WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 1041-1063. Research Article ISSN 2277– 7105

# IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF DIMER IMPURITIES IN ANTIMUSCARINIC AGENT DARIFENACIN HYDROBROMIDE

**P. Badarinadh Gupta[1,2,*], B. Venkateswara Rao[2], S. Paul Douglas[2], Hemant Kumar Sharma[1] and D. Vijaya Bharathi[1]**

[1]APL Research Centre-II(A Division of Aurobindo Pharma Ltd), Survey No: 71&72, Indrakaran(V), Sangareddy (M), Medak Dist., Hyderabad – 502 329, Andhra Pradesh, India.

[2]Department of Engineering Chemistry, A. U. College of Engineering (A), Andhra University, Visakhapatnam– 530 003, Andhra Pradesh, India.

Article Received on
03 April 2017,

Revised on 23 March 2017,
Accepted on 13 May 2017

DOI: 10.20959/wjpr20176-8561

***Corresponding Author**
**P. Badarinadh Gupta**
APL Research Centre-II(A Division of Aurobindo Pharma Ltd), Survey No: 71&72, Indrakaran(V), Sangareddy (M), Medak Dist., Hyderabad – 502 329, Andhra Pradesh, India.

## ABSTRACT

Darifenacin hydrobromide, antimuscarinic agent with actions similar to those of atropine and has greater selectivity for the muscarinic receptors of the urinary bladder, is used to treat symptoms of urge urinary incontinence, urgency, and frequency. It is an antagonist on the effects of muscarinic receptors in cholinergically mediated functions including stimulation of salivary secretion. The present study describes the identification, isolation and characterization of two process related dimer impurities in darifenacin hydrobromide using high performance liquid chromatography (HPLC) analysis. Impurities were identified using liquid chromatography coupled with time of flight mass spectrometry (LC-MS/TOF). Proposed structures were unambiguously confirmed by characterization using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), mass spectroscopy and elemental analysis.

**KEYWORDS:** Darifenacin, Isolation and Characterization of dimer impurities, NMR.

## INTRODUCTION

Darifenacin Hydrobromide, is a potent muscarinic M3 receptor antagonist, used for the treatment of overactive bladder with symptoms of urge urinary incontinence. Overactive

bladder is a chronic and debilating condition, caused by the untimely contraction of the bladder muscle and resulting in urinary urgency.[1-8] The chemical name of Darifenacin hydrobromide (Fig. 1) is (s)2-{1-[2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide hydrobromide, having molecular mass of 426.55 with molecular formula $C_{28}H_{30}N_2O_2$.

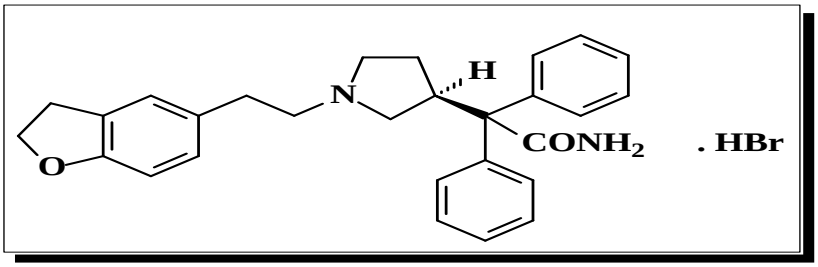



**Fig. 1**

Blockage of destructor muscle activity manifests in an increase in urine volume that the bladder can contain, reduction of urination frequency and decrease in pressure and urgency associated with the urge to urinate and thereby episodes of incontinence are reduced. Darifenacin works by blocking the M3 muscarinic acetylchloline receptor, which is responsible for bladder muscle contractions. It thereby decreases the urgency to urinate.[9-11] But it should not used in people with urinary retention. Darifenacin is approved in US and Canada as Enablex and Emselex in Europe is used to treat urinary incontinence as medication. Darifenacin has a clinically significant effect on bladder function and control.[12] The formation of new impurities which were obtained during the process development of Darifenacin hydrobromide has been studied in this research work. An earlier study in literature discloses the structure of Darifenacin dimer-1 and some spectral information of Darifenacin dimer impurity and some of the related impurities[13-15] have been identified and accordingly analytical methods developed for chromatographic separations. As per the stringent regulatory requirements, the impurities above threshold of ≥0.05% must be characterized. Based on all the spectral data, the impurities are characterized as Darifenacin dimer 2-[(3S)-1,1-bis[2-(2,3-dihydrobenzofuran5-yl)ethyl]-2,3,4,5-tetrahydropyrrol-3-yl]-2,2-diphenylacetamide,formate and Darifenacin dimer-1 as N-[2-(2,3-dihydrobenzofuran-5yl)ethyl]-2-[(3s)-1-[2(2,3-dihydrobenzofuran-5-l)ethyl]-pyrrolidin-3-yl]-2,2-diphenylacetamide. To the best of our knowledge, these impurities complete structural eluciadation information have not been reported in literature till date. Present work deals with identification, isolation, structural elucidation of impurities under a variety of ICH

recommended test conditions[16] A comprehensive study was undertaken to isolate and characterize these impurities by the spectroscopic techniques.

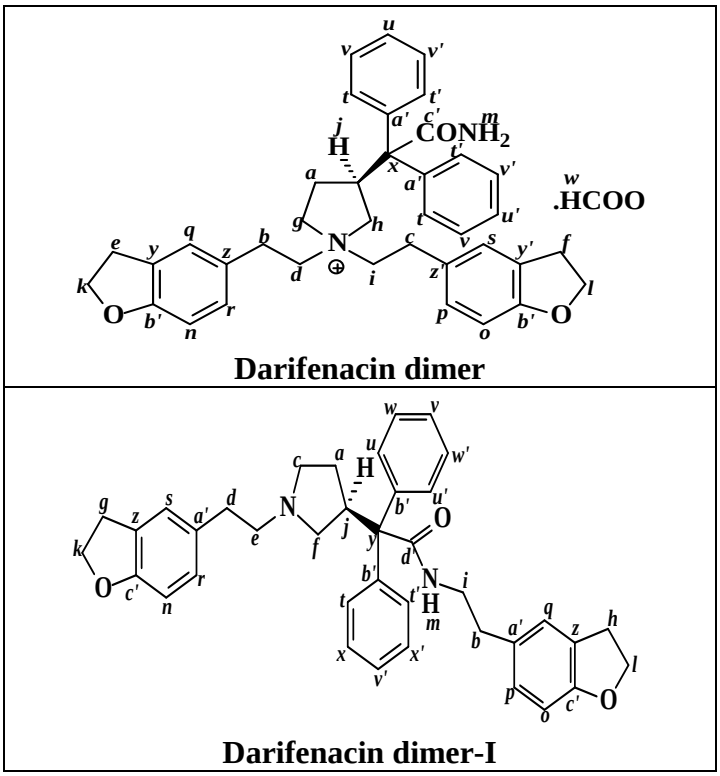



**Fig: 2 Impurities structure and its numbering for structural elucidation**

## 2. MATERIAL AND METHODS

### 2.1. Chemicals, Reagents and Samples

The investigated samples of Darifenacin hydrobromide and impurities were gifted from APL Research Centre-II Laboratories (A division of Aurobindo Pharma Ltd., Medak district, India). AR grade materials like Potassium dihydrogen orthophosphate, Ortho phosphoric acid, acetonitrile and potassium bromide (IR spectroscopy grade) were procured from Merck (India) limited, pure milli-Q water was used with the help of millipore purification system.

### 2.2. High Performanance liquid Chromatography (HPLC)

HPLC Chromatographic separations were performed on with Waters alliance 2695 separation module equipped with 2996 photodiode array detector with Empower pro data handling system [Waters Corporation, MILFORD, MA 01757, USA]. The analysis was carried out on Xbridge C18, 250mm long, 4.6mm i.d, 5 µm particle size column. Mobile phase A was phosphate buffer pH 3.5 (It was prepared by dissolving 2.72 g of potassium dihydrogen orthophosphate in 1000 ml water and 2ml of TEA and pH adjusted to 3.5 0±0.05 using diluted OPA solution). Mobile phase B was acetonitrile. Diluent was prepared by mixing

water and acetonitrile in the ratio of 70:30 v/v. Injection volume was 20µl, flow rate was kept as 1.0 ml/min and column oven temperature was maintained at 30°C. UV detection was carried out at 210 nm and data acquisition time was 50 min. Pump was in gradient mode and the program was as follows:

Time (min) / A (v/v): B (v/v); T0.01/75:25, T25/60:40, T35/45:55, T50/45:55, T52/75:25, T60/75:25.

### 2.3. Preparative Liquid Chromatography

A Shimadzu LC-8A preparative liquid chromatograph equipped with SPD-10A VP, UV-Vis detector [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] was used. Hyperprep HS C18, 500 mm long, 30 mm i.d., (make: Thermo Scientific) packed with 10 µm particle size was employed for isolation of the impurity. Mobile phase A was 1.0% ammonium acetate solution, pH adjusted to 5.0±0.1 with glacial acetic acid and mobile phase B was acetonitrile. Flow rate was 35 ml/min and UV detection was carried out at 210 nm. Various methods were tried to isolate the all impurities, using 1% ammonium formate / 0.2% acetic acid with acetnitrile / methanol combinations. Out of all experiments, we found 1% Ammonium formate with acetonitrile buffer suits to isolate the impurities in pure form. Finally the sample desalted / eluted with 0.01% formic acid with acetonitrile in the ratio of 30:70. The gradient program was as follows, Time (min)/A(v/v):B(v/v);T0.01/100:0, T10/90:10, T 25/80:20, T40/ 70:30, T55/ 60:40, T65/ 55:45, T80/ 50:50 T100/ 40:60, T120/ 20:80.

### 2.4. MS/LC-MS

MS/LC-MS analysis was carried out using a Perkin Elemer triple quadruple mass spectrometer (API 2000, PE SCIEX) coupled with a Shimadzu HPLC equipped with SPD10 AT VP UV-Vis detector and LC 10 AT VP pump(Foster city, CA). Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5kv and temperature was set at 375ºC. The auxillary gas and curtain gas used was high pure Nitrogen. Zero air was used as nebulizer gas. LC-MS spectra were acquired from m/z 100-1000 in 0.1 amu steps with 2.0s dwell time. The analysis was carried out by using Atlantis DS, 250mm long, 4.6mm i.d., 5µm particle diameter column. Mobile phase-A consists of 0.1% formic acid in $H_2O$ and mobile phase-B consists of 0.1% formic acid in methanol. UV detection was carried out 210nm, temperature maintained at 45ºC and the flow rate was kept as 0,7ml/min. Data acquisition was 60min. The gradient programme was as follows,

Time (min)/A(v/v): B(v/v); T0.01/100:0, T10/100:0, T20/80:20, T30/60:40, T50/40:60, T52/0:100, T60/0:100.

### 2.5. NMR Spectroscopy

$^{1}H$ NMR and $^{13}C$-NMR and all 2D experiments were performed on Agilent 500 MHz NMR spectrometer using deuterated chloroform ($CDCl_3$) as solvent and tetramethylsilane (TMS) as internal standard at 25°C temperature. For 1H NMR, 2D-NMR and $^{13}C$-NMR experiments, the operating frequencies were 500.1315 MHz and 125.4748 MHz; number of scans was 32 and 1062 respectively. LB 0.3 Hz parameter was used for data processing.

