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# Supporting Information to

## Synthesis and Characterization of Impurities in the Production Process of Lopinavir

**Ambati V. RAGHAVA REDDY, Srinivas GARAGA, Chandiran TAKSHINAMOORTHY, Andra NAIDU**

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**Fig. 1S.** $^{1}$H-NMR spectrum of Lopinavir dimer **7**

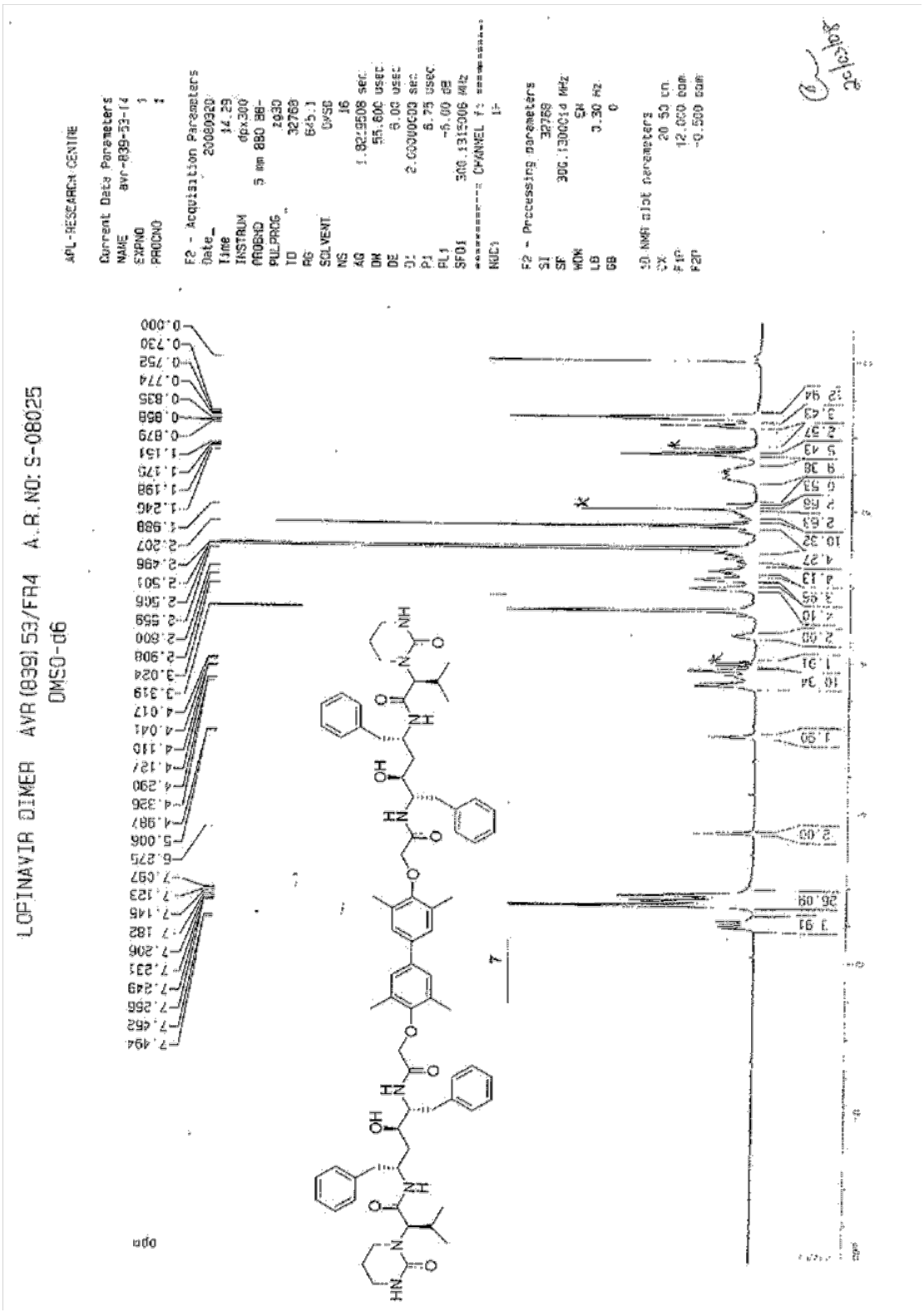


**Fig. 2S.** $^{13}$C-NMR spectrum of Lopinavir dimer **7**

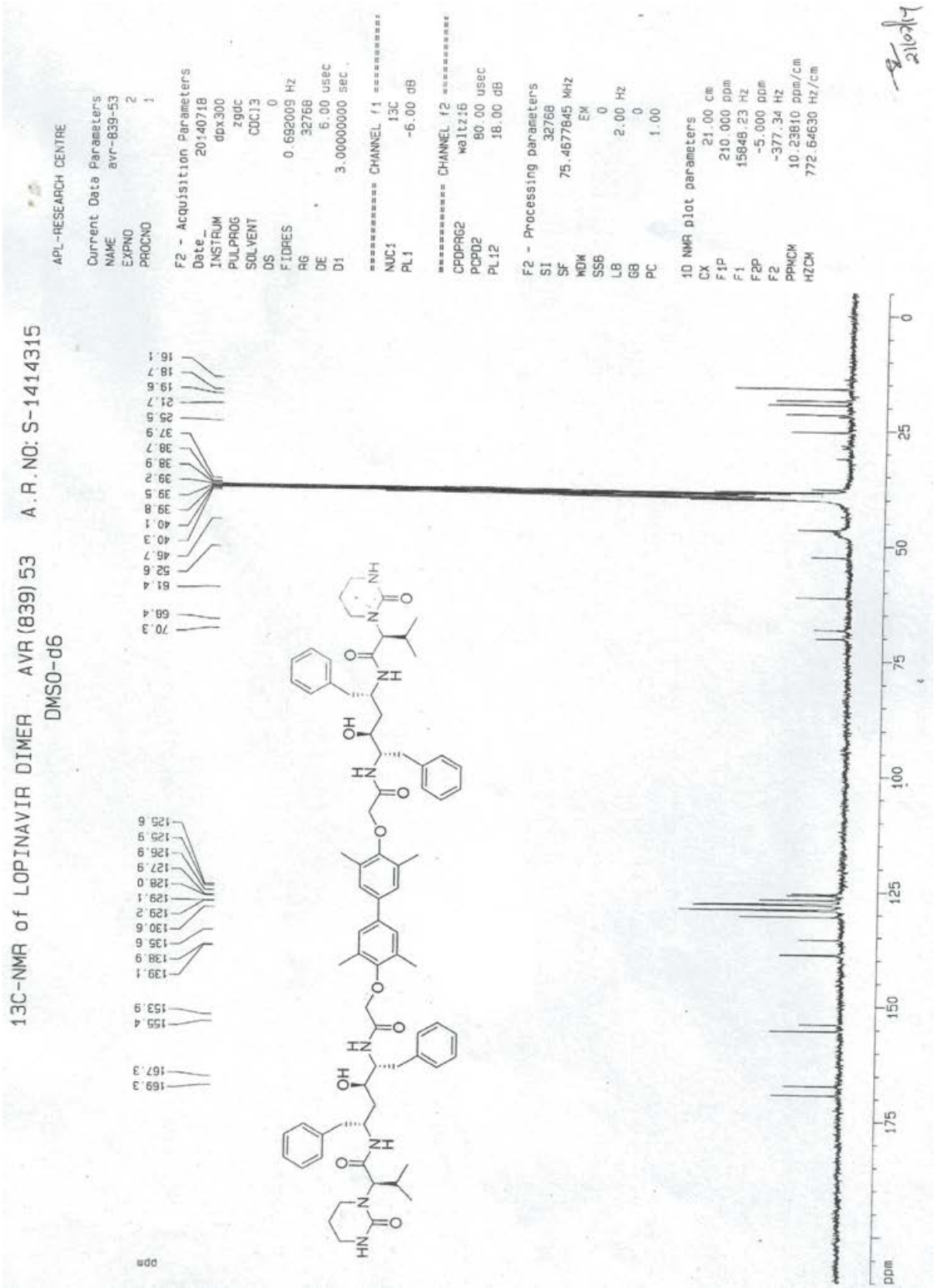


**Fig. 3S.** MS spectrum of Lopinavir dimer **7**

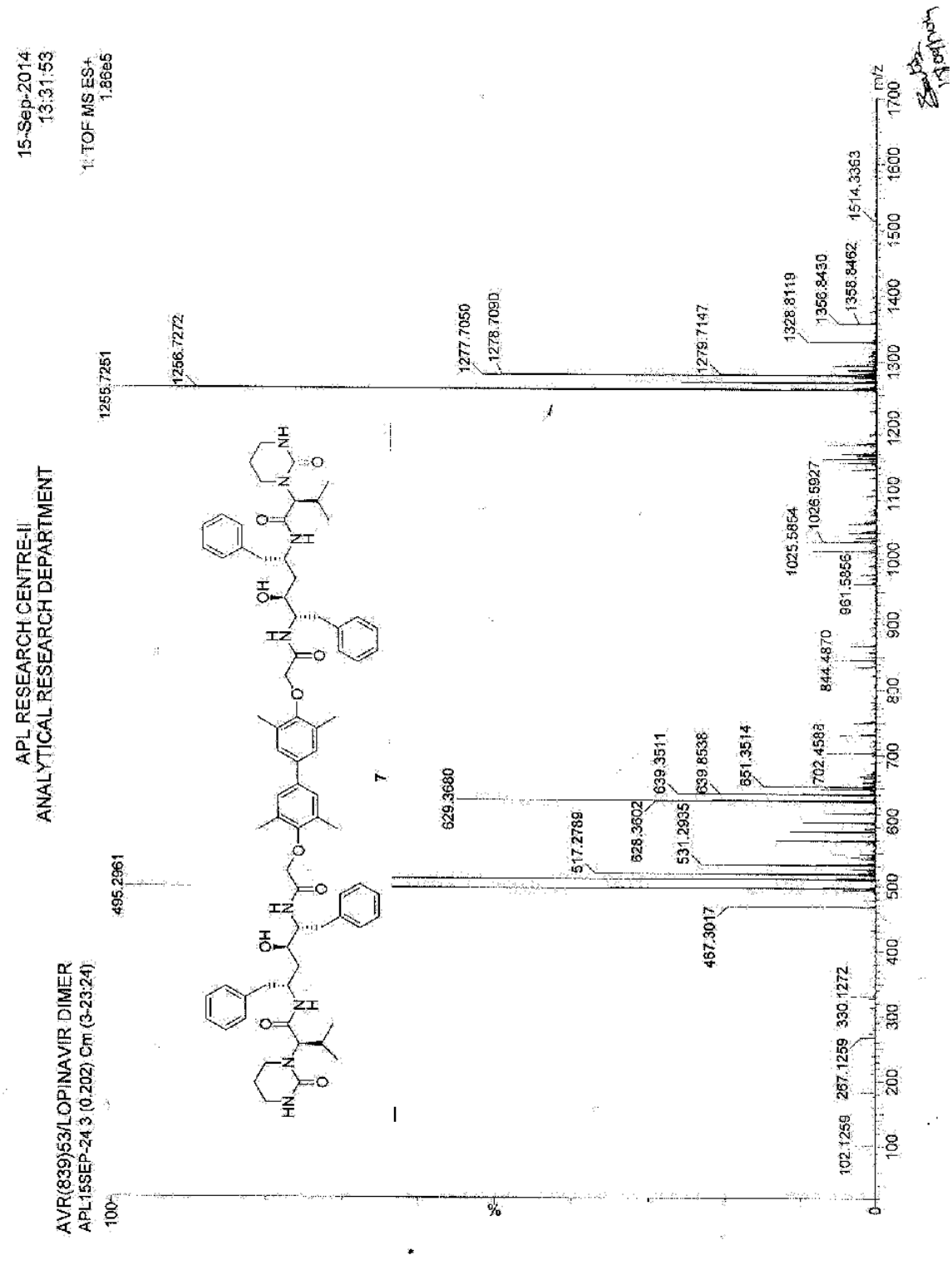


**Fig. 4S.** IR spectrum of Lopinavir dimer **7**

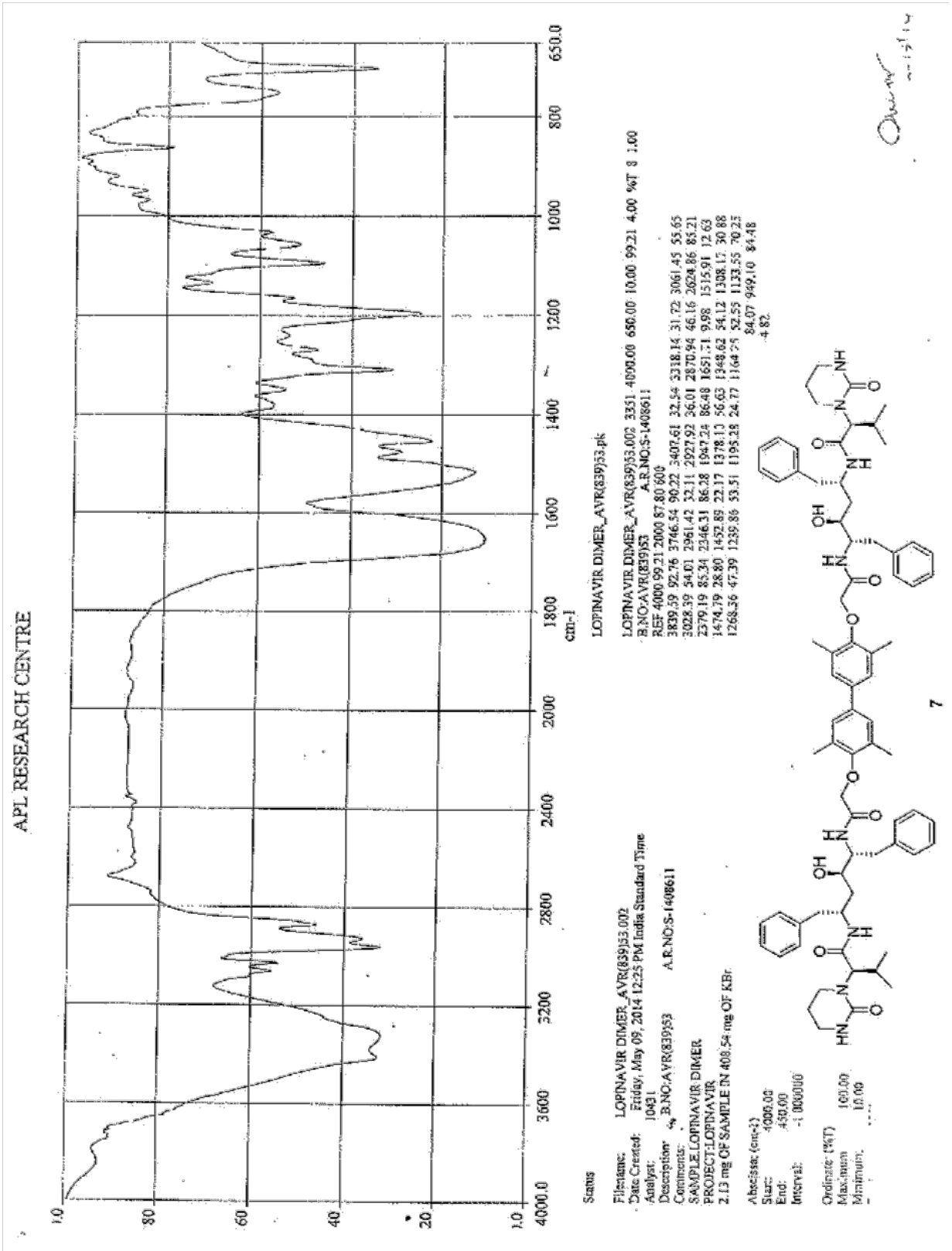
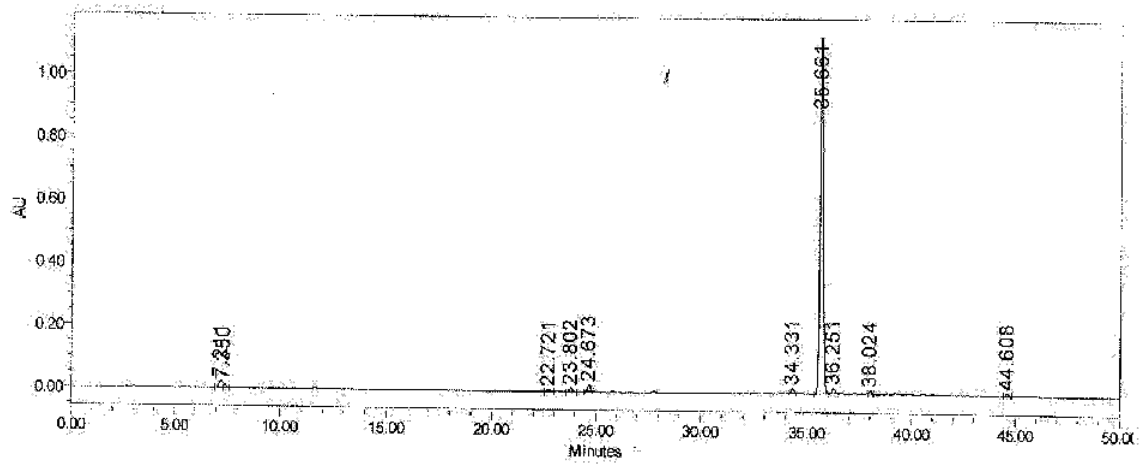


**Fig. 5S.** HPLC of Lopinavir dimer **7**

APL RESEARCH CENTRE-II
ANALYTICAL RESEARCH DEPARTMENT

