation

Inject diluent as a blank.

System suitability solution (0.1 % solution)

A tablets powder equivalent to 40 mg of Aliskiren hemifumarate weighed and transferred to 20 ml volumetric flask. To it, 10 ml of diluent added and sonicated for 15 minutes to dissolve. Volume made up to mark with diluent. 0.1 ml of solution further diluted to 100 using same diluent.

Test solution preparation

A tablets powder equivalent to 40 mg of Aliskiren hemifumarate weighed and transferred to 20 ml volumetric flask. Add 10 ml of diluent and sonicated for 15 minutes to dissolve. Volume made up to mark with diluent. Filter resulting solution using 0.45 µ nylon filter and inject.

Partial Method Validation for Mass compatible Analytical method:

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present; these might include impurities, degradation products and formulation matrix. Force degradation study was performed on tablet formulation as per method for demonstration of the specificity of proposed method.

Preparation for specificity study

Test solution acid stress

A tablets powder equivalent to 40 mg of Aliskiren hemifumarate weighed and transferred to 20 ml volumetric flask. To it, 1 ml of 0.1N hydrochloric acid solution added and placed on water bath at 80°C for 4 hr. After 4 hr, the mixture was allowed to cool and neutralized with 0.1N sodium hydroxide solution. To the resulting solution 10 ml of diluent added and sonicated for 15 minutes to dissolve. Volume made up to mark with diluent.

Test solution alkaline stress

A tablets powder equivalent to 40 mg of Aliskiren hemifumarate weighed and transferred to 20 ml volumetric flask. To it, 1 ml of 0.1N sodium hydroxide solution added and placed on water bath at 80°C for 4 hr. After 4 hr, the mixture was allowed to cool and neutralized with 0.1N hydrochloric acid solution. To the resulting solution 10 ml of diluent added and sonicated for 15 minutes to dissolve. Volume made up to mark with diluent.

Test solution Oxidative stress

A tablets powder equivalent to 40 mg of Aliskiren hemifumarate weighed and transferred to 20 ml volumetric flask. To it, 1 ml of 10% H2O2 solution added and placed on water bath at 80°C for 4

hr. After 4 hr, the mixture was allowed to cool and to the resulting solution 10 ml of diluent added and sonicated for 15 minutes to dissolve. Volume made up to mark with diluent.

Test solution humidity stress

A tablets powder equivalent to 40 mg of Aliskiren hemifumarate weighed and transferred to 20 ml volumetric flask. To it, 1 ml of purified water added and placed on water bath at 80°C for 4 hr. After 4 hr, the mixture was allowed to cool and to the resulting solution 10 ml of diluent added and sonicated for 15 minutes to dissolve. Volume made upto mark with diluent.

Determination of Limit of quantification (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Test solution of Aliskiren 1.0 ppm (0.05% with respect to test concentration) was consider for LOQ level and area obtained with six replicate of injections were used for determination of LOQ level.

Procedure

A blank preparation, System suitability solution and test solutions (i.e acid stress, alkaline stress, Oxidative stress and humidity stress) injected in high performance liquid chromatography and record the chromatogram. A solution preparation for determination of Limit of quantification (LOQ) was injected for as per proposed method and record chromatogram.