### 2.6. FT-IR Spectroscopy

IR spectra ware recorded as KBr pellet on Perkin Elmer Instrument model-Spectrum one.

## 3. RESULTS AND DISCUSSION

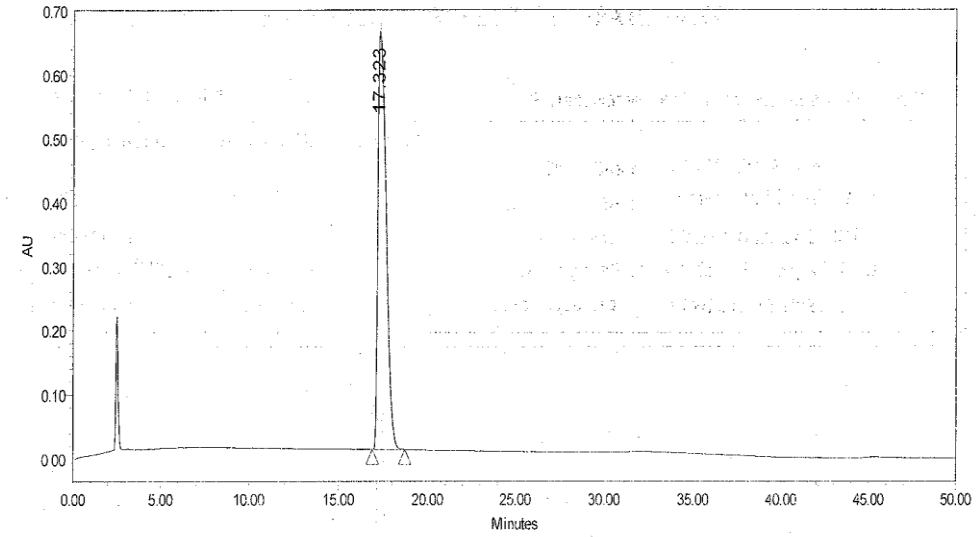
### 3.1. Detection of Impurities

HPLC analysis using the method described in Section 2.2 revealed the presence of two impurities at specified RT & RRTs with respect to Darifenacin peak tabulated in Table- 1. Aim of the research work is to identify and characterize the two impurities.

**Table: 1 Impurities RT & RRT**

| Impurity | 1 | 2 |
|---|---|---|
| RT | 32.92 | 36.34 |
| RRT(~) | 1.90 | 2.09 |

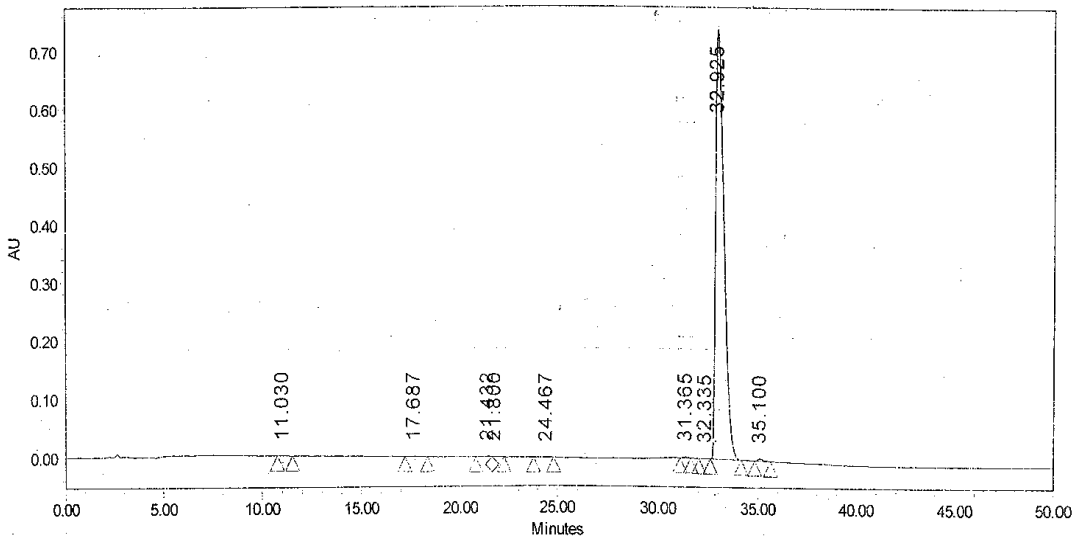
These impurities were isolated in small quantities by preparative isolation and co-injected into the HPLC for confirmation of relative retention time. The typical HPLC chromatograms of Darifenacin hydrobromide and the isolated impurity chromatograms were shown in Fig. 3, Fig. 3A and Fig. 3B, Structural elucidation and formation of impurities were discussed in the following sections. The chemical structures of these impurities along with numbering were shown in Fig. 2.




Peak Results

| | RT | Area (µV*sec) | % Area | Name |
|---|---|---|---|---|
| 1 | 17.323 | 20829434 | 100.00 | Darifenacin |

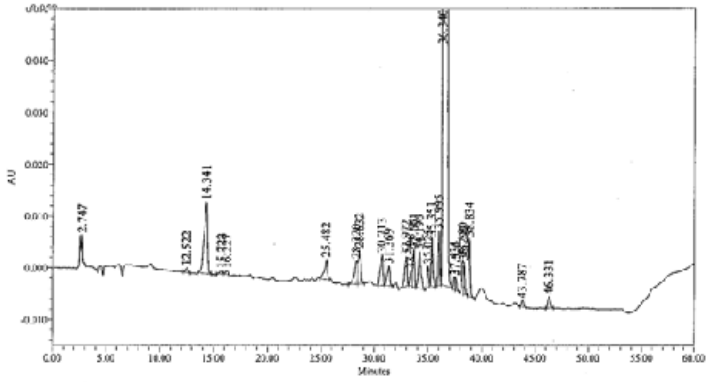
**Fig. 3 (A Typical representative HPLC chromatogram of Darifenacin drug substance)**




Peak Results

| | RT | Area (µV*sec) | % Area | Name |
|---|---|---|---|---|
| 1 | 11.030 | 13349 | 0.06 | Peak1 |
| 2 | 17.687 | 23446 | 0.11 | Peak2 |
| 3 | 21.432 | 31000 | 0.15 | Peak3 |
| 4 | 21.800 | 19453 | 0.09 | Peak4 |
| 5 | 24.467 | 11557 | 0.05 | Peak5 |
| 6 | 31.365 | 24179 | 0.11 | Peak6 |
| 7 | 32.335 | 9732 | 0.05 | Peak7 |
| 8 | 32.925 | 21150143 | 99.05 | Darifenacin Dimer |
| 9 | 35.100 | 71099 | 0.33 | Peak9 |

**Fig. 3A (A Typical representative HPLC chromatogram of Darifenacin dimer)**




SampleName Batch No.: RAT(1536)82; Date Acquired 05/03/2016 16:02:46 IST; Vial 4; Injection 1; Injection Volume 20.00; Run Time 60.00; Date Processed 03/03/2015 12:20:14 IST; Processed Channel Descr. MWD: Signal A, 210 nm/Bw4 nm

| | Retention Time (min) | Area (µV*sec) | % Area | RT Ratio | Name | Int Type |
|---|---|---|---|---|---|---|
| 1 | 2.747 | 13472 | 0.04 | 0.076 | Peak1 | BB |
| 2 | 12.522 | 14391 | 0.05 | 0.345 | Peak2 | BB |
| 3 | 14.341 | 308316 | 1.02 | 0.395 | Peak3 | BB |
| 4 | 15.722 | 11049 | 0.04 | 0.433 | Peak4 | BB |
| 5 | 16.227 | 11295 | 0.04 | 0.447 | Peak5 | BB |
| 6 | 25.482 | 71826 | 0.24 | 0.701 | Peak6 | BB |

| | Retention Time (min) | Area (µV*sec) | % Area | RT Ratio | Name | Int Type |
|---|---|---|---|---|---|---|
| 7 | 28.320 | 82587 | 0.27 | 0.779 | Peak7 | BV |
| 8 | 28.632 | 119659 | 0.40 | 0.788 | Peak8 | VB |
| 9 | 30.713 | 91636 | 0.30 | 0.845 | Peak9 | BB |
| 10 | 31.369 | 62719 | 0.21 | 0.863 | Peak10 | BB |
| 11 | 32.972 | 82055 | 0.27 | 0.907 | Peak11 | BB |
| 12 | 33.396 | 33489 | 0.11 | 0.919 | Peak12 | BV |
| 13 | 33.661 | 83077 | 0.27 | 0.926 | Peak13 | VV |
| 14 | 34.196 | 125960 | 0.42 | 0.941 | Peak14 | VB |
| 15 | 35.025 | 41904 | 0.14 | 0.964 | Peak15 | BB |
| 16 | 35.351 | 90867 | 0.30 | 0.973 | Peak16 | BB |
| 17 | 35.995 | 89594 | 0.30 | 0.991 | Peak17 | BB |
| 18 | 36.340 | 28592788 | 94.51 | 1.000 | Darifenacin Dimer-I | BB |
| 19 | 37.431 | 19386 | 0.06 | 1.030 | Peak19 | BV |
| 20 | 37.536 | 24055 | 0.08 | 1.033 | Peak20 | VB |
| 21 | 38.190 | 68966 | 0.23 | 1.051 | Peak21 | BV |
| 22 | 38.292 | 45111 | 0.15 | 1.054 | Peak22 | VV |
| 23 | 38.834 | 113537 | 0.38 | 1.069 | Peak23 | VB |
| 24 | 43.787 | 16720 | 0.06 | 1.205 | Peak24 | BB |
| 25 | 46.331 | 39121 | 0.13 | 1.275 | Peak25 | VB |

**Fig. 3B (A Typical representative HPLC chromatogram of Darifenacin dimer-1)**

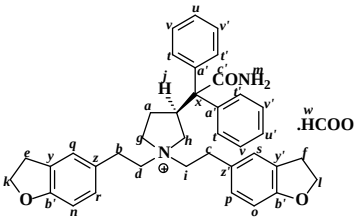
### 3.2. Characterization of impurities

#### 3.2.1. Darifenacin dimer

The ESI mass spectrum of Darifenacin dimer impurity displayed a protonated molecular ion m/z 573.3 amu [M+H]+ in positive ion mode, which corresponds to base molecular ion mass of 572 amu. There was no mass observed in negative ion mode confirms the structure could be in protonated stage. The proposed structure (Table- 2) of this impurity was supported by the presence of major fragments at m/z 147.1 obtained from the Mass & MS/MS spectra shown in Fig. 4A & Fig. 4B. Existence of fragment m/z 147.1 was supportive that Darifenacin dimer impurity having additional 2,3-dihydro ethyl benzofuran moiety. The main ambiguity is the attachment of 2,3-dihydro ethyl benzofuran moiety either on nitrogen atom of tetrahydro pyrrole moiety or on the amide nitrogen atom. As per the $^{1}$H-NMR data (Fig. 4C, 4D & 4E), additional protons related to 2,3 dihydro benzofuran moiety and amide protons were observed at δ6.32 & 6.61ppm in correlation with Darifenacin hydrobromide API. So attachment could not be on amide moiety. To confirm further 2D-NMR experiments

(COSY, HSQC, HMBC, TOCSY) were performed. Based on HMBC ($J_{2-3}$) bond correlations observed between methylene protons of tetrahydro pyrrole moiety with methylene protons of 2,3-dihydro ethyl benzofuran moiety confirms the attachment of ethylbenzofuran moiety. The complete proton, carbon assignments including 2D-NMR interpretations were tabulated below in Table. 3. The $^{13}$C-NMR, PENDANT NMR spectra were shown in Fig. 4F and 4G. The HSQC 2D-NMR spectra were shown in Fig 4H. The COSY 2D-NMR spectrum, where the through bond proton correlations were observed was shown in Fig. 4I. The HMBC 2D-NMR spectrum was shown in Fig. 4J and 4K. The TOCSY NMR (Fig. 4L) data confirms the overall correlations between the moieties. Based on the elemental analysis, theoretical values C, 73.78; H, 6.63; N, 4.53 and observed values C, 73.76; H, 6.62; N, 4.51 suggest that the elemental composition of this impurity is $C_{38}H_{41}N_2O_3$.