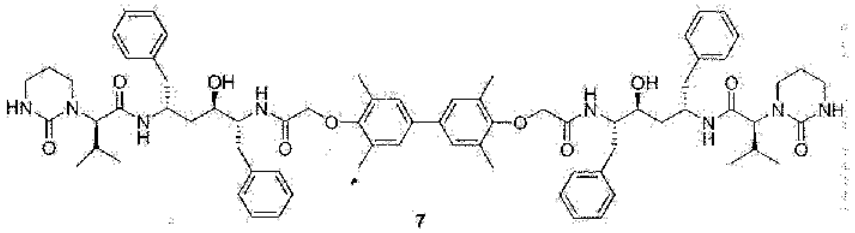
INSTRUMENT ID RCII_AE153 PROJECT NAME SEP_2014\HPLC Inprocess\RCII_AE153

Sample ID: AVR(839)53/Lopinavir Dimer
Vial: 6
Injection: 1
Injection Volume: 20.00 ul
Run Time: 60.0 Minutes
Proc. Chnl. Descr.: Detector A 210nm
Date Acquired: 16/09/2014 17:46:46 IST
Acq. Method Set: Lopinavir RS_MET
Date Processed: 20/09/2014 11:47:15 IST
Processing Method: Lopinavir RS_Imp




Peak Results

| | RT | Area | % Area | Name |
|---|---|---|---|---|
| 1 | 7.21 | 274546 | 2.17 | Peak1 |
| 2 | 7.35 | 85976 | 0.68 | Peak2 |
| 3 | 22.72 | 74734 | 0.59 | Peak3 |
| 4 | 23.80 | 132511 | 1.05 | Peak4 |
| 5 | 24.67 | 218154 | 1.72 | Peak5 |
| 6 | 34.33 | 150833 | 1.19 | Peak6 |
| 7 | 35.66 | 11411325 | 90.11 | Lopinavir Dimer |
| 8 | 36.25 | 169056 | 1.33 | Peak8 |
| 9 | 38.02 | 72141 | 0.57 | Peak9 |
| 10 | 44.61 | 74947 | 0.59 | Peak10 |



