SUPPORTING INFORMATION

Molecular Markers for Pyrethrin Autoxidation in Stored Pyrethrum Crop: Analysis and Structure Determination

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Representative chromatograms

Autoxidation studies:

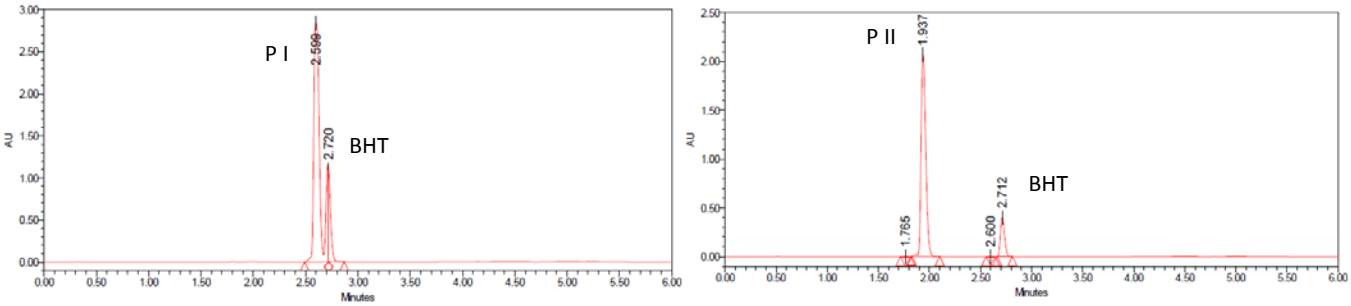


Figure S1 - UPLC chromatograms of pyrethrin I (PI) and pyrethrin II (PII) autoxidation solutions at the zero time

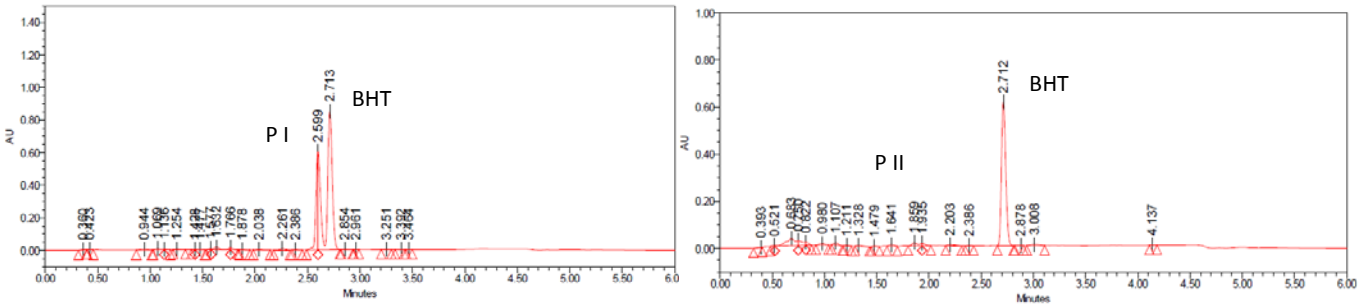


Figure S2 - UPLC chromatograms of pyrethrin I (PI) and pyrethrin II (PII) autoxidation solutions at 96 hours

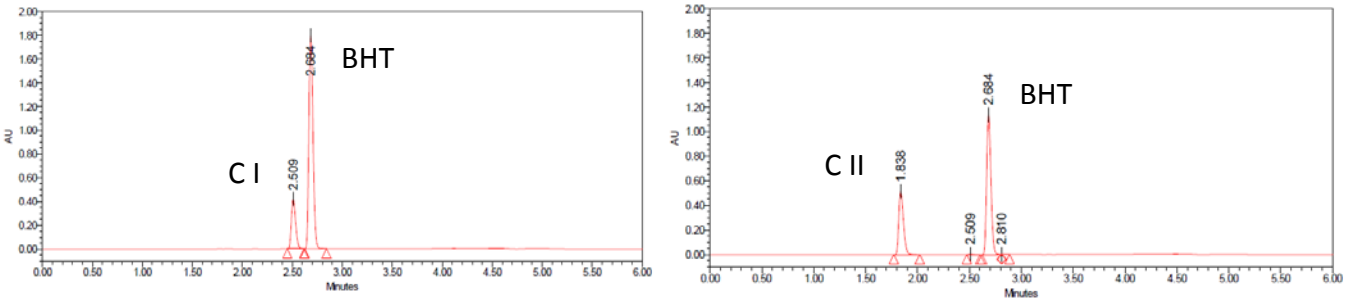


Figure S3 - UPLC chromatograms of cinerin I (CI) and cinerin II (CII) autoxidation solutions at the zero time