**Table- 2 Darifenacin dimer chemical name, structure, origin and control**

| **DARIFINACIN DIMER** |
|---|
| **CHEMICALNAME:** 2-[(3s)-1,1-bis[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-2,3,4,5-tetrahydropyrrol-3-yl]-2,2-diphenylacetamide, formate. |
|  |
| **MF:** $C_{38}H_{41}N_2O_3.HCO_2$ |
| |
| ***Origin & Control****: Darifenacin Dimer impurity forms during the condensation reaction of Darifenacin by condensing one more molecule of 5-(2-Bromoethyl)-2,3-dihydrobenzofuran (Bromoethyl DBF, Intermediate-B) to Darifenacin, which is already formed.* |

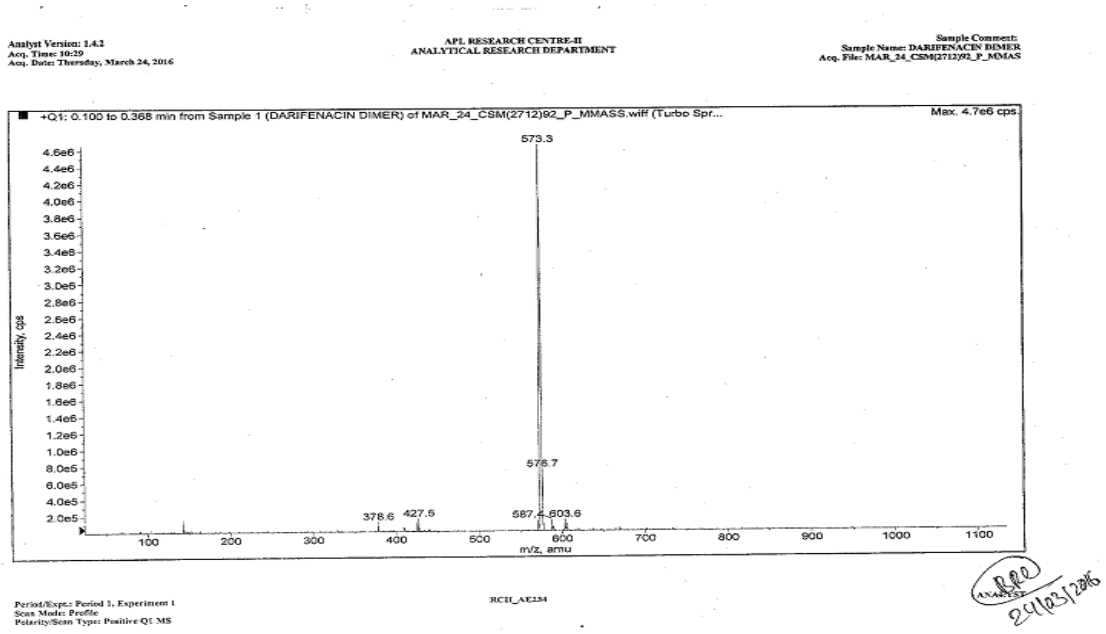



**Fig. 4A Darifenacin dimer Mass spectrum**

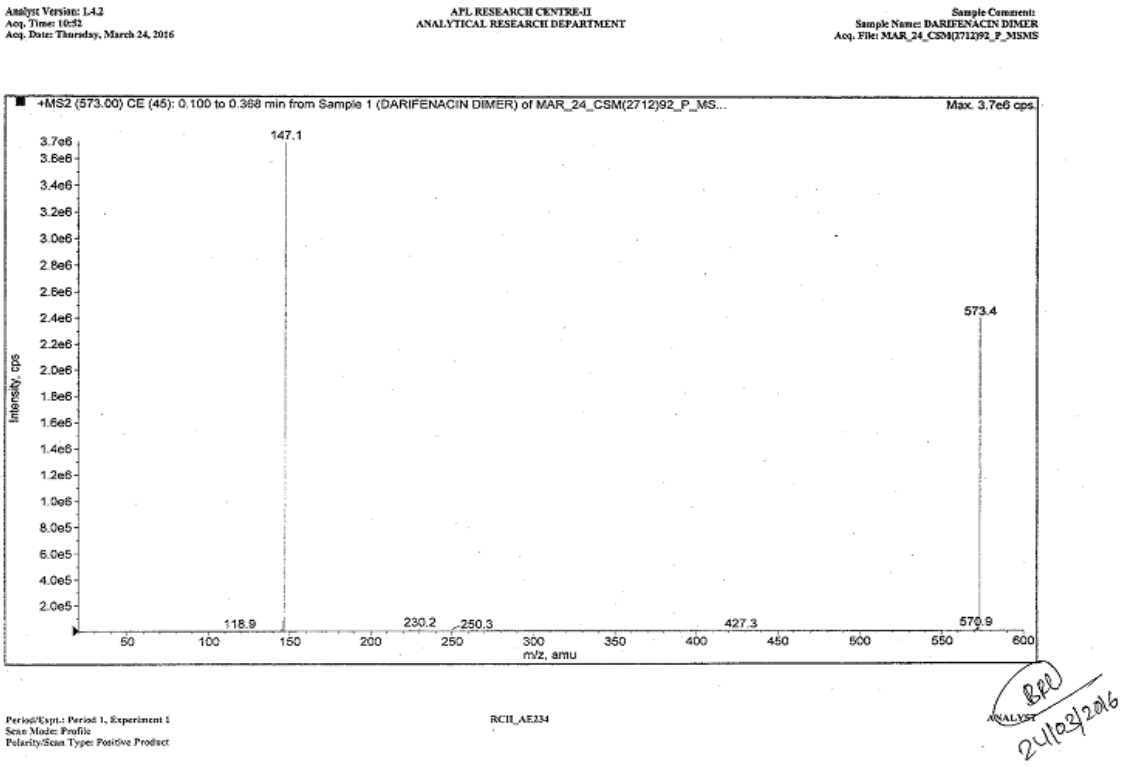



**Fig. 4B Darifenacin dimer MS/MS spectrum**

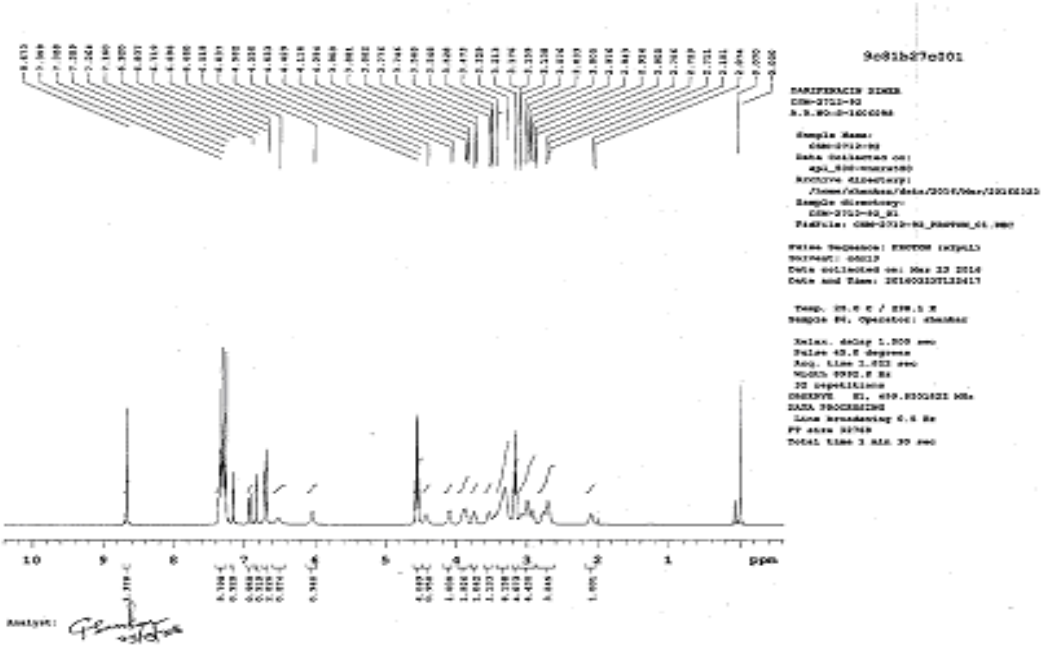


**Fig. 4C Darifenacin dimer NMR spectrum**

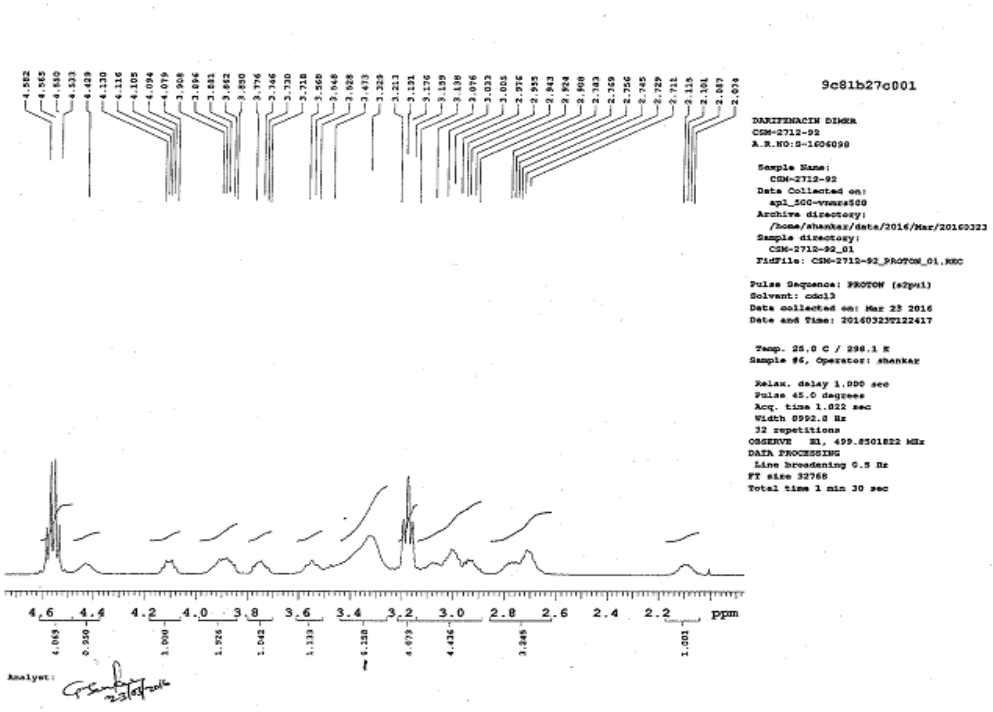


**Fig. 4D Darifenacin dimer NMR expansion spectrum**

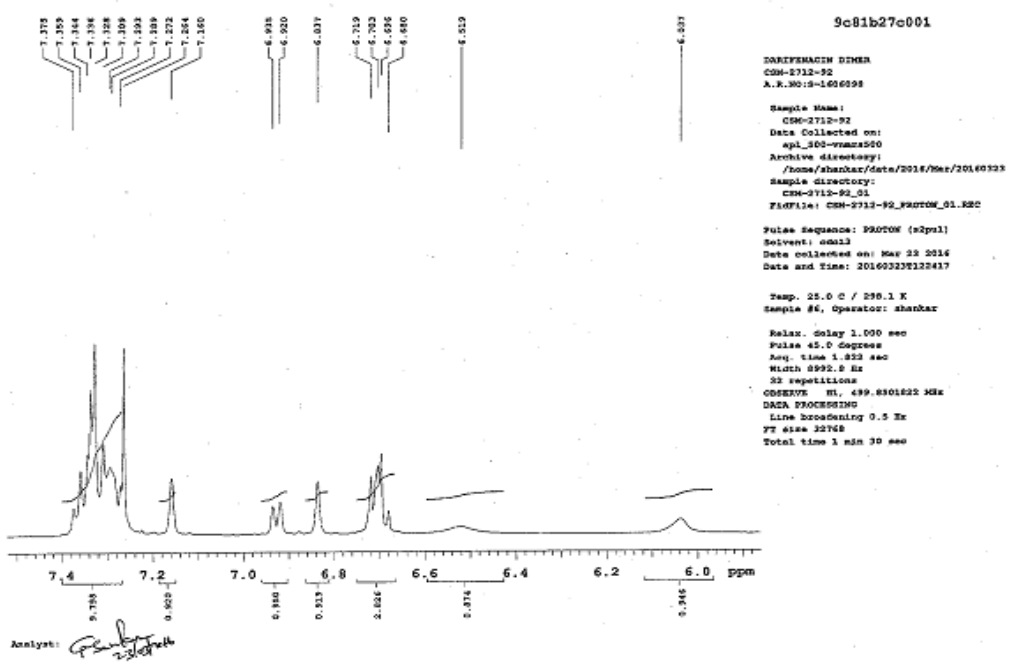



**Fig. 4E Darifenacin dimer NMR expansion spectrum**

**Table. 3 Darifenacin dimer complete $^{1}$H-NMR Interpretation**

| *Position* | *$\delta^{1}$H (ppm)* | *$\delta^{13}$C (ppm)* | *COSY* | *HMBC* |
|---|---|---|---|---|
| *a* | *2.08 & 2.72 (2m, 2H)* | *26.66* | *g, j* | *g* |
| *b* | *2.70 (m, 2H)* | *28.96* | *d* | *r, q* |
| *c* | *2.92 & 2.98 (2m, 2H)* | *29.25* | *i* | *i, p, s* |
| *d* | *3.04 (m, 2H)* | *62.09* | *b* | *b, g, i* |
| *e & f* | *3.15 (m, 4H)* | *29.51 & 29.54* | *k, l* | *k, l, q, s* |
| *g* | *3.34 & 4.00 (2m, 2H)* | *63.00* | *a* | *i* |
| *h* | *3.52 & 3.82 (t, 2H)* | *65.83* | *j* | *a, g, i* |
| *i* | *3.69 & 3.79 (2m, 2H)* | *60.98* | *c* | *c, g* |
| *j* | *4.26 (m, 1H)* | *42.02* | *a, h* | *g* |
| *k & l* | *4.53 (m, 4H)* | *71.33 & 71.36* | *e, f* | *e, f* |
| *m* | *6.32 & 6.61 (2brs, 2H)* | - | - | - |
| *n & o* | *6.68 (m, 2H)* | *109.56, 109.59* | *p, r* | *e, f, q, s* |
| *p & r* | *6.90 & 6.72 (2d, 2H)* | *128.14 & 128.28* | *n, o* | *b, c, q, s* |
| *q & s* | *6.83 & 7.12 (2s, 2H)* | *125.19 & 125.46* | *p, r* | *c, e, f, p, r* |
| *t, t', u, u', v, v'* | *7.26-7.35 (m, 10H)* | *127.62,127.93, 128.71, 128.73,* | - | - |

| | | | | |
|---|---|---|---|---|
| *w* | *8.61 (s, 1H)* | *167.39* | - | - |
| *x* | - | *62.60* | - | *a, t, t'* |
| *y & y'* | - | *126.18 & 126.61* | - | *b, c, e, f, n, o, p, r* |
| *z & z'* | - | *128.03, 128.21* | - | *e, f, k, l, n, o* |
| *a'* | - | *140.78, 141.31* | - | *v, v'* |
| *b'* | - | *159.53* | - | *e, f, k, l, n, o, p, r, q , s* |
| *c'* | - | *175.30* | - | - |