**7**

**Fig. 6S.** $^{1}$H-NMR spectrum of Lopinavir carboxymethyl analog **8**

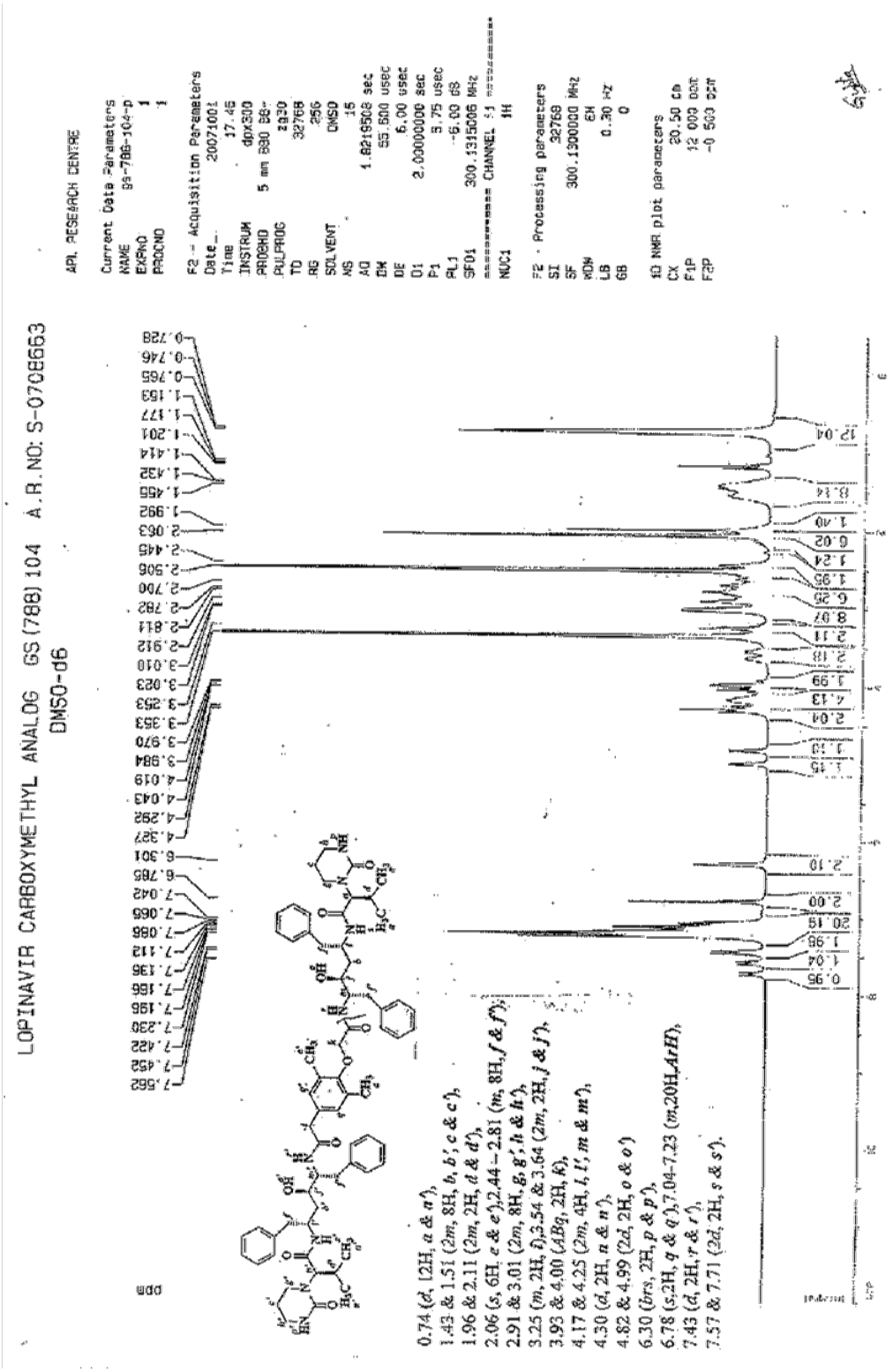


**Fig. 7S.** $^{13}$C-NMR spectrum of Lopinavir carboxymethyl analog **8**

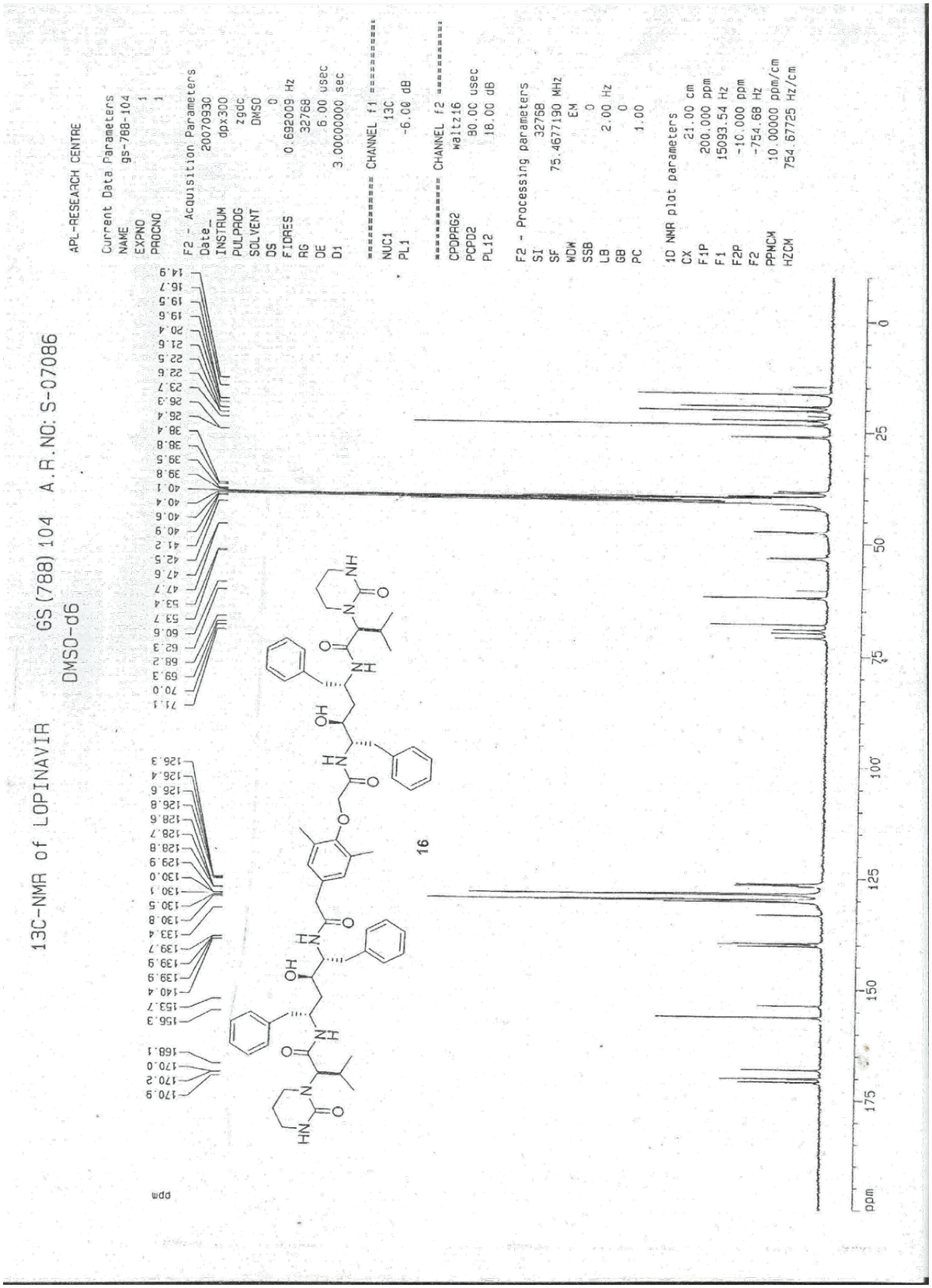


**Fig. 8S.** MS spectrum of Lopinavir carboxymethyl analog **8**

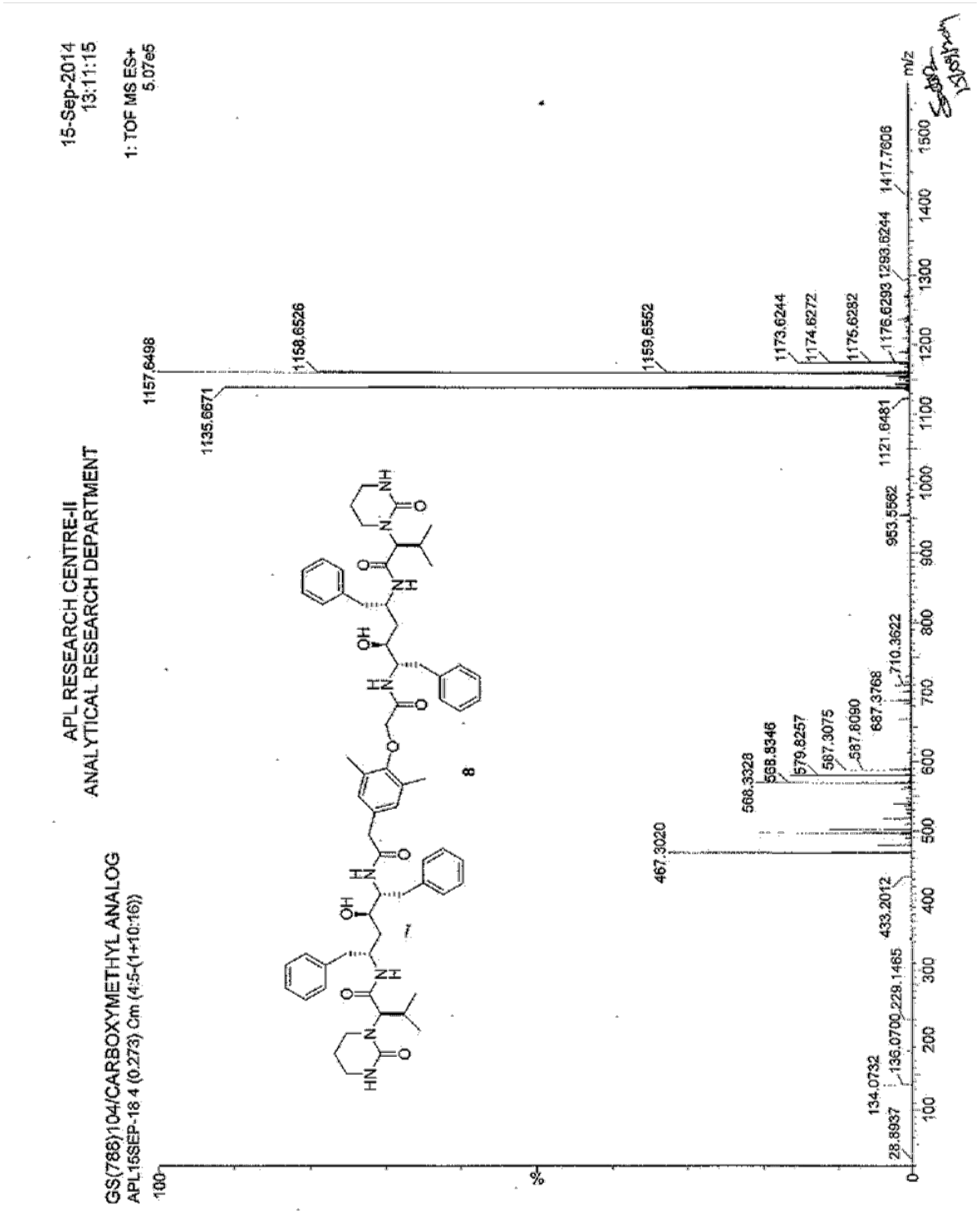


**Fig. 9S.** IR spectrum of Lopinavir carboxymethyl analog **8**

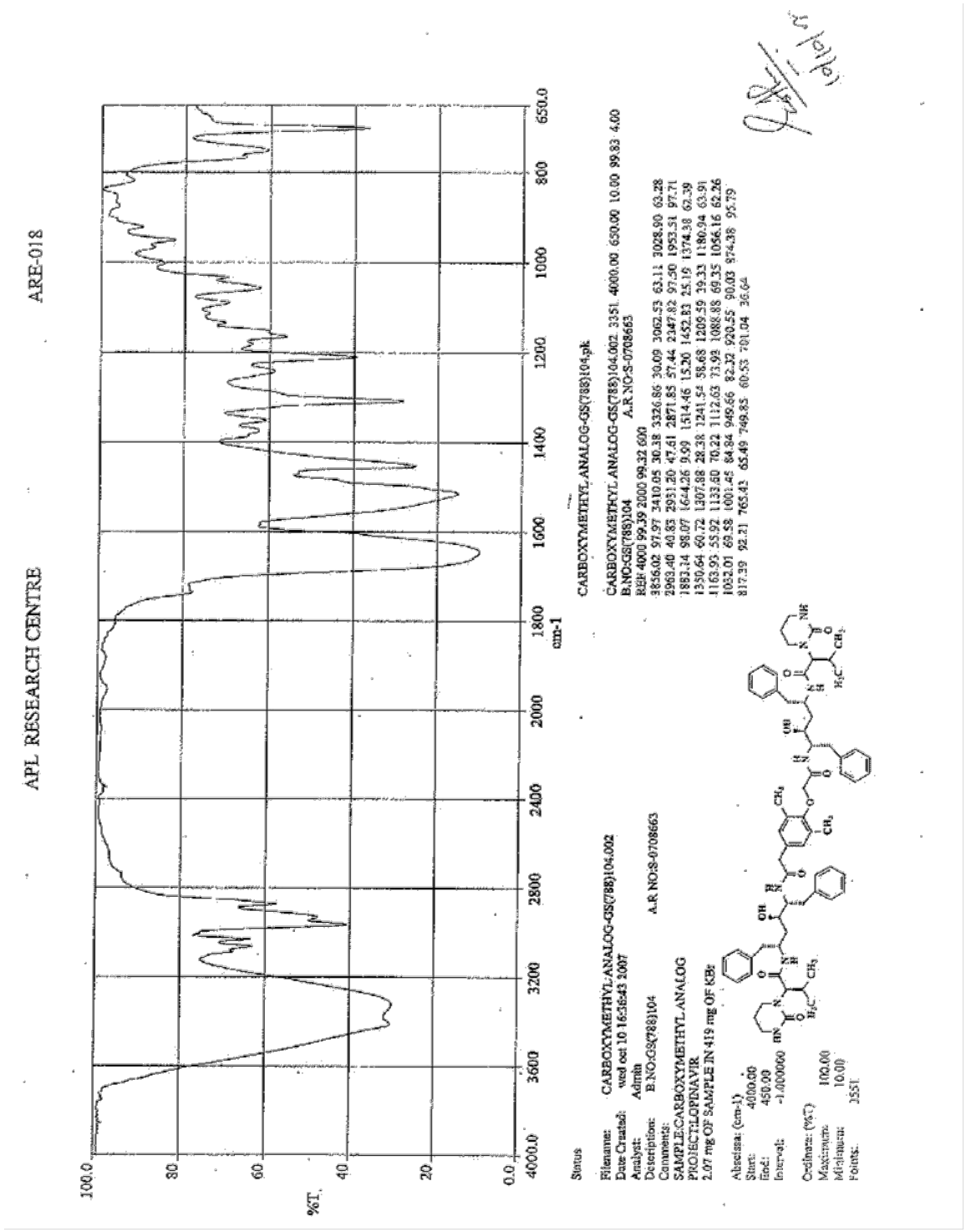
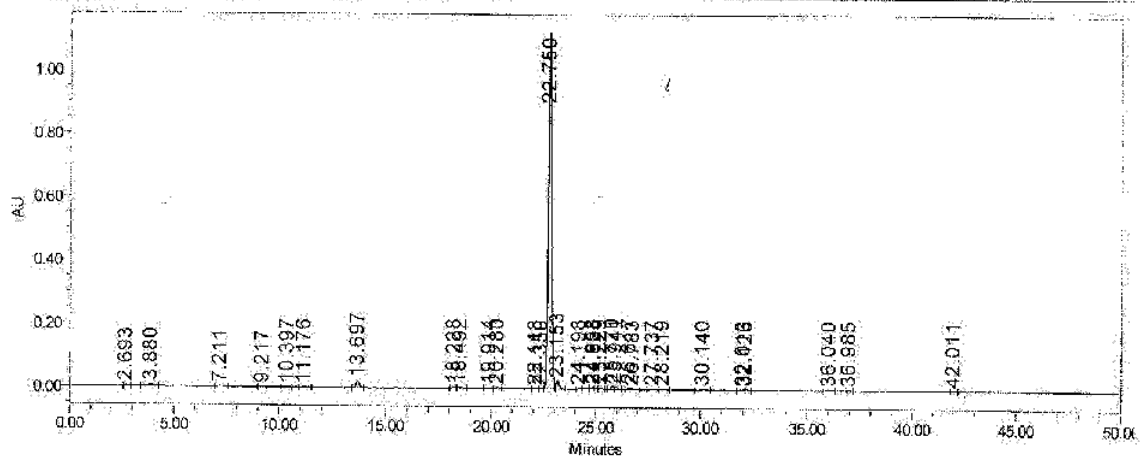