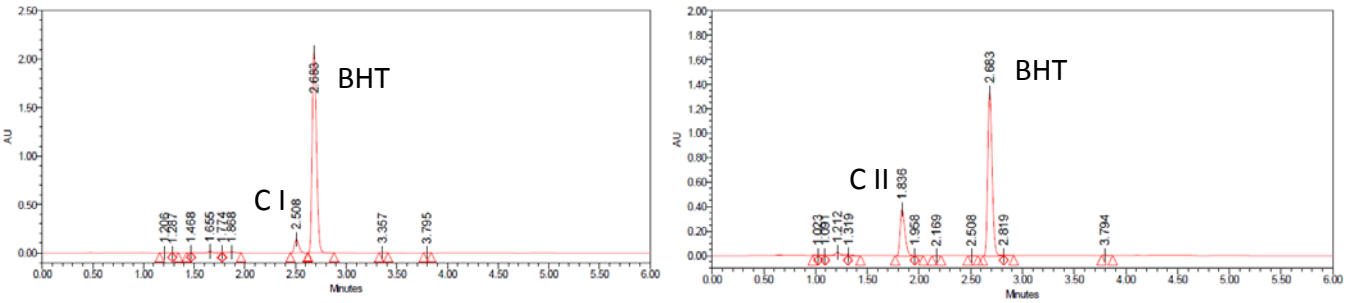


Figure S4 - UPLC chromatograms of cinerin I (CI) and cinerin II (CII) autoxidation solutions at 96 hours

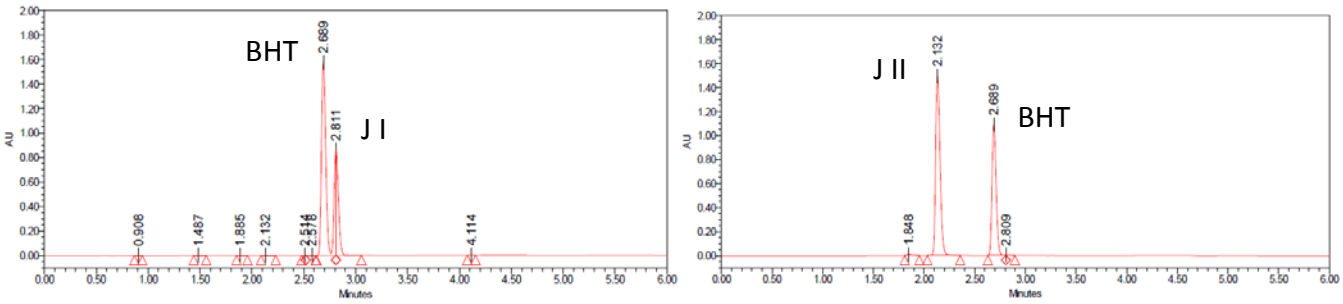


Figure S5 - UPLC chromatograms of jasmolin I (JI) and jasmolin II (JII) autoxidation solutions at the zero time

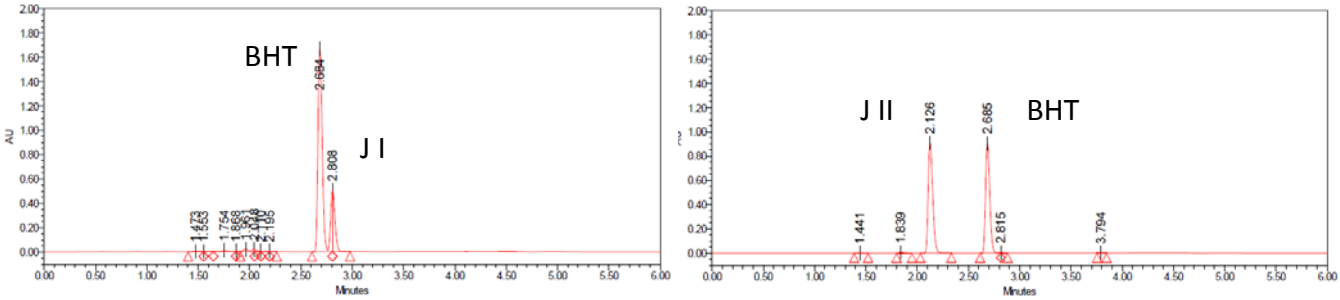


Figure S6 - UPLC chromatograms of jasmolin I (JI) and jasmolin II (JII) autoxidation solutions at 96 hours