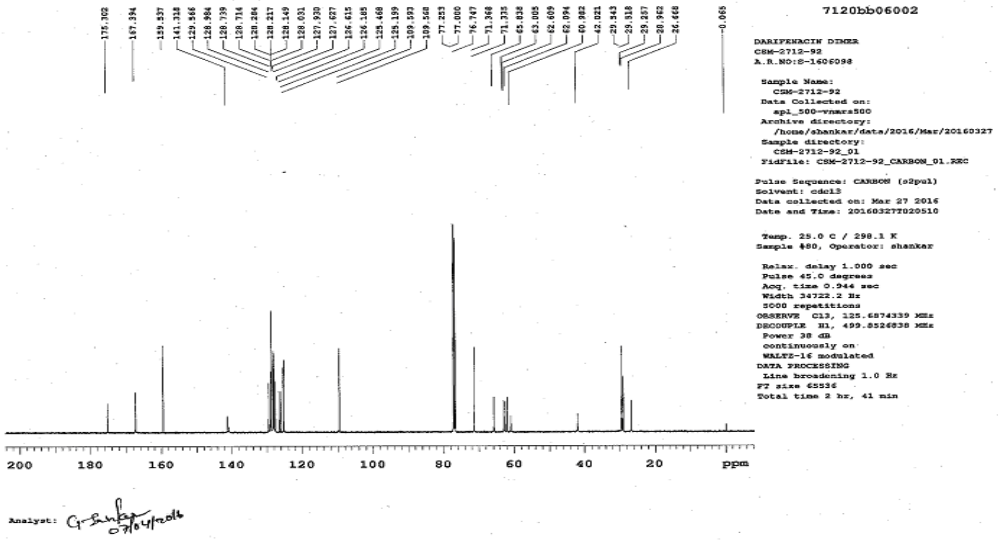



**Fig. 4F Darifenacin dimer complete $^{13}$C-NMR spectrum**

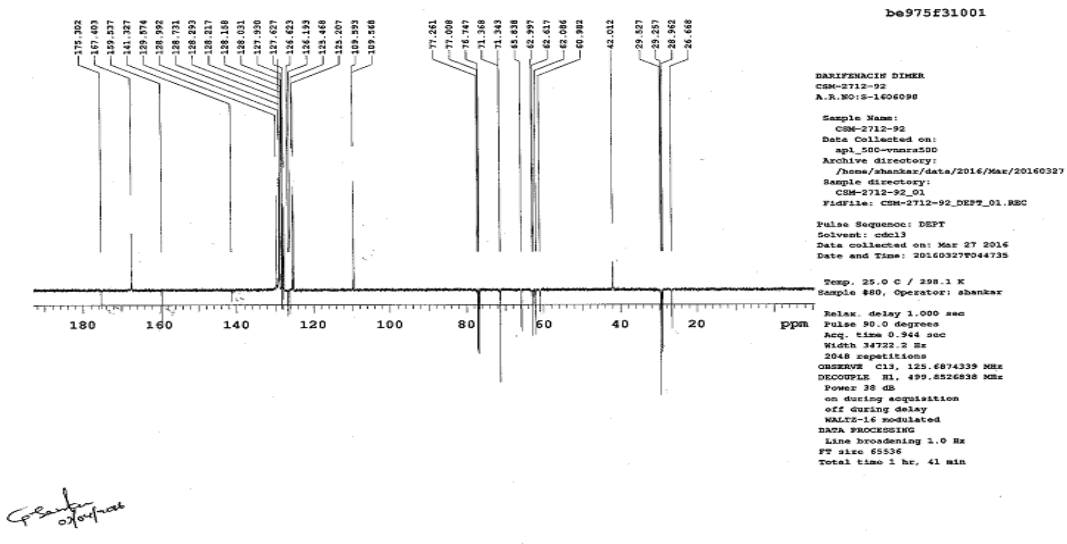



**Fig. 4G Darifenacin dimer PENDANT NMR spectrum**

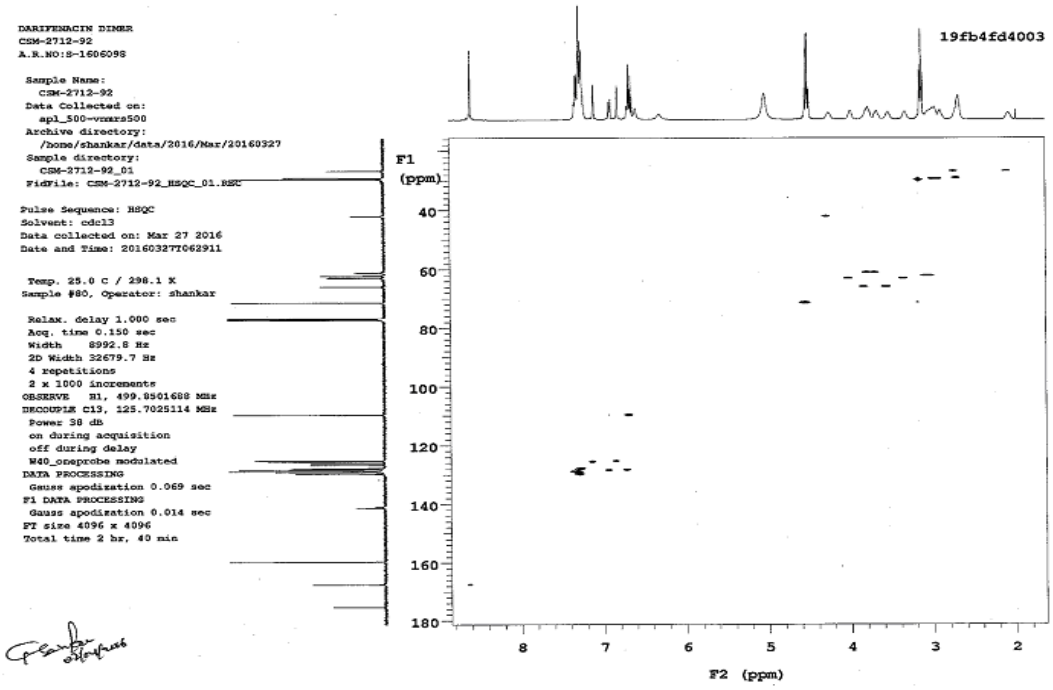



**Fig. 4H Darifenacin dimer HSQC NMR spectrum**

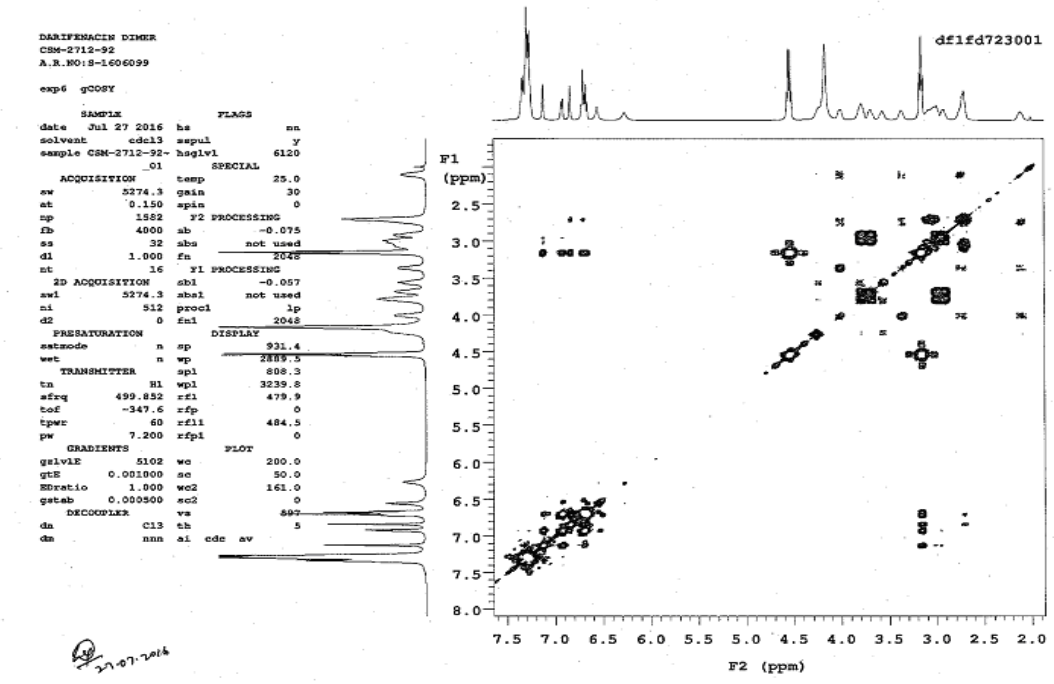



**Fig. 4I Darifenacin dimer COSY NMR spectrum**

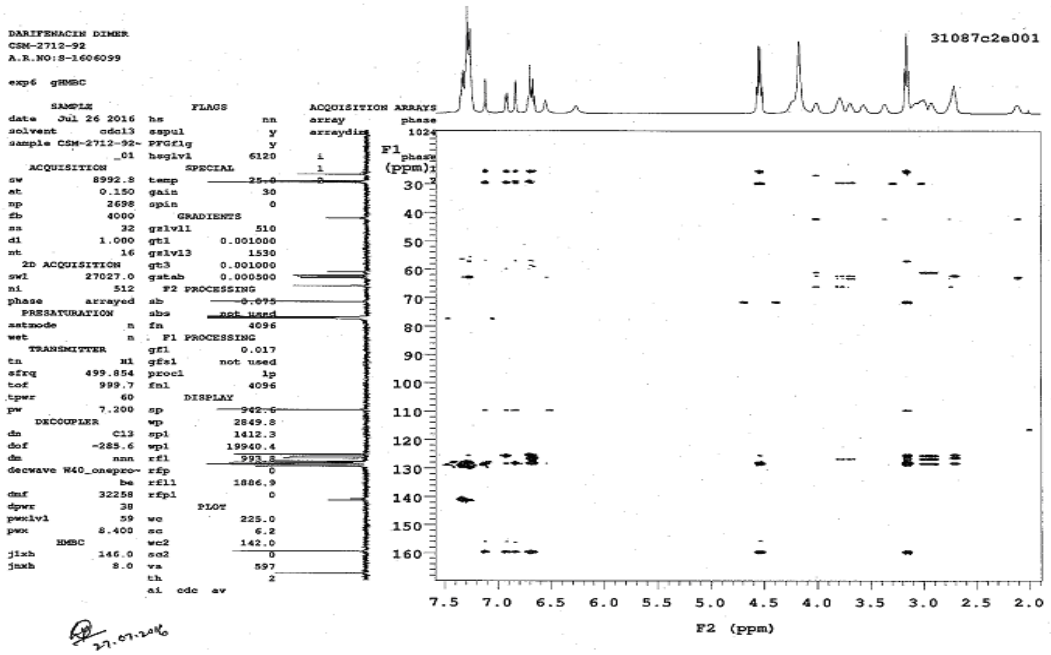



**Fig. 4J Darifenacin dimer HMBC NMR spectrum**

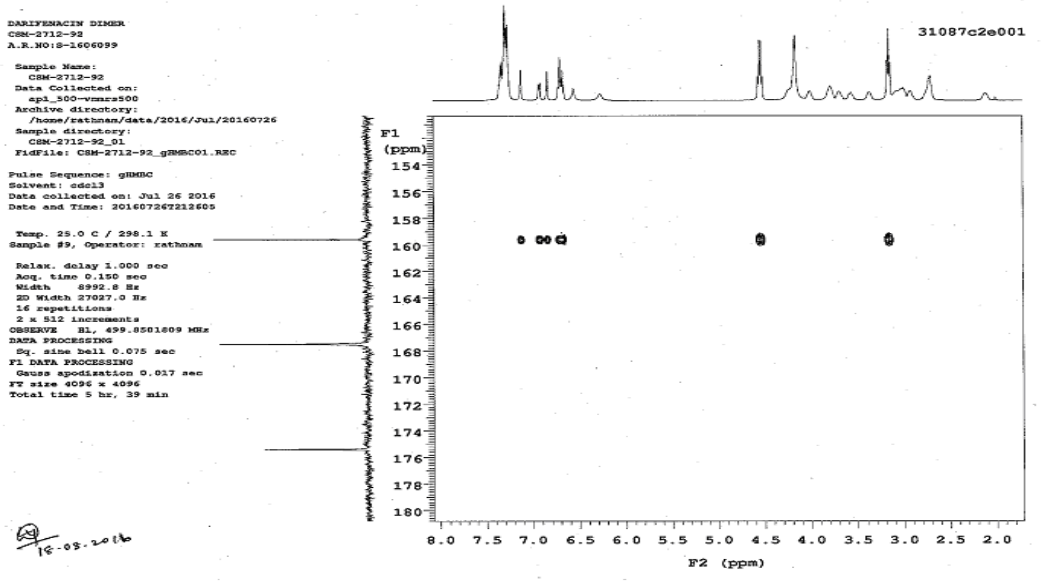



**Fig. 4K Darifenacin dimer HMBC expansion NMR spectrum**

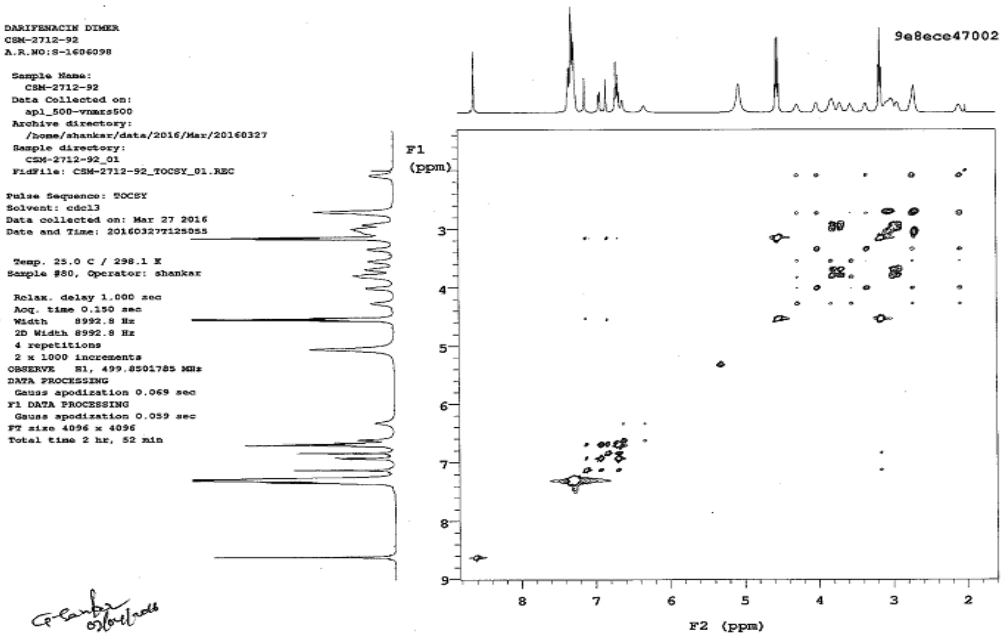
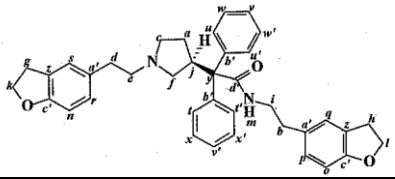



**Fig. 4L Darifenacin dimer TOCSY NMR spectra**

### 3.2.2. Darifenacin dimer-1

**Table- 4 Darifenacin dimer-1 chemical name, structure, origin and control**

| **DARIFINACIN DIMER-1** |
|---|
| **CHEMICALNAME:** n-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-2-[(3s)-1-[2-(2,3-dihydro-benzofuran-5-yl)ethyl]-pyrrolidin-3-yl]-2,2-diphenyl acetamide |
| **Molecular Structure**  |
| **MF:** $C_{38}H_{40}N_2O_3$ |
| |
| ***Origin & Control****: Darifenacin Dimer impurity forms during the condensation reaction of Darifenacin by condensing one more molecule of 5-(2-Bromoethyl)-2,3-dihydrobenzofuran (Bromoethyl DBF, Intermediate-B) to Darifenacin, which is already formed.* |