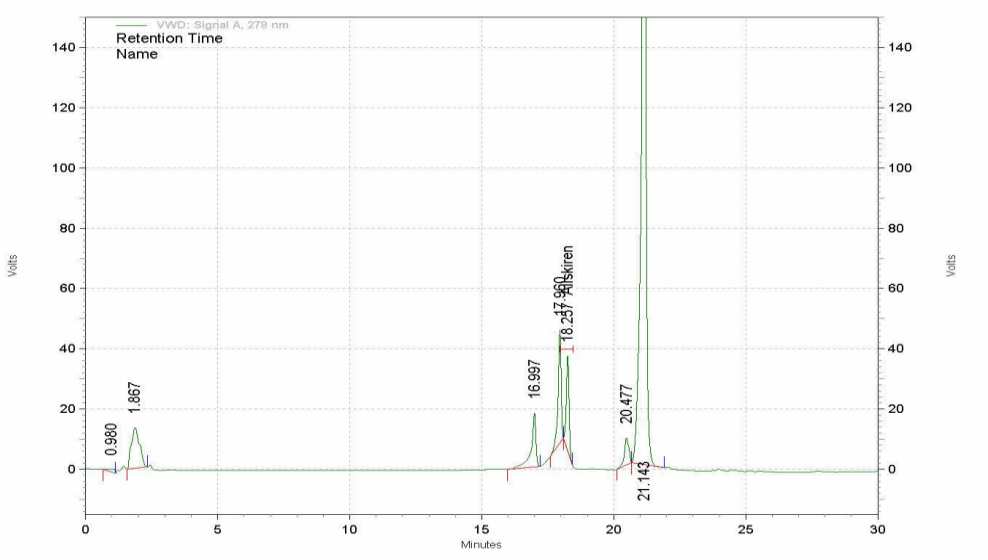
Preparation and identification of Aliskiren Lactose adducts

In a 25 mL round-bottom flask, 60 mg of ALS and 72 mg of Lactose (i.e reaction mole ratio 1:0.7) were transferred and to it 10 mL of 1-propanol was added as reaction media. Reaction mixture was refluxed at 80°c using water bath for 4 hours under controlled condition. A reaction mixture turns to red brown colored as reaction progressed. The resulting reaction mixture was cooled to room temperature and filtered. The residue washed with 5 ml of 1-propanol and the combined filtrate was collected in another 25 ml round-bottom flask. The combined filtrate was evaporated under vacuum to a thick solution. The brown sticky solid material referred as adduct mixture and used for HPLC analysis using proposed method to confirm reaction progress. The reaction mixture was dissolved in 50% methanol to obtain 200 ppm of solution and injected for HPLC analysis using proposed method. A solution of 1000 ppm of same reaction mixture was analyzed on LCMS with minor changed in chromatographic condition, to obtain similar separation to the HPLC method.

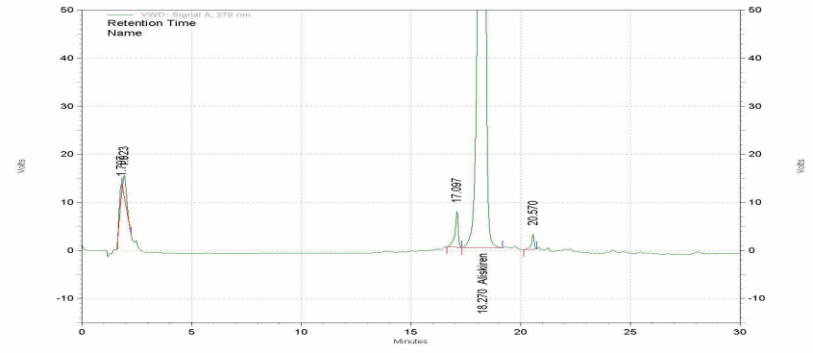
RESULTS AND DISCUSSION

Specificity

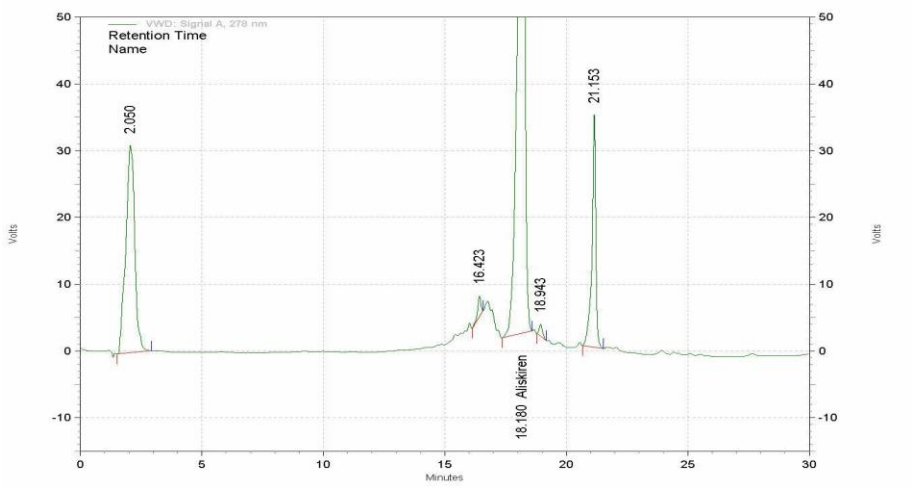
Based on recorded chromatogram (figure -2), data evaluation was carried out. Chromatographic parameter like capacity factor, theoretical plate and asymmetry were summarized in table-1. Test results of stress study are summarized in table-2, which shows acceptable mass balance by area normalization method. Interference of degradation impurity was evaluated using peak purity index of the Aliskiren peak and % degradation were calculated by area normalization method.