Table S1. Percentage decrease of pyrethrin esters over time in AIBN-promoted autoxidation reactions.

time (hrs)	Percentage decrease					
	P I	P II	J I	J II	C I	C II
0	0.0	0.0	0.0	0.0	0.0	0.0
2	31.1	55.0		31.1	9.5	2.6
4	58.7	51.3	13.4	25.2	9.2	7.6
6	49.3	54.5	2.5	40.8	21.3	
8			11.2	37.1	16.0	18.3
24	97.6	80.3	29.7	41.1		22.0
26	98.8	91.3			43.0	23.0
28			18.2	52.8		
30						21.3
32	98.6	92.7	24.8	46.1		
48			36.8	46.8	41.6	43.8
96	97.2	99.8	39.7	55.4	70.9	37.5

Retention time: 28.92-29.07 min – no match.

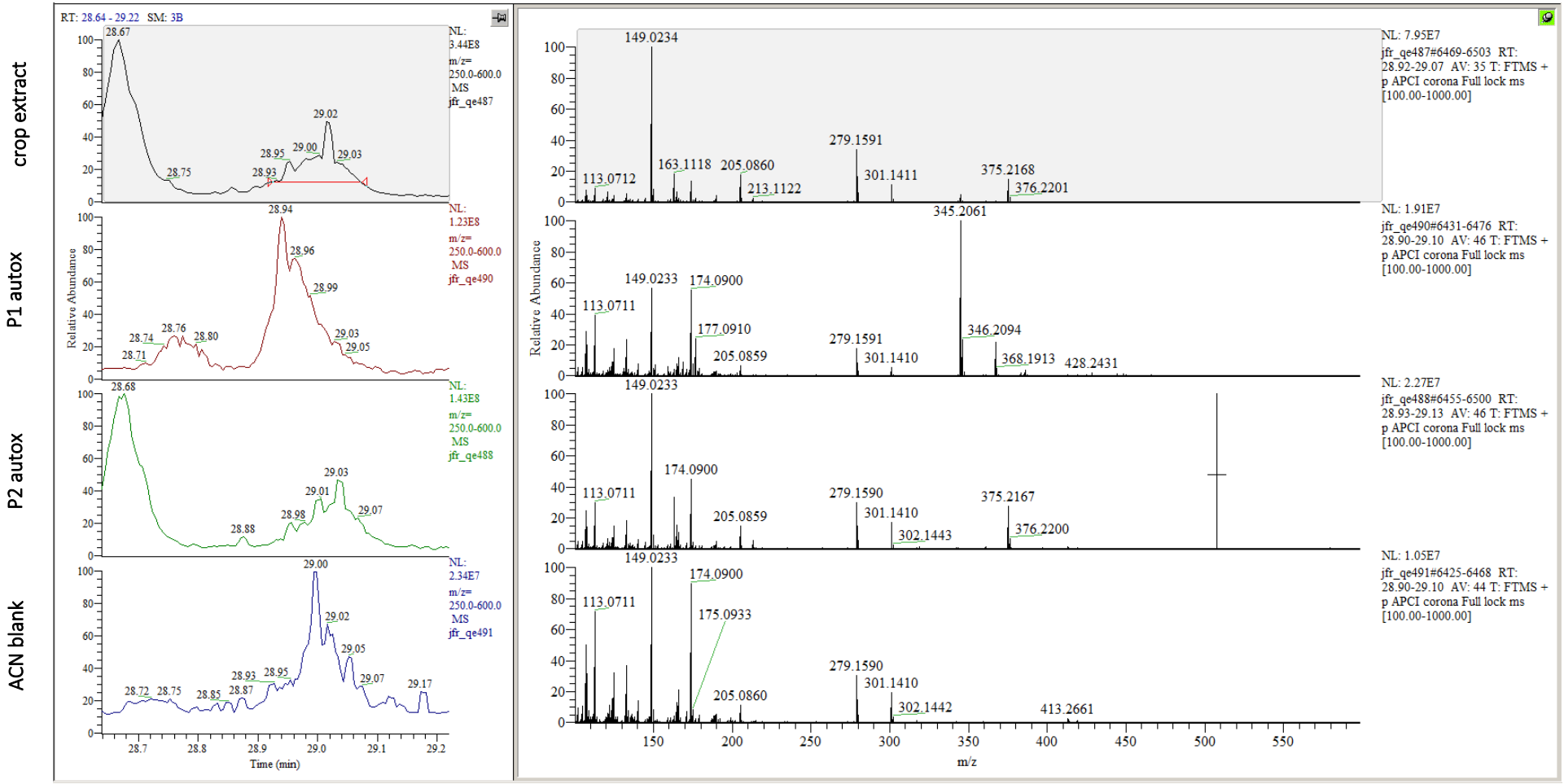


Figure S7. LCMS analysis of peak at 29.0 min

Retention time: 28.64-28.70 min – residual pyrethrin II.