The ESI mass spectrum of Darifenacin dimer-1(Table. 4) impurity displayed a protonated molecular ion m/z 573.2 amu [M+H]+ in positive ion mode, which corresponds to molecular ion mass of 572 amu. Both Darifenacin dimer and Darifenacin dimer-1 impurities shows similar mass of 572 molecular ion. NMR data shows similar number of protons but showed some protons chemical shift differences in comparison with Darifenacin dimer impurity. This impurity NMR data also showed additional protons corresponding to ethyl benzofuran moiety. The main ambiguity is the attachment of 2,3-dihydro ethyl benzofuran moiety either

on nitrogen atom of tetrahydro pyrrole moiety or on the amide nitrogen atom. In $^{1}$H-NMR, amide (-$NH_2$) protons were not observed, but showed amide -NH proton at δ6.08 ppm. To confirm further 2D-NMR experiments (COSY, HSQC, HMBC, TOCSY) were performed. Based on HMBC data, correlations observed between amide carbonyl carbon with methylene protons 2,3-dihydro ethyl benzofuran moiety confirms the attachment of ethyl benzofuran moiety at amide position. Whereas Darifenacin dimer impurity does not show any HMBC correlations between amide carbonyl carbon with methylene protons of tetrahydro pyrrole moiety confirms the attachment. Based on all spectral data, both impurities are positional isomers. The complete proton, carbon assignments including 2D-NMR interpretations were tabulated below in Table. 5. The $^{1}$H-NMR spectra was shown in Fig. 5A. The $^{13}$C-NMR, PENDANT NMR spectra were shown in Fig. 5B, 5C. The HSQC 2D-NMR spectra were shown in Fig. 5D. The HMBC 2D-NMR spectrum was shown in Fig. 5E and 5F. The COSY 2D-NMR spectrum was shown in Fig. 5G. The TOCSY NMR spectrum was shown in Fig. 5H. The mass spectrum was shown in Fig. 5I. The IR spectrum was shown in Fig. 5J. The complete NMR, MS spectral data was useful to elucidate the Darifenacin dimer-1 impurity structure as N-[2-(2,3-dihydrobenzofuran-5yl)ethyl]-2-[(3s)-1-[2(2,3-dihydrobenzofuran-5-l)ethyl]-pyrrolidin-3-yl]-2,2-diphenylacetamide. Based on the elemental analysis, theoretical values C, 79.72; H, 6.99; N, 4.89 and observed values C, 79.70; H, 6.98; N, 4.88 suggest that the elemental composition of this impurity is $C_{38}H_{40}N_2O_3$.

**Table. 5 Darifenacin dimer-1 complete $^{1}$H-NMR Interpretation**

| *Position* | *$\delta^{1}$H (ppm)* | *$\delta^{13}$C (ppm)* | *COSY* | *HMBC* |
|---|---|---|---|---|
| *a* | *1.82 & 2.30 (2m, 2H)* | *28.52* | *c, j* | *c, f, j* |
| *b* | *2.61 (t, 2H)* | *34.85* | *i* | *i, p, q* |
| *c* | *2.63 & 3.13 (2t, 2H)* | *53.64* | *a* | *a, e, f* |
| *d* | *2.74 (m, 2H)* | *32.82* | *e* | *e, r, s* |
| *e* | *2.82 (m, 2H)* | *57.01* | *d* | *d, c, f* |
| *f* | *2.92 & 3.14 (2m, 2H)* | *57.08* | *j* | *c, e, j* |
| *g & h* | *3.10 (t, 4H)* | *29.77 & 29.79* | *k, l* | *k, l, q, s* |
| *i* | *3.39 & 3.50 (2m, 2H)* | *41.13* | *b, m* | *b* |
| *j* | *3.61 (m, 1H)* | *44.27* | *a, j* | *a, c, f* |
| *k & l* | *4.51 (t, 4H)* | *71.25 & 71.26* | *g, h* | *g, h* |
| *m* | *6.08 (brs, 1H)* | - | *i* | - |

| | | | | |
|---|---|---|---|---|
| *n & o* | *6.60 & 6.65 (2d, 2H)* | *109.20 & 109.26* | *p, r* | *g, h, s, q* |
| *p & r* | *6.67 & 6.85 (dd, 2H)* | *128.11 & 128.21* | *n, o* | *b, q, s* |
| *q & s* | *6.82 & 6.97 (2s, 2H)* | *125.18 & 125.26* | - | *b, g, h, p, r* |
| *t, t' & u, u'* | *7.09 & 7.17 (2d, 4H)* | *129.13 & 129.42* | *x, x', w, w'* | *x, x', w, w', v, v'* |
| *v, v', w, w', x, x'* | *7.22-7.28 (m, 6H)* | *127.35 128.39, 128.52, 128.63* | - | *t, t', u, u'* |
| *y* | - | *63.56* | - | *a, f, j, t, t', u, u'* |
| *z* | - | *127.14* | - | *g, h, k, l, n, o* |
| *a'* | - | *130.27 & 130.42* | - | *b, d, e, i, n, o* |
| *b'* | - | *143.24 & 143.31* | - | *t, t', u, u', w, w', x, x'* |
| *c'* | - | *158.82* | - | *g, h, k, l, o, p, q, s* |
| *d'* | - | *173.01* | - | *i, j* |