**Fig. 10S.** HPLC of Lopinavir carboxymethyl analog **8**

APL RESEARCH CENTRE-II
ANALYTICAL RESEARCH DEPARTMENT

INSTRUMENT ID:RCII_AE153 PROJECT NAME:SEP_2014\HPLC Inprocess\RCII_AE153

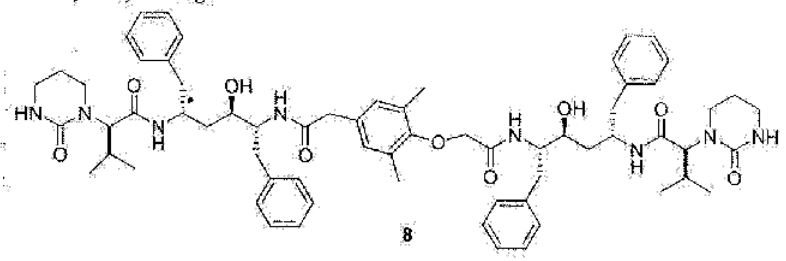
Sample ID: GS(788)104/Carboxy Methyl Analouge
Vial: 5
Injection : 1
Injection Volume: 20.00 ul
Run Time: 60.0 Minutes
Proc. Chnl. Descr.: Detector A 210nm
Date Acquired: 16/09/2014 16:45:40 IST
Acq. Method Set: Lopinavir RS_MET
Date Processed: 17/09/2014 9:50:49 IST
Processing Method Lopinavir RS_Imp