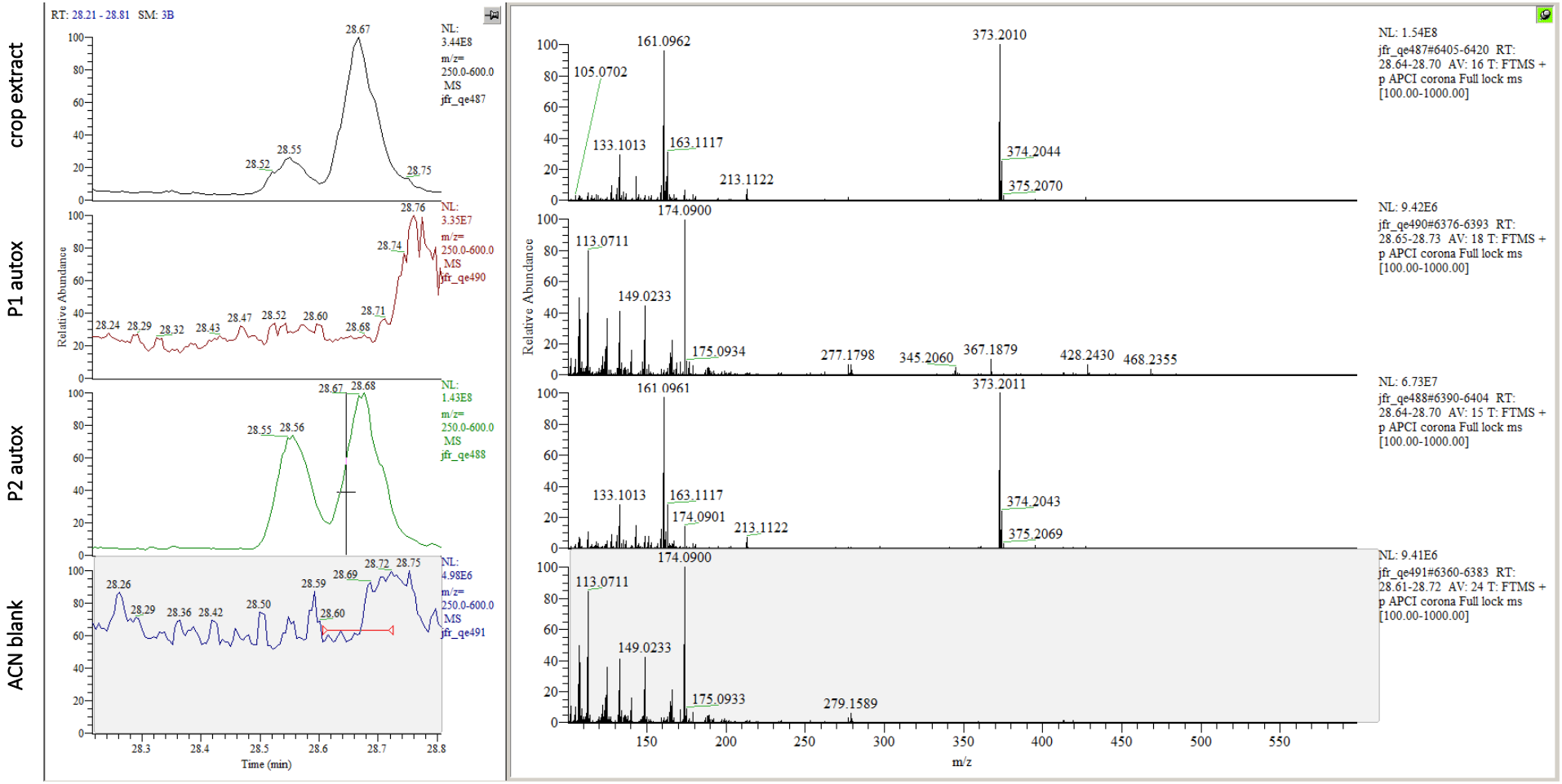


Figure S8. LCMS analysis of peak at 28.7 min

Retention time: 28.50-28.60 min – residual cinerin II.