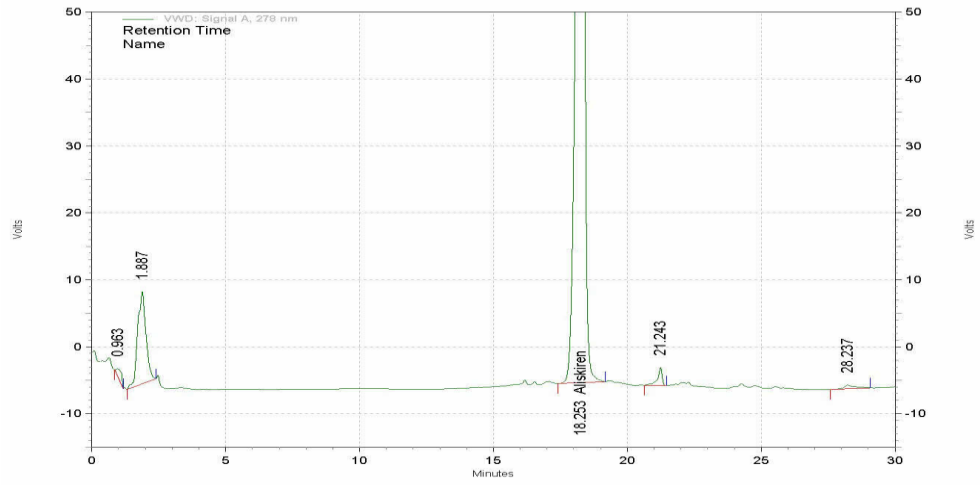
Chromatogram depicting Acid stress study



Chromatogram depicting Alkaline stress study



Chromatogram depicting Oxidative stress study



Chromatogram depicting Humidity stress study

Figure -2: Chromatograms depicting stress study

Table -1 Summary for chromatographic parameter

System suitability Test	Results obtained
Retention time of Fumaric acid peak	1.9
Retention time of Aliskiren peak	18.2
Capacity factor for Aliskiren peak	>8.0
Theoretical Plate (Aliskiren peak)	>5000
Asymmetry (Aliskiren peak)	<2.0

Table -2 Summary of Stress study of Tablet formulation

Stress condition	Peak purity	% of drug content	% impurity level	Impurity relative retention time (r.r.t.) with respect to Aliskiren peak
Acid stress	>0.99	5.28	80.33	1.15
			5.08	0.93
			6.94	0.98
Alkaline stress	>0.99	97.95	1.51	0.94
			0.54	1.13

Oxidative stress	>0.99	46.79	47.18 5.61	1.04 1.15
humidity stress	>0.99	99.13	0.59 0.29	1.16 1.55

Determination of Limit of Quantification:

% RSD results obtained based on area obtained from chromatogram of LOQ preparation is 3.25%, which shows that well acceptance for sensitivity of the proposed method.

Identification of Aliskiren Lactose adducts:

Chromatogram obtained shows that mixture consist of 43.8 % of lactose adduct and 56.2 % of Aliskiren by area normalization (Figure-3). Mass spectra (MS2) are presented in Figure-4. The mass spectrum obtained confirms molecular weight of 876.6 for peak at retention 12.7. Proposed structures for lactose adduct at retention 12.7 are shown in Figure-5. The molecular mass of unknown peak having retention time of 12.7 is confirm with the Aliskiren–lactose condensation product formed by the elimination of one mole of water molecule from the parent compounds (Scheme-1).