Peak Results

| | RT | Area | % Area | Name |
|---|---|---|---|---|
| 1 | 2.69 | 32836 | 0.25 | Peak1 |
| 2 | 3.88 | 94634 | 0.71 | Peak2 |
| 3 | 7.21 | 36460 | 0.27 | Peak3 |
| 4 | 9.22 | 14442 | 0.11 | Peak4 |
| 5 | 10.40 | 12431 | 0.09 | Peak5 |
| 6 | 11.18 | 16135 | 0.12 | Peak6 |
| 7 | 13.70 | 221218 | 1.67 | Peak7 |
| 8 | 18.23 | 36948 | 0.28 | Peak8 |
| 9 | 18.49 | 54964 | 0.41 | Peak9 |
| 10 | 19.91 | 21387 | 0.16 | Peak10 |
| 11 | 20.28 | 30730 | 0.23 | Peak11 |
| 12 | 22.12 | 18842 | 0.14 | Peak12 |
| 13 | 22.35 | 9062 | 0.07 | Peak13 |
| 14 | 22.75 | 11842238 | 89.22 | Carboxymethyl Analog |
| 15 | 23.15 | 252985 | 1.91 | |
| 16 | 24.20 | 21774 | 0.16 | |
| 17 | 24.86 | 29406 | 0.22 | |
| 18 | 24.93 | 25044 | 0.19 | |
| 19 | 25.23 | 42410 | 0.32 | |
| 20 | 25.77 | 88694 | 0.67 | |

| | RT | Area | % Area | Name |
|---|---|---|---|---|
| 21 | 25.94 | 81625 | 0.61 | Peak21 |
| 22 | 26.62 | 82017 | 0.62 | Peak22 |
| 23 | 26.78 | 22058 | 0.17 | Peak23 |
| 24 | 27.74 | 39205 | 0.30 | Peak24 |
| 25 | 28.22 | 39229 | 0.30 | Peak25 |
| 26 | 30.14 | 39969 | 0.30 | Peak26 |
| 27 | 32.01 | 15886 | 0.12 | Peak27 |
| 28 | 32.13 | 16221 | 0.12 | Peak28 |
| 29 | 36.04 | 18955 | 0.14 | Peak29 |
| 30 | 36.99 | 9466 | 0.07 | Peak30 |
| 31 | 42.01 | 6078 | 0.05 | Peak31 |



**8**

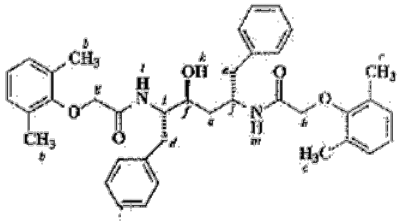
**Fig. 11S.** Data sheet Lopinavir diamide **9**

APL RESEARCH CENTRE

## *LOPINAVIR DIAMIDE*

**CHEMICAL NAME** : *(2S,3S,5S)-2,5-Bis[(2,6-DIMETHYLPHENOXY-ACETYL)AMINO]-3-HYDROXY-1,6-DIPHENYLHEXANE*

**STRUCTURAL FORMULA** :



**MOLECULAR FORMULA** : $C_{38}H_{44}N_2O_5$

**MOLECULAR WEIGHT** : 608

**DESCRIPTION** : An off-white powder.

**RELATIVE RETENTION TIME*** : ~ 1.59
*[w.r.t. LOPINAVIR, HPLC]*

**SPECTRAL DATA****

- **$^1$H NMR (300 MHz) in *DMSO-$d_6$*** : *δ(ppm)*; 1.65 (*m*, 2H, *a*), 2.14 & 2.16 (*2s*, 12H, *b* & *c*), 2.78 & 2.85 (*2d*, 4H, *d* & *e*), 3.70 (*m*, 1H, *f*), 3.95 & 4.05 (*ABg*, 2H, *g*), 4.11 (*s*, 2H, *h*), 4.35 (*m*, 2H, *i* & *j*), 5.08 (*d*, 1H, *k*), 6.93 – 7.27 (*m*, 16H, *ArH*), 7.52 & 7.86 (*2d*, 1H each, *l* & *m*).
- **MASS (PE SCIEX-API 2000) ESI** : m/z; 609.2 [$(MH)^+$]
  *in +ve ion mode*
- **INFRARED ABSORPTION SPECTRUM (IR)** : *Characteristic absorption spectrum enclosed.*
  *in KBr*