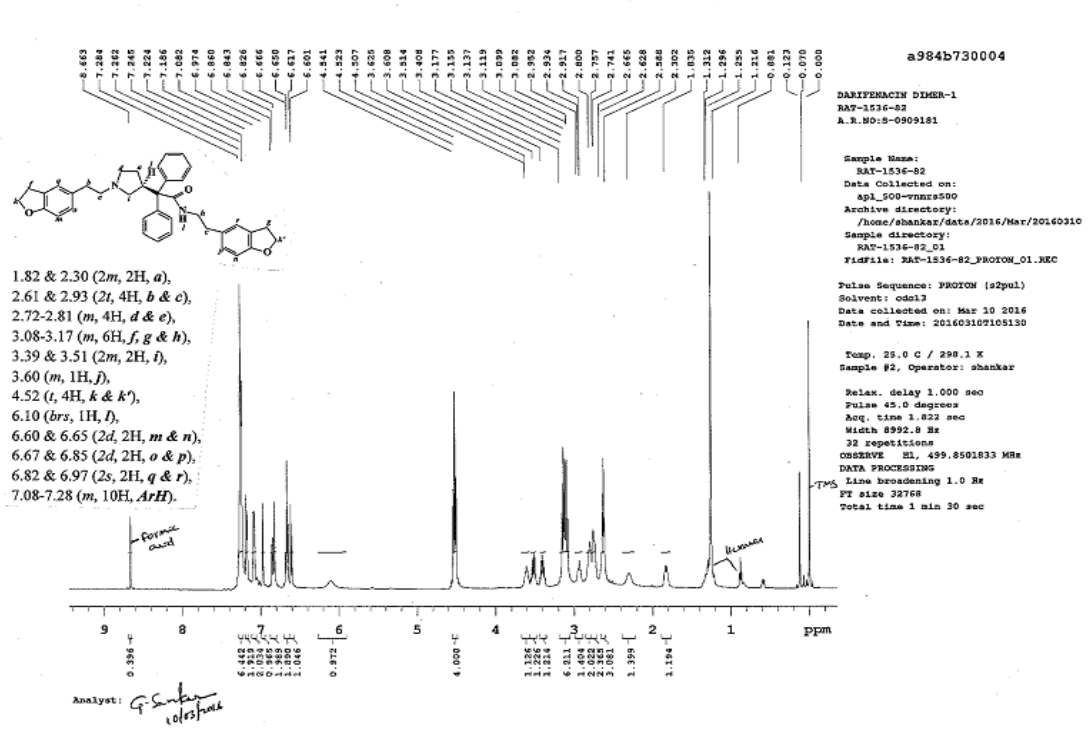



**Fig. 5A Darifenacin dimer-1 $^{1}$H-NMR spectra**

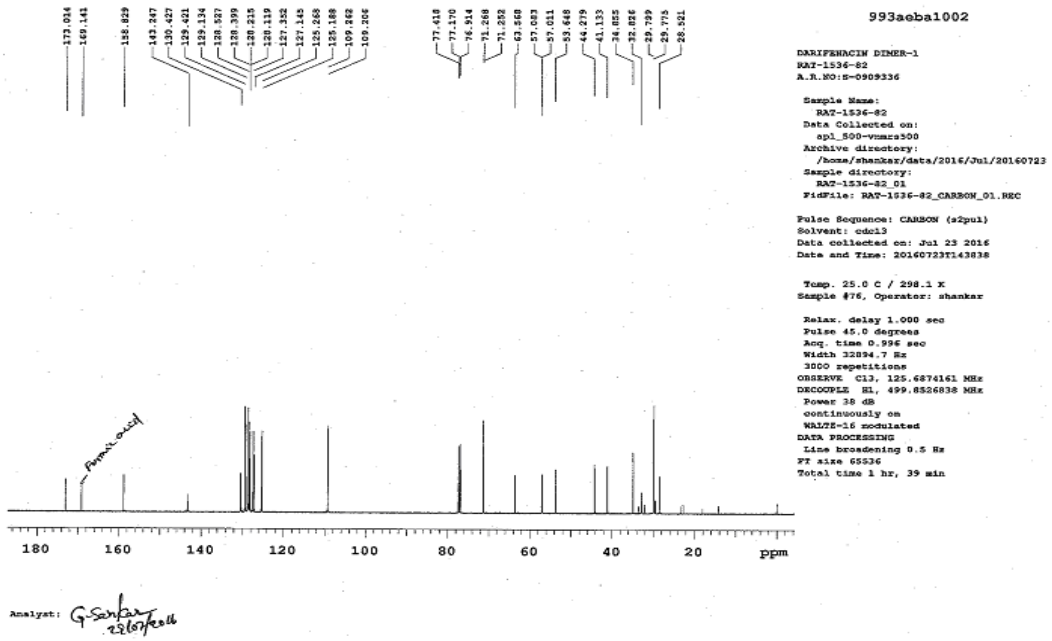



**Fig. 5B Darifenacin dimer-1 $^{13}$C-NMR spectrum**

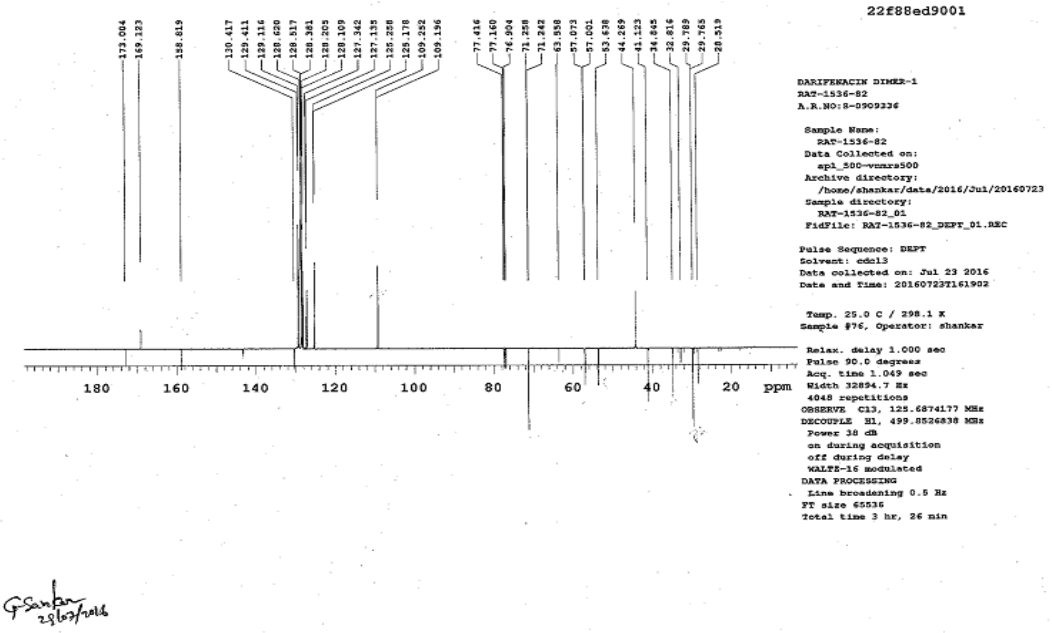



**Fig. 5C Darifenacin dimer-1 PENDANT-NMR spectrum**

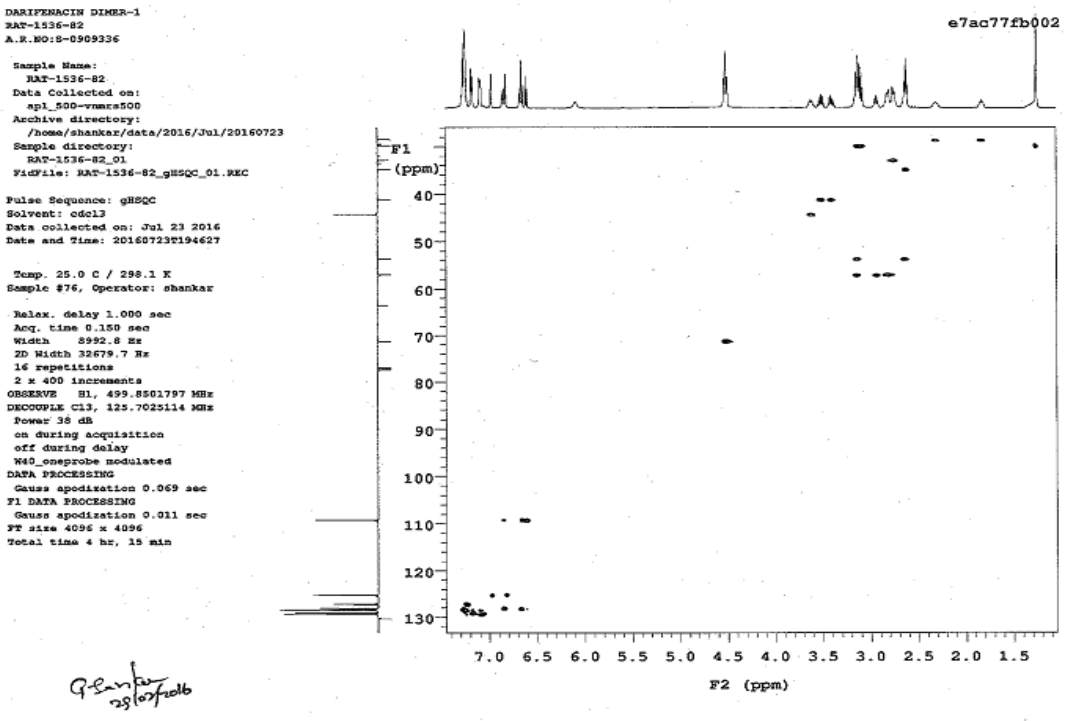



**Fig. 5D Darifenacin dimer-1 HSQC-NMR spectrum**

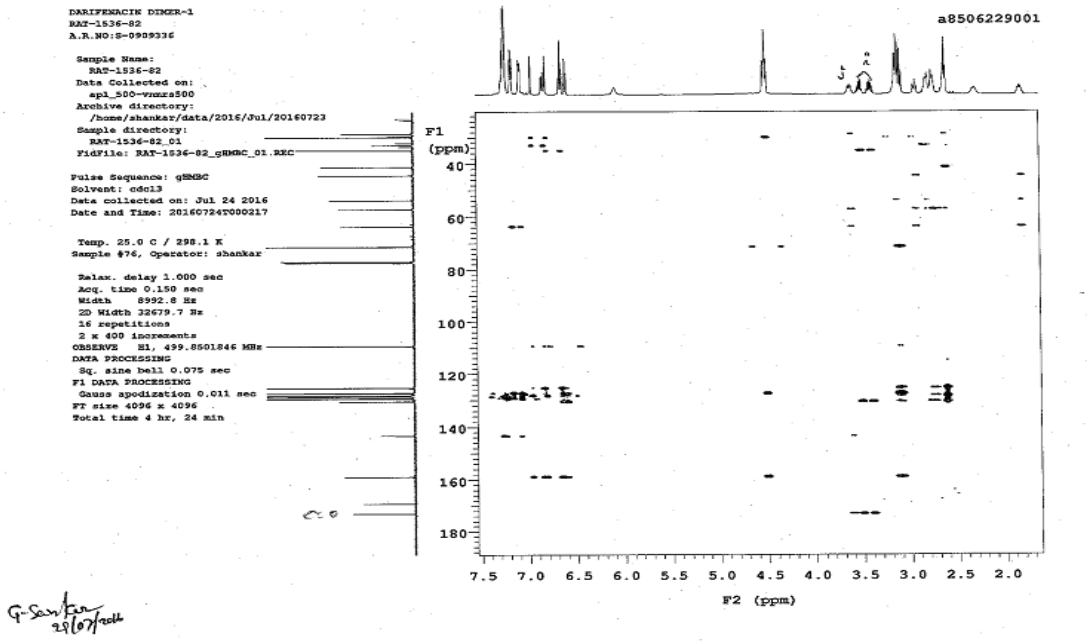



**Fig. 5E Darifenacin dimer-1 HMBC-NMR spectrum.**

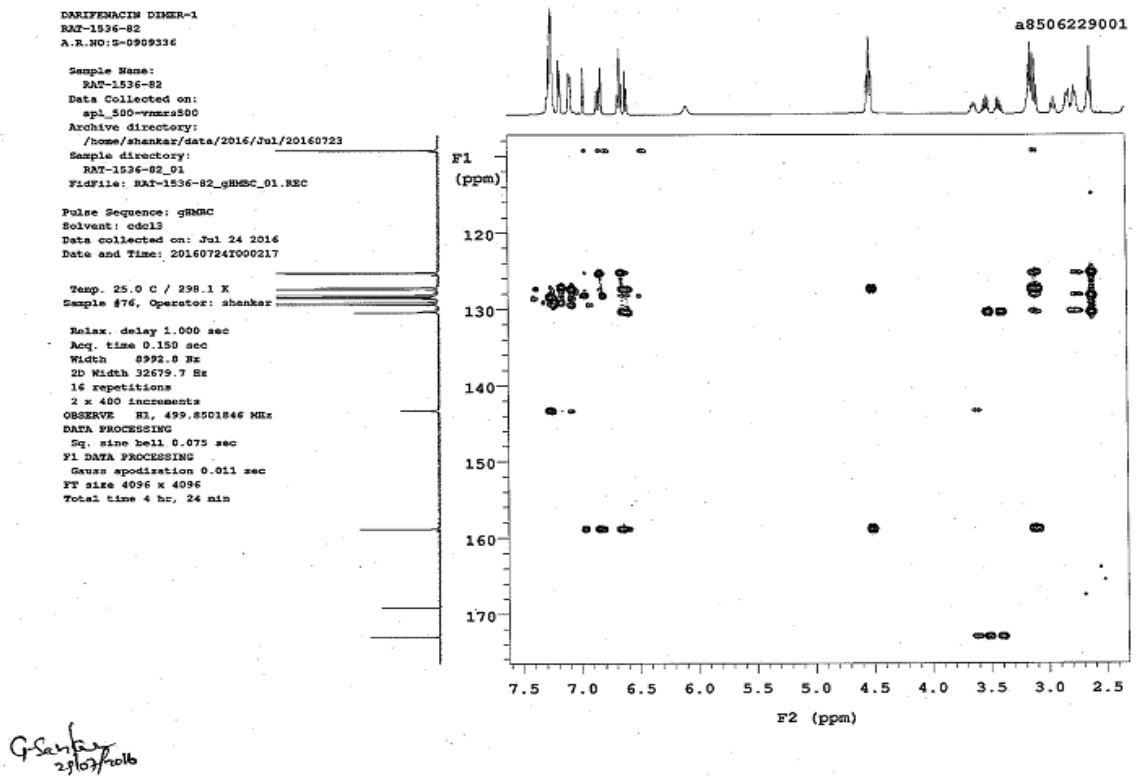



**Fig. 5F Darifenacin dimer-1 HMBC-NMR expansion spectrum**

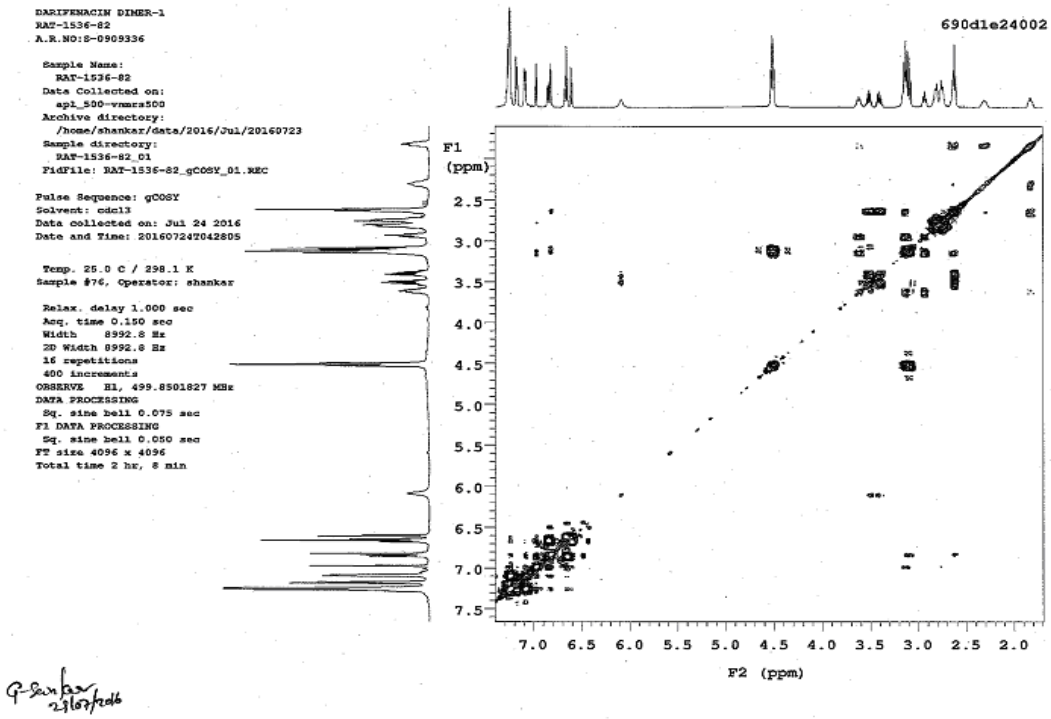



**Fig. 5G Darifenacin dimer-1 COSY-NMR spectrum.**

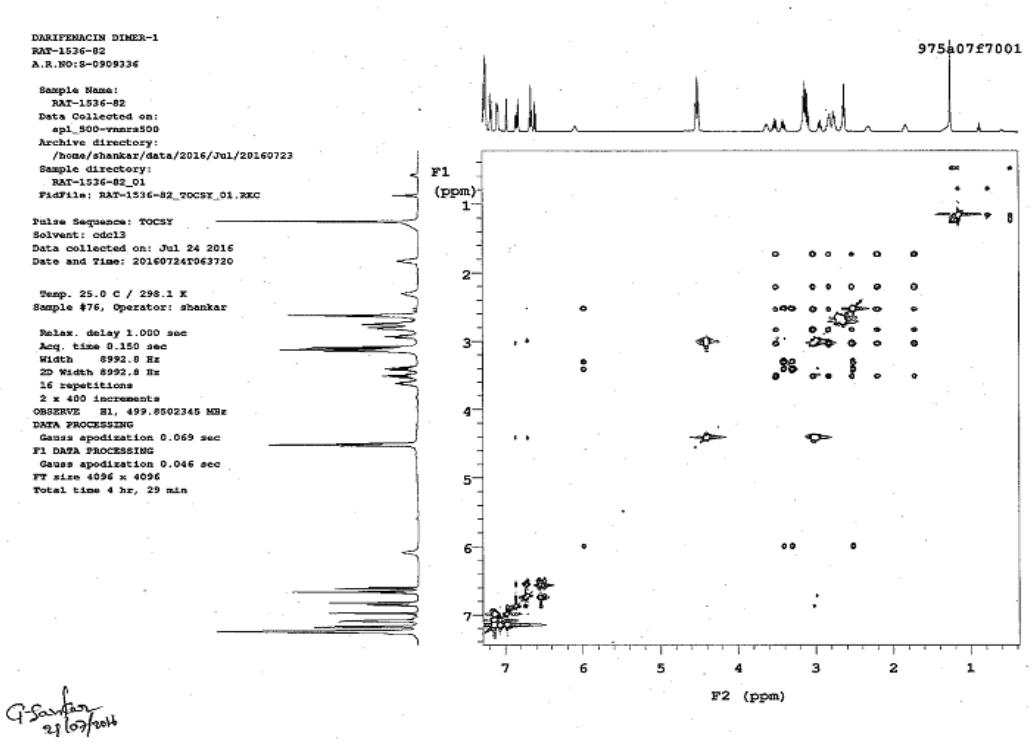



**Fig. 5H Darifenacin dimer-1 TOCSY-NMR spectrum**

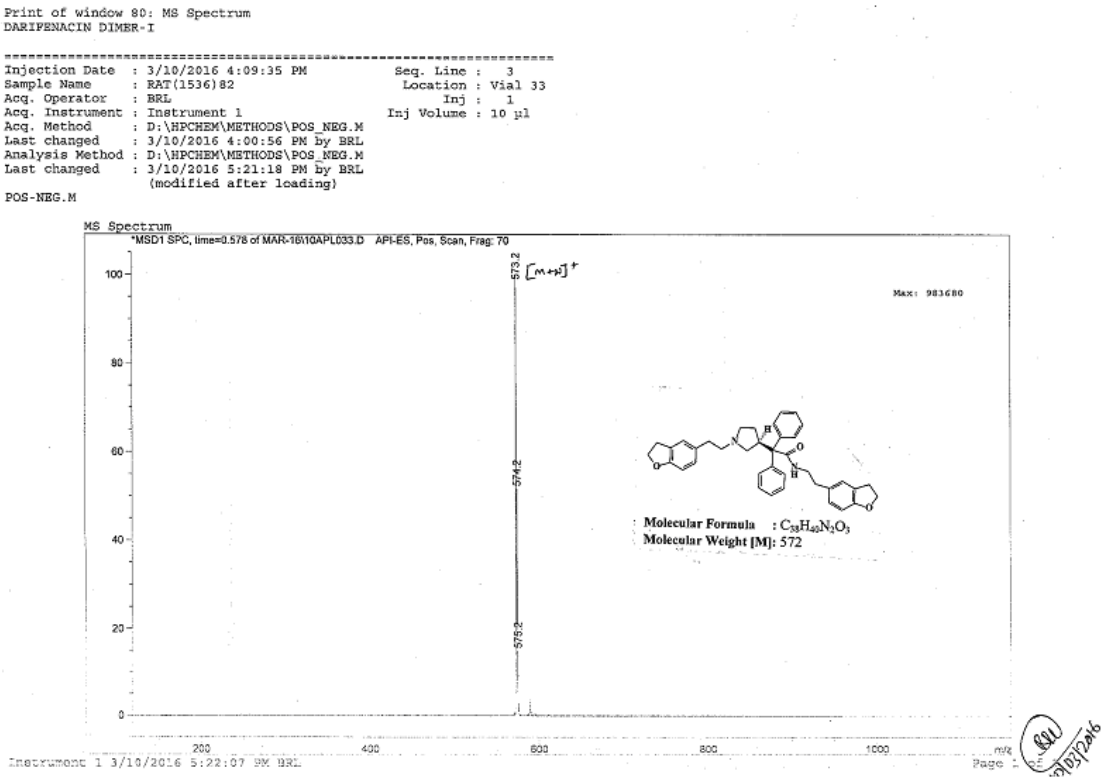



**Fig. 5I Darifenacin dimer-1 Mass spectrum.**

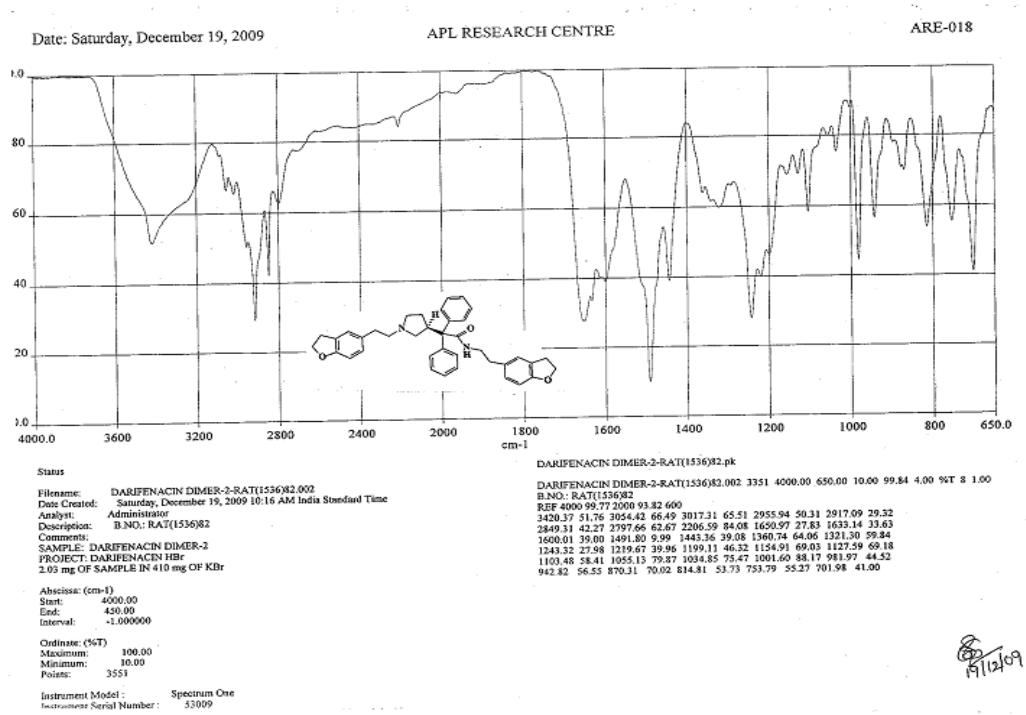



**Fig. 5J Darifenacin dimer-1 IR spectrum**

## 4.0 CONCLUSION

In Darifenacin hydrobromide two impurities were identified by LC/MS and isolated small quantity of impurities