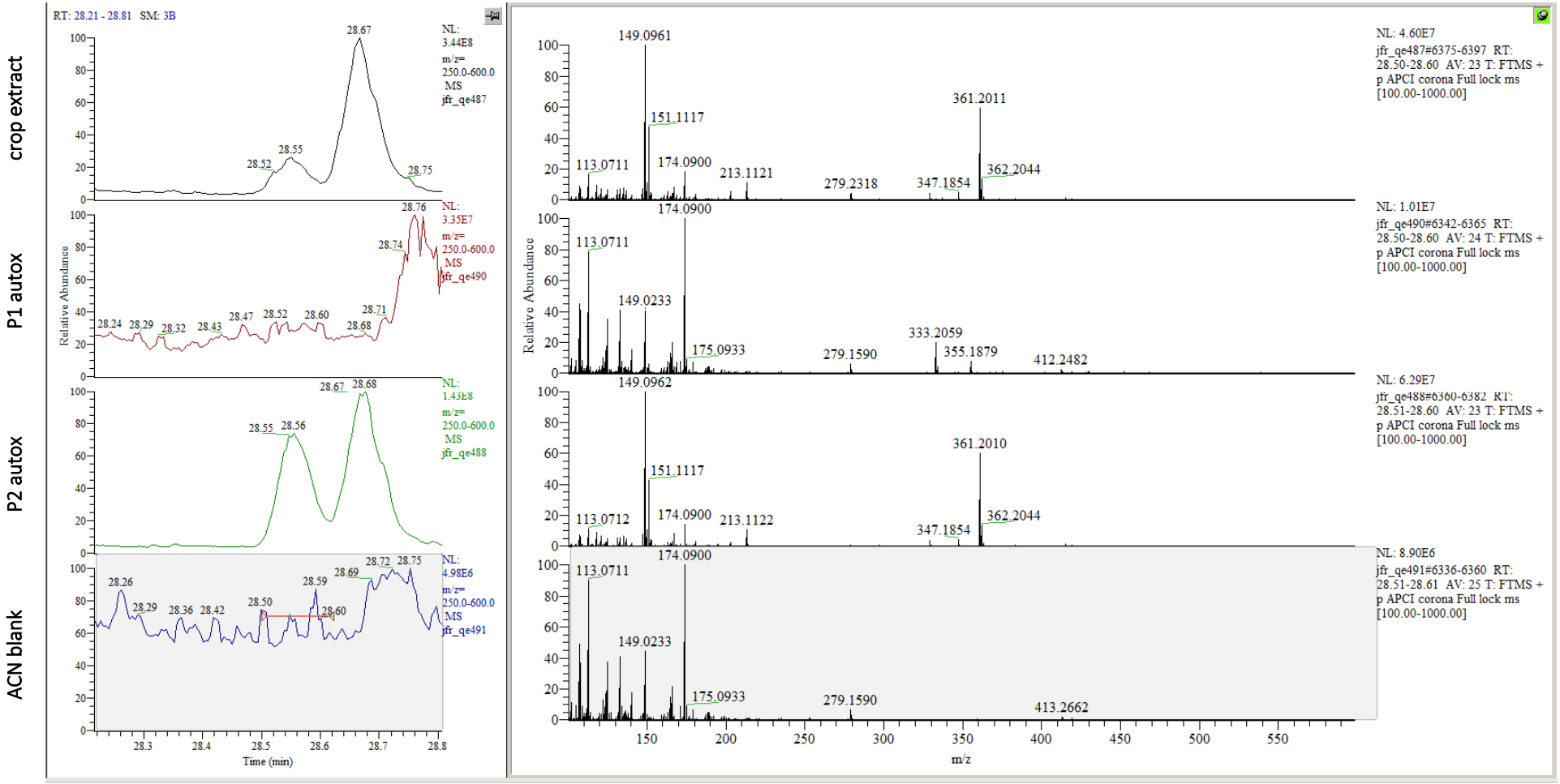


Figure S9. LCMS analysis of peak at 28.6 min

Retention time: 28.13-28.18 min – peak 5, matches in both PI and PII autoxidation mixtures.