* *Refer enclosed STP*

** *Batch No.: AVR(193)74*

**Fig. 12S.** $^{1}$H-NMR spectrum of Lopinavir diamide **9**

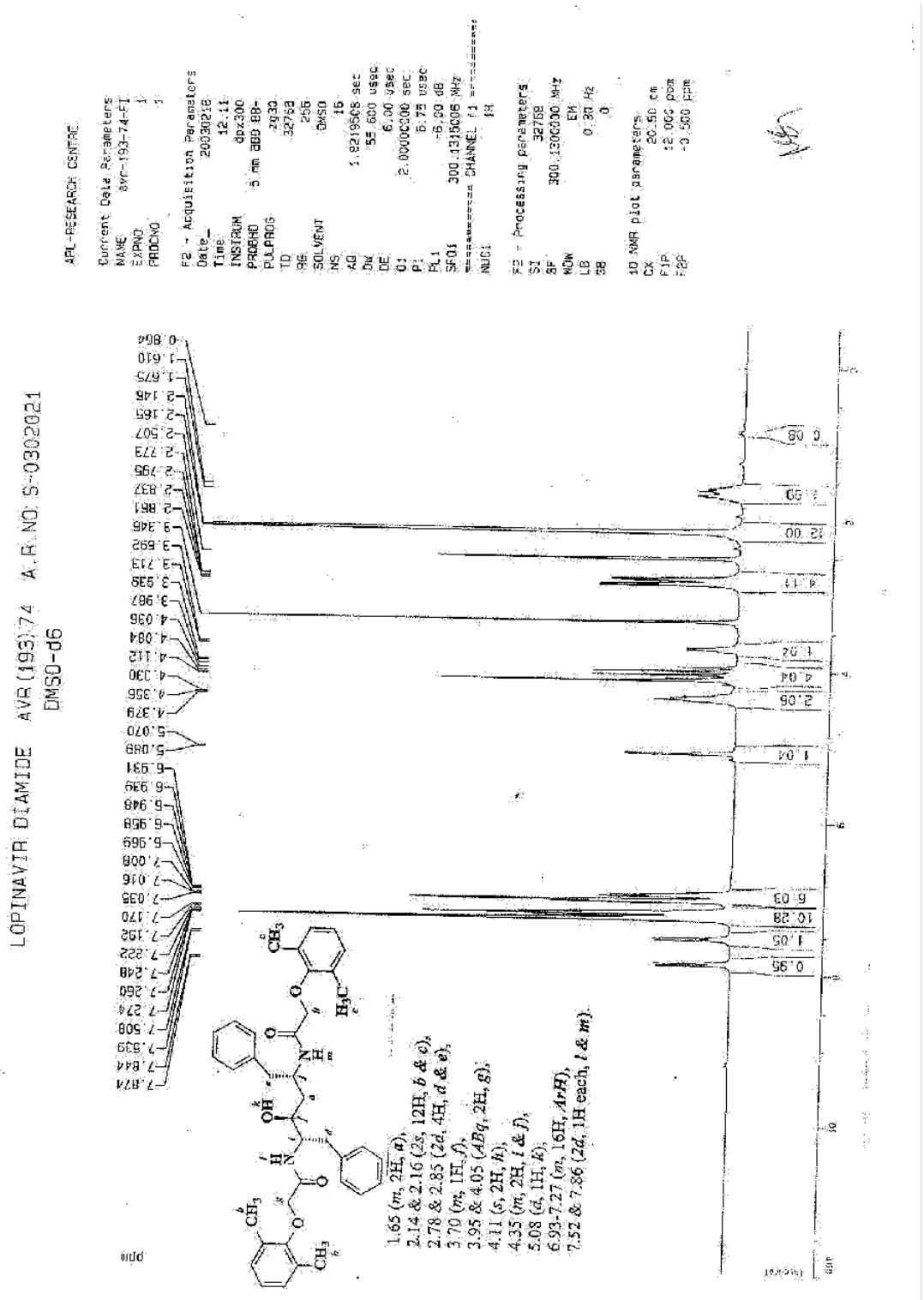


**Fig. 13S.** MS spectrum of Lopinavir diamide **9**

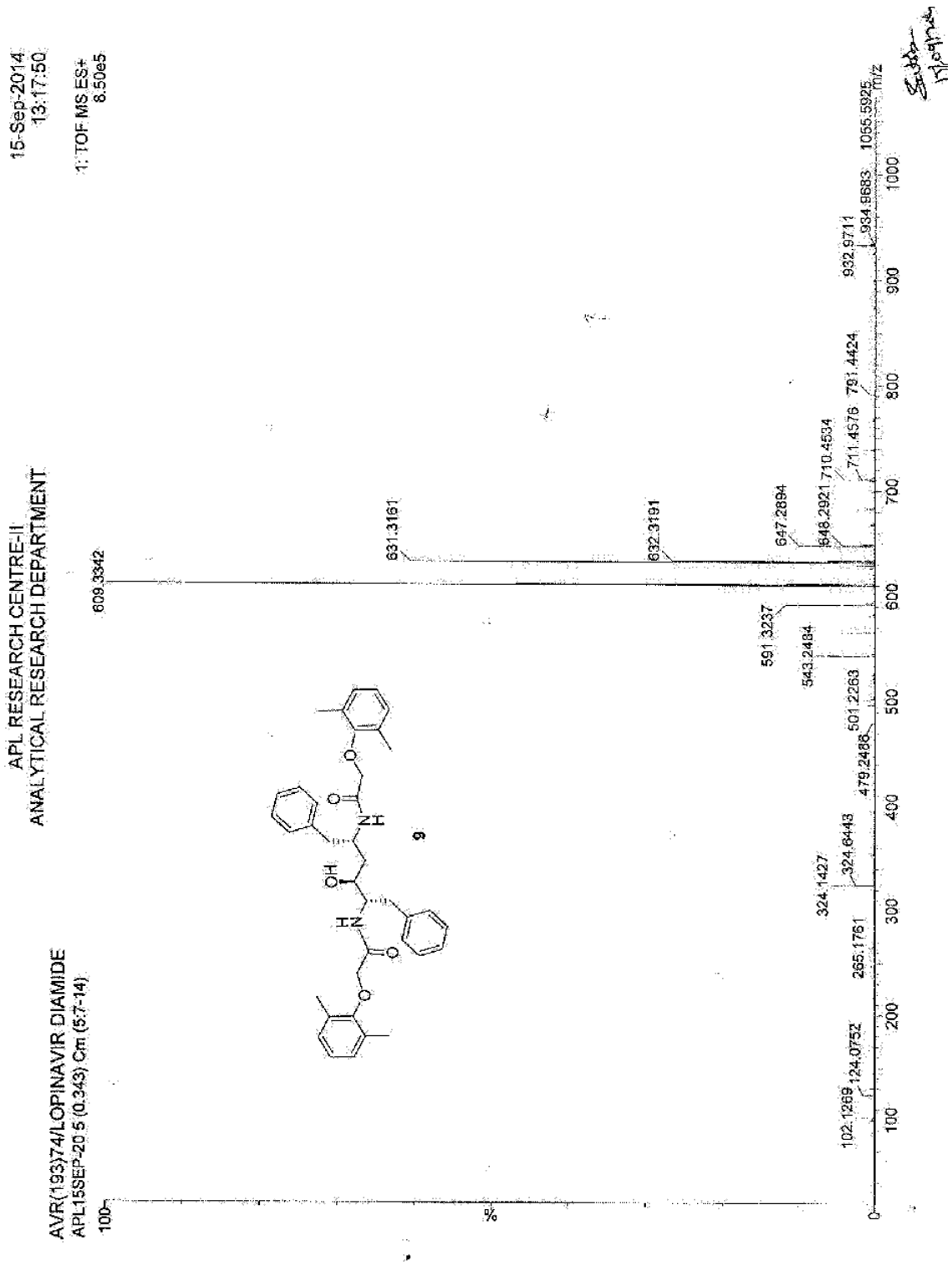


**Fig. 14S.** IR spectrum of Lopinavir diamide **9**

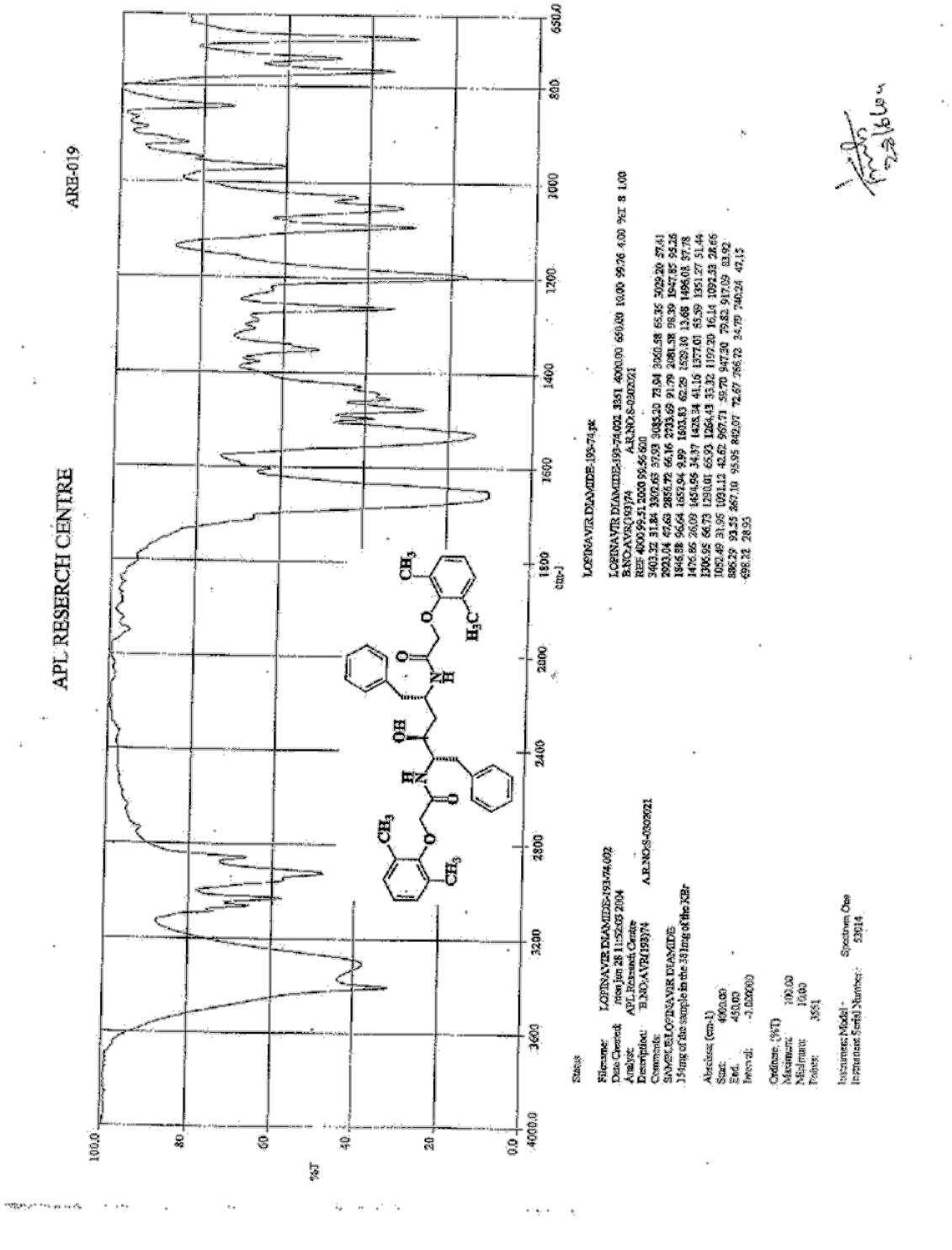
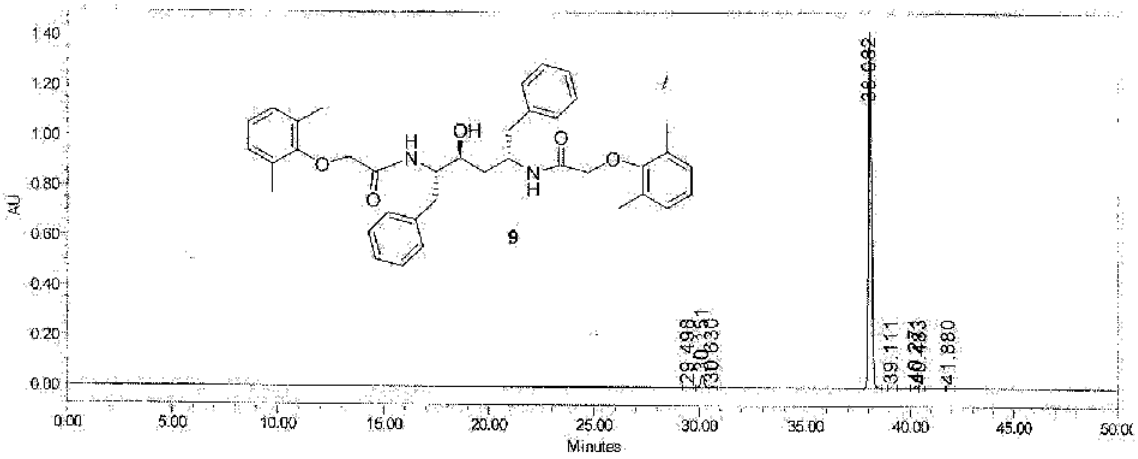


**Fig. 15S.** HPLC of Lopinavir diamide **9**

APL RESEARCH CENTRE-II
ANALYTICAL RESEARCH DEPARTMENT

INSTRUMENT ID RCII_AE153 PROJECT NAME SEP_2014\HPLC Inprocess\RCII_AE153

Sample ID: AVR(193)74/ Lopinavir Diamide
Vial: 4
Injection: 1
Injection Volume: 20.00 ul
Run Time: 60.0 Minutes
Proc. Chnl. Descr.: Detector A 210nm
Date Acquired: 16/09/2014 15:44:31 IST
Acq. Method Set: Lopinavir RS_MET
Date Processed: 17/09/2014 9:46:03 IST
Processing Method: Lopinavir RS_Imp




Peak Results

| | RT | Area | % Area | Name |
|---|---|---|---|---|
| 1 | 29.50 | 6873 | 0.05 | Peak1 |
| 2 | 30.15 | 551668 | 3.62 | Peak2 |
| 3 | 30.63 | 47114 | 0.31 | Peak3 |
| 4 | 38.08 | 14491483 | 95.01 | Lopinavir Diamide |
| 5 | 39.11 | 23493 | 0.15 | Peak5 |
| 6 | 40.27 | 90185 | 0.59 | Peak6 |
| 7 | 40.48 | 19307 | 0.13 | Peak7 |
| 8 | 41.88 | 22912 | 0.15 | Peak8 |

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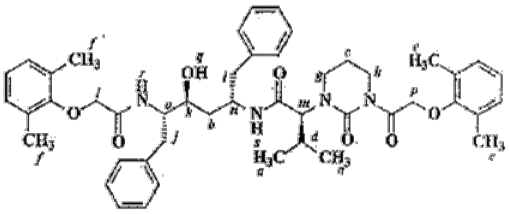
**Fig. 16S.** Data sheet of Diacylated Lopinavir **10**