by preparative HPLC and characterized by NMR (2D-NMR), MS & FT-IR. as Darifenacin dimer 2-[(3S)-1,1-bis[2-(2,3-dihydrobenzofuran5-yl)ethyl]-2,3,4,5-tetrahydropyrrol-3-yl]-2,2-diphenylacetamide,formate and Darifenacin dimer-1 as N-[2-(2,3-dihydrobenzofuran-5yl)ethyl]-2-[(3s)-1-[2(2,3-dihydrobenzofuran-5-l)ethyl]-pyrrolidin-3-yl]-2,2-diphenylacetamide.

## 5.0 REFERENCES

1. Cross P E & Mackenzie ARD, EP 0388054, Chem Abstr, 1990; 114: 247125.
2. Barry K, William J H, Paul V M, Sylvia R, David A S, Richard F V & William W, Anal Chem, 1996; 68: 395.
3. Robert M W & Carolyn M N, Life Sciences, 1999; 64: 395.
4. Yamada S, Maruyama S, Takaji Y, Uchida S & Oki T, Life Sciences, 2006; 80,127, 42: 75–86.
5. Smith C M & Wallis R M, Journal of Receptor and Signal transduction Research, 1997; 17: 177.
6. Choppin A & Eglen R M, Brit J Pharmacol, 2001; 133: 1035.
7. Beaumont K C, Cussans N J, Nicholas D J & Smith D A, Xenobiotica, 1998; 28: 63.

8. C R Chapple Expert Opin. Investig. Drugs, 2004; 13(11): 1493-1500.
9. Beaumont K C, Cussans N J, Nicholas D J & Smith D A, Xenobiotica, 1998; 28: 63.