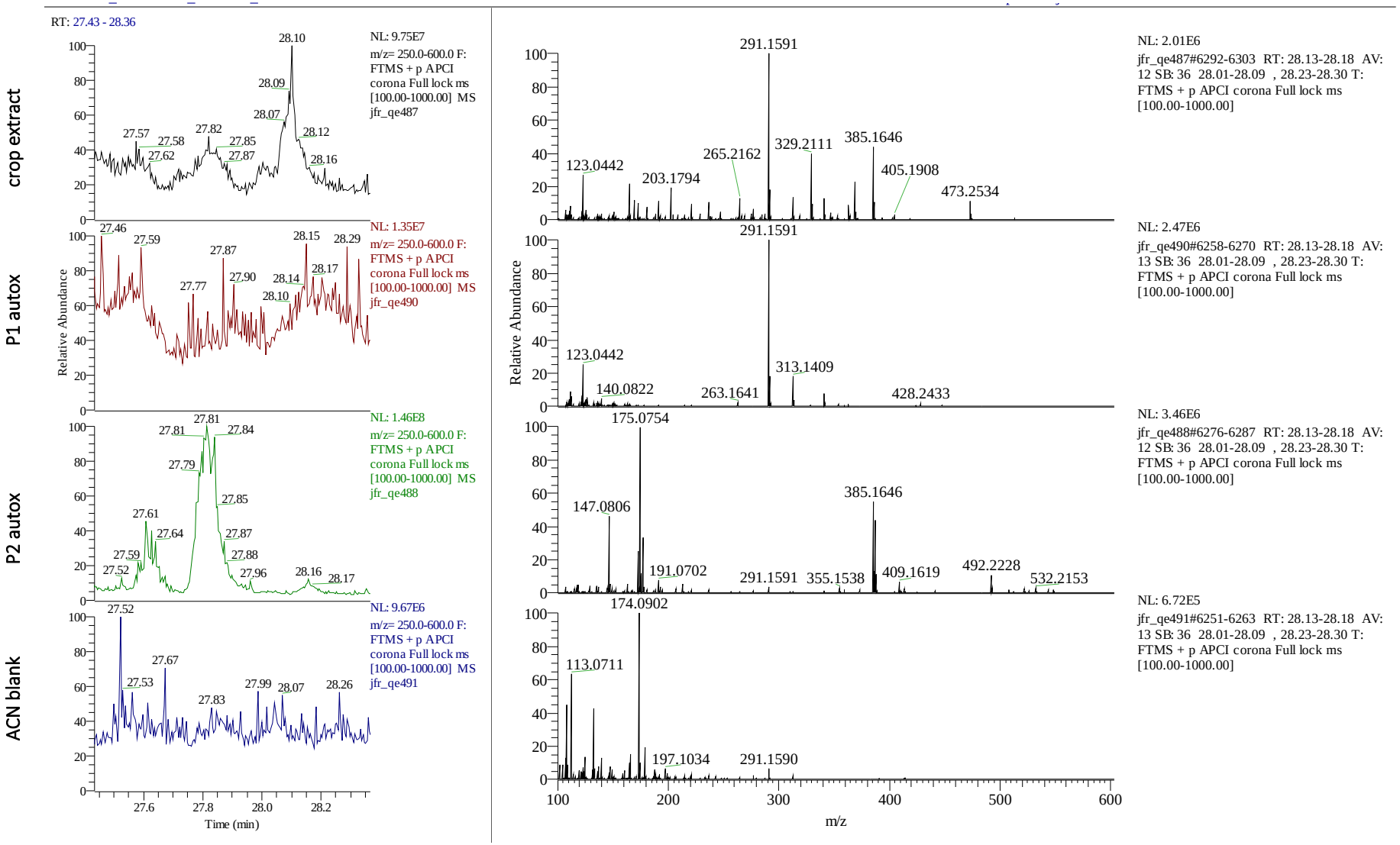


Figure S10. LCMS analysis of peak at 28.1 min

Retention time: 27.77-27.85 min – peak 4, match in PII autoxidation mixture.