APL RESEARCH CENTRE

## *DIACYLATED LOPINAVIR*

| | | |
|---|---|---|
| **CHEMICAL NAME** | : | *(2S,3S,5S)-2-(2,6-DIMETHYLPHENOXY)-ACETYL)AMINO-3-HYDROXY-5-[2S-(1-TETRAHYDROPYRIMID-3-(2,6-DIMETHYLPHENOXYACETYL)-2-ONYL]-3-METHYLBUTANOYL]AMINO-1,6-DIPHENYLHEXANE* |
| **STRUCTURAL FORMULA** | : |  |
| **MOLECULAR FORMULA** | : | $C_{47}H_{53}N_4O_7$ |
| **MOLECULAR WEIGHT** | : | 790 |
| **DESCRIPTION** | : | A white powder. |
| **RELATIVE RETENTION TIME*** *[w.r.t. LOPINAVIR, HPLC]* | : | ~ 1.68 |
| **SPECTRAL DATA**** | | |
| ➢ **$^1$H NMR (300 MHz) in *DMSO-$d_6$*** | : | *δ(ppm)*; 0.74 (*2d*, 6H, *a*),<br>1.45 – 1.71 (*2m*, 4H, *b* & *c*),<br>2.02 (*m*, 1H, *d*),<br>2.15 & 2.24 (*2s*, 12H, *e* & *f*),<br>2.47, 2.97 & 3.53 (*3m*, 4H, *g* & *h*),<br>2.71 & 2.79 (*2m*, 4H, *i* & *j*),<br>3.62 (*m*, 1H, *k*),<br>4.05 & 4.15 (*ABq*, 2H, *l*),<br>4.24 (*d*, 1H, *m*),<br>4.28 & 4.37 (*2m*, 2H, *n* & *o*)<br>4.87 & 4.96 (*ABq*, 2H, *p*),<br>5.01 (*d*, 1H, *q*),<br>6.93 – 7.26 (*m*, 16H, *ArH*),<br>7.49 & 7.73 (*2d*, 2H, *r* & *s*). |
| ➢ **MASS (PE SCIEX-API 2000) ESI** *in +ve ion mode* | : | m/z; 791.4 [$(MH)^+$] |
| ➢ **INFRARED ABSORPTION SPECTRUM (IR)** *in KBr* | : | *Characteristic absorption spectrum enclosed.* |