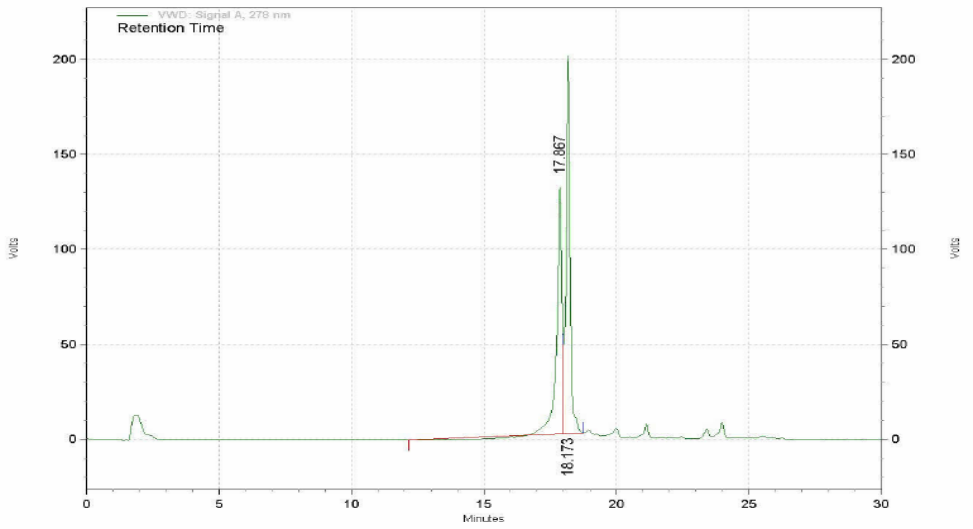


Figure -3: Chromatogram depicting Aliskiren adduct mixture

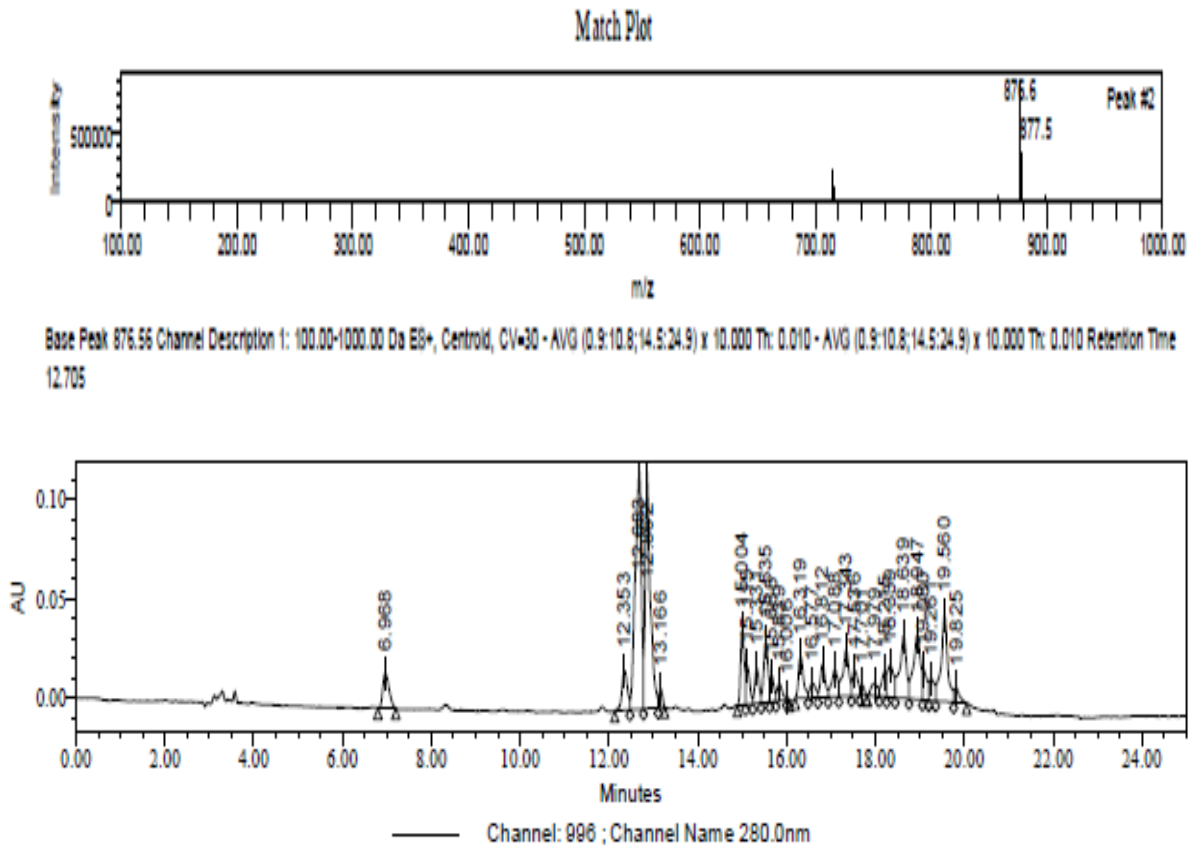


Figure – 4: LCMS Data for Identification of Lactose adduct

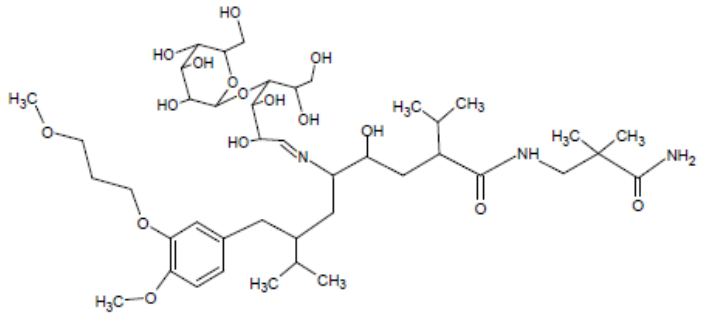
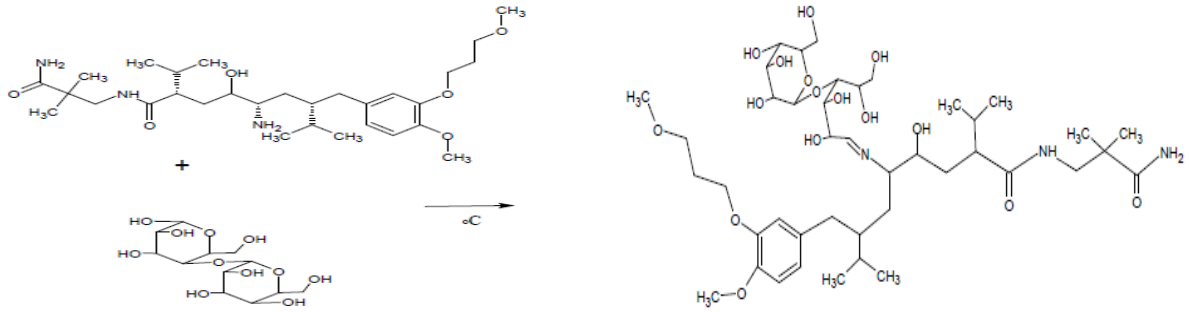


Figure-5: Structure of Lactose adduct



Scheme -1: Reaction mechanism-formation for Lactose adduct

CONCLUSION

Test results obtained from partial validation using proposed HPLC method shows that method is specific to degradation products and sensitive to 0.05% LOQ level. The developed method found specific, sensitive at 0.05% level and can be used for early stage development activity such as pre-formulation study and impurity identification process, with deliberated changes in methodology based on requirement. The proposed method is compatible to mass spectroscopy, so this method is applicable to Quality by Design prospective like peak picking, peak tracking and retention modeling to optimize the chromatographic conditions.

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