10. Merli V, Canavesi A, Daverio P & Vailati A, PCT Int Appl QO 2007076157, 2003; Chem Abstr, 2007; 147: 143270.
11. Merli V, Canavesi A, Daverio P & Vailati A, PCT Int Appl QO 2007076159, 2003; Chem Abstr, 2007; 147: 118125.
12. N. Ohtake, T. Mase J. Med. Chem., 2000; 43(26): 5017-5029.
13. K. Srinivas, S. Rajeswar Reddy, G. M. Reddy, P.K.Dubey, A. K. Chakravarthy and P. Pratap Reddy, Synthesis and characterization of novel and potential impurities of Darifenacin, A Potent muscarinic M3 receptor antagonist. Rasayan J. Chem., 2009; 2(1): 151-155.
14. Chinmoy pramanik, Kiran Bapat, Ashok Chaudhari, Narendra K. Tripathy and Mukund K. Gurjar. A new solvent system (cyclopentyl methyl ether-water) in process development of Darifenacin HBr. Org.process res dev, 2012; 16: 1591-1597.
15. WO2008100651 A3, Preparation of darifenacin and its salts, Nov 20, 2008.
16. International Conference on Harmonization (ICH) of Technical requirements for Registrations of Pharmaceuticals for Human Use, Topic Q1A (R2): Stability testing of New Drug substances and products, Geneva, 2003.