* *Refer enclosed STP*

** *Batch No.: MSK(741)34*

**Fig. 17S.** $^{1}$H-NMR spectrum of Diacylated Lopinavir **10**

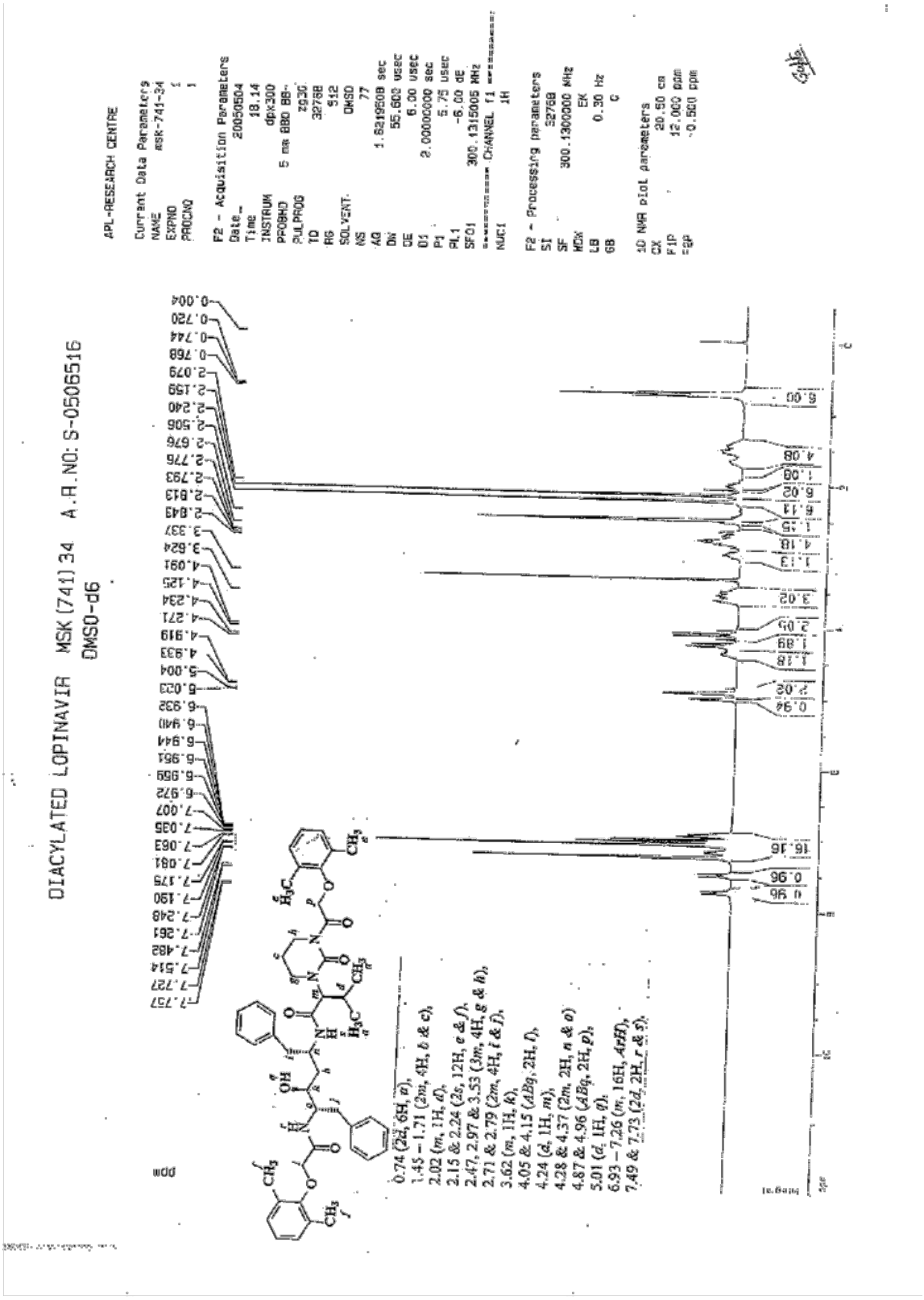


**Fig. 18S.** MS spectrum of Diacylated Lopinavir **10**

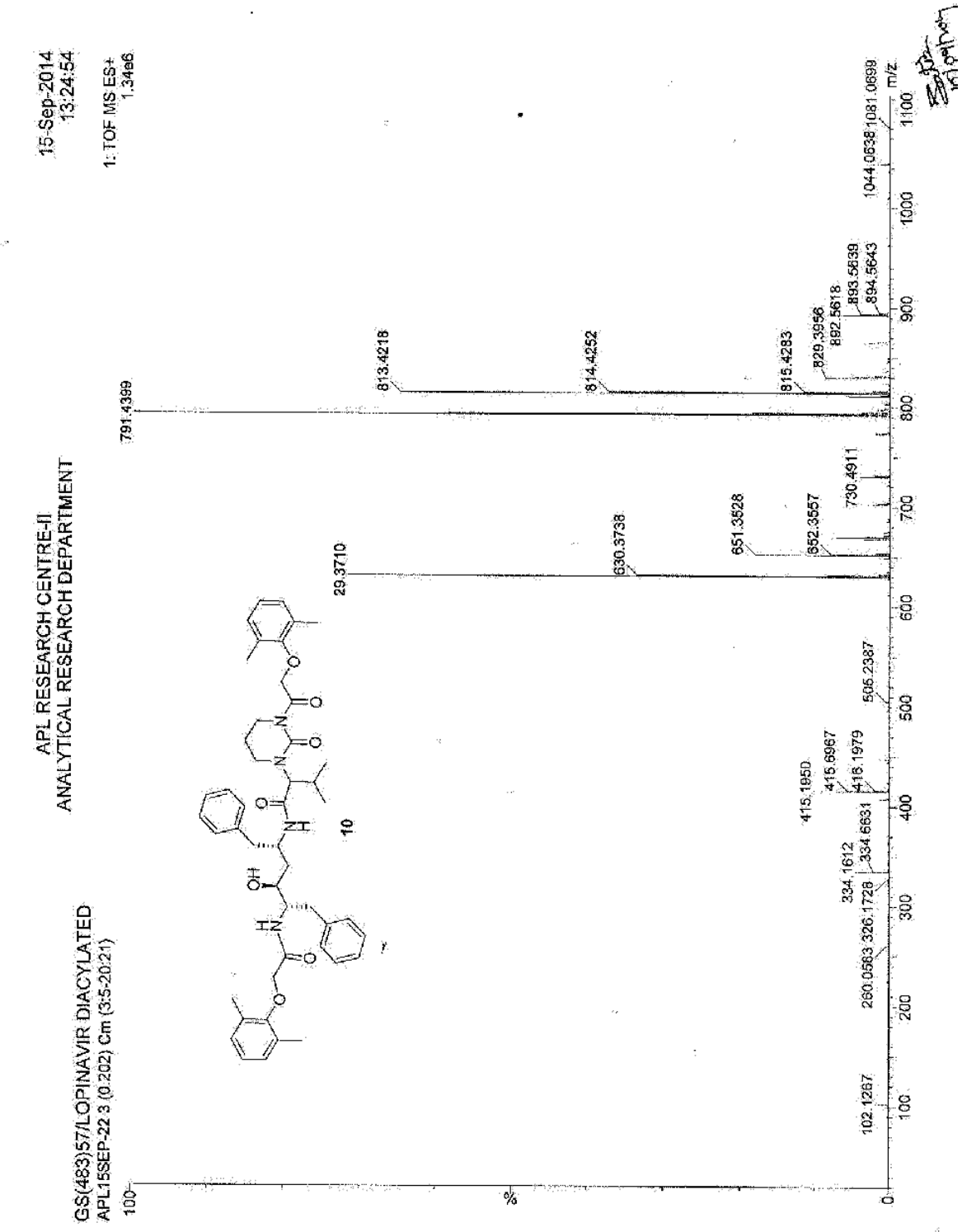


**Fig. 19S.** IR spectrum of Diacylated Lopinavir **10**

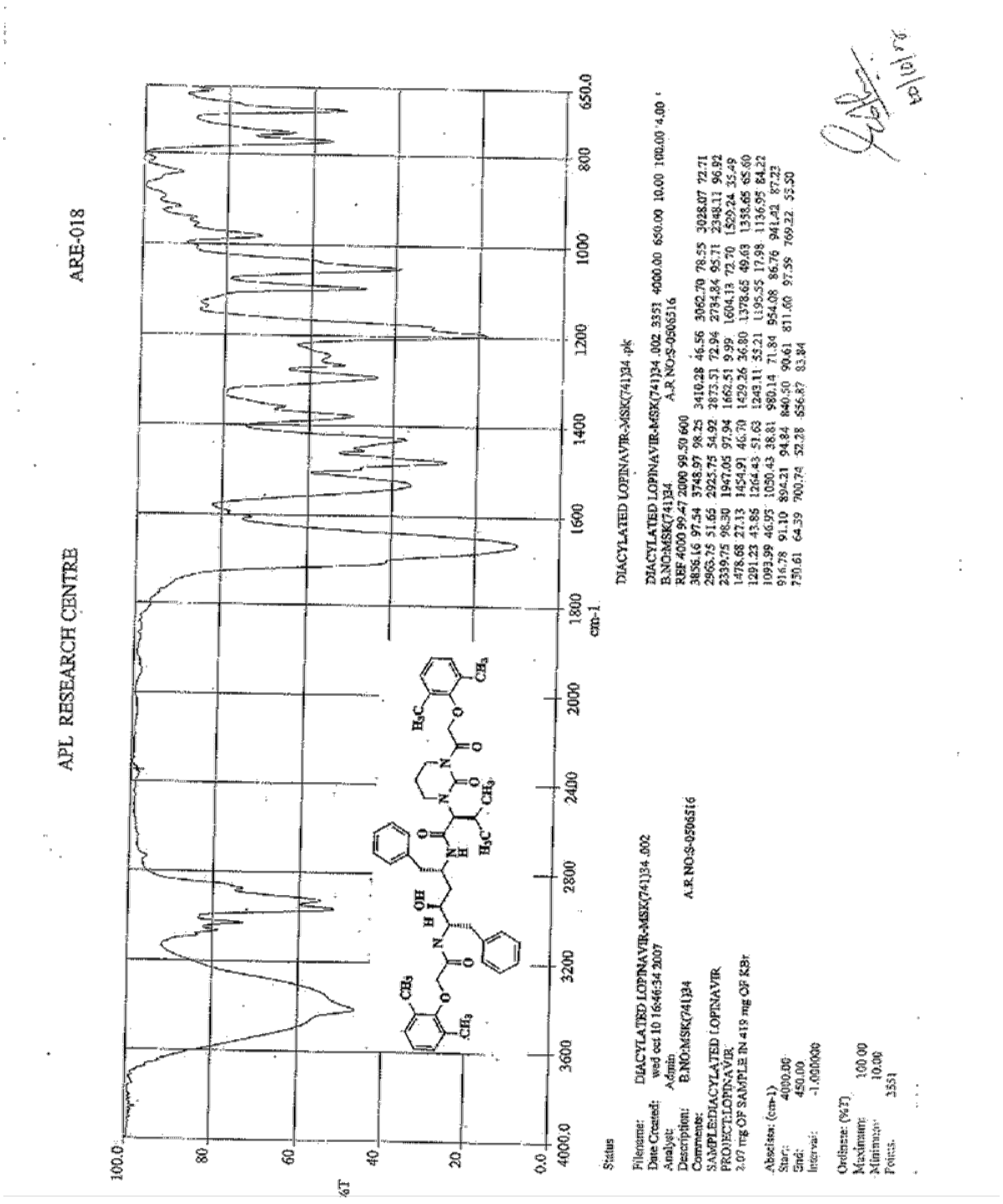
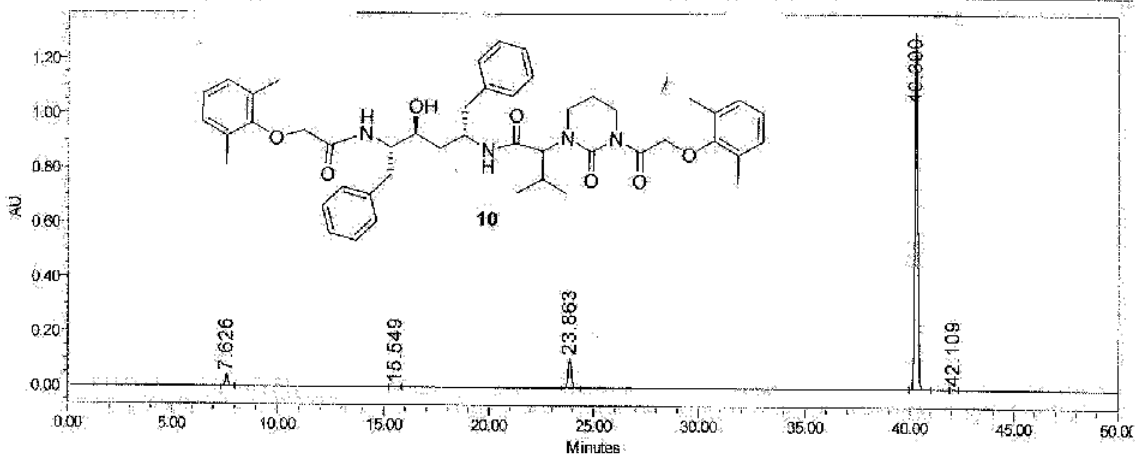


**Fig. 20S.** HPLC of Diacylated Lopinavir **10**

APL RESEARCH CENTRE-II
ANALYTICAL RESEARCH DEPARTMENT

INSTRUMENT ID:RCII_AE153 PROJECT NAME:SEP_2014\HPLC Inprocess\RCII_AE153

| | | | |
|---|---|---|---|
| Sample ID: | GS(483)57/Diacylated Lopinavir | | |
| Vial: | 3 | Proc. Chnl. Descr.: | Detector A 210nm |
| Injection : | 1 | Date Acquired: | 16/09/2014 14:43:24 IST |
| Injection Volume: | 20.00 ul | Acq. Method Set: | Lopinavir RS_MET |
| Run Time: | 60.0 Minutes | Date Processed: | 17/09/2014 9:43:52 IST |
| | | Processing Method | Lopinavir RS_Imp |




Peak Results

| | RT | Area | % Area | Name |
|---|---|---|---|---|
| 1 | 7.63 | 372056 | 2.73 | Peak1 |
| 2 | 15.55 | 6180 | 0.05 | Peak2 |
| 3 | 23.86 | 1212943 | 8.89 | Peak3 |
| 4 | 40.30 | 12047255 | 88.26 | Diacylated Lopinavir |
| 5 | 42.11 | 11675 | 0.09 | Peak5 |

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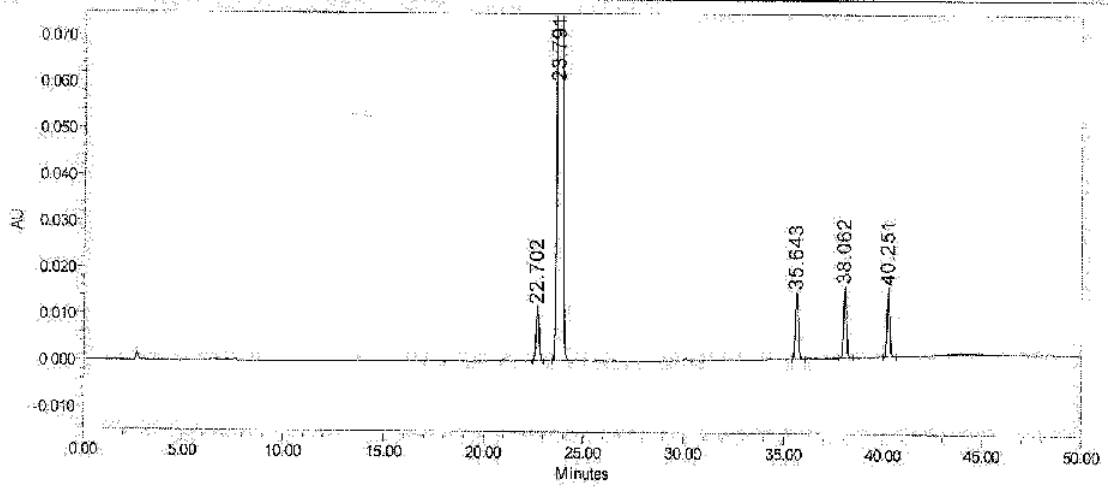
**Fig. 21S.** HPLC of Impurity mixture

**APL RESEARCH CENTRE-II**
**ANALYTICAL RESEARCH DEPARTMENT**

INSTRUMENT ID RCII_AE153 PROJECT NAME SEP_2014\HPLC Inprocess\RCII_AE153

| | | | |
|---|---|---|---|
| Sample ID: | Impurity Mixture | | |
| Vial: | 8 | Proc. Chnl. Descr.: | Detector A 210nm |
| Injection: | 1 | Date Acquired: | 16/09/2014 19:49:03 IST |
| Injection Volume: | 20.00 ul | Acq. Method Set: | Lopinavir RS_MET |
| Run Time: | 60.0 Minutes | Date Processed: | 17/09/2014 9:59:52 IST |
| | | Processing method | Lopinavir RS_Imp |




Peak Results

| | RT | USP Plate Count | USP Tailing | USP Resolution | RT Ratio | Name |
|---|---|---|---|---|---|---|
| 1 | 22.70 | 114994 | 1.10 | | 0.95 | Carboxymethyl Analog |
| 2 | 23.79 | 107900 | 1.07 | 3.84 | 1.00 | Lopinavir |
| 3 | 35.64 | 292739 | 1.10 | 42.05 | 1.50 | Lopinavir Dimer |
| 4 | 38.06 | 337120 | 1.02 | 9.02 | 1.60 | Lopinavir Diamide |
| 5 | 40.25 | 445067 | 1.03 | 8.54 | 1.69 | Diacylated Lopinavir |

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