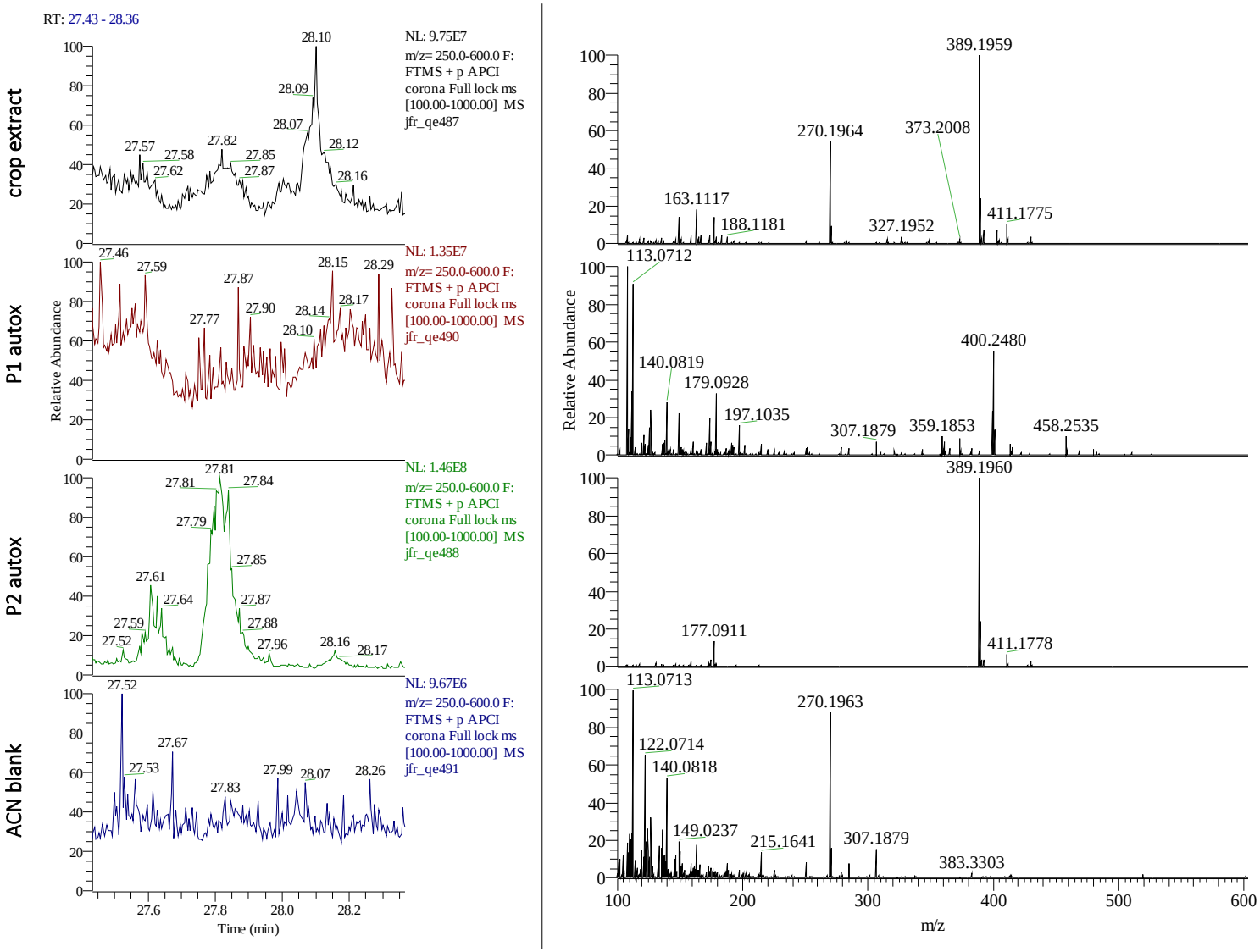


Figure S11. LCMS analysis of peak at 27.8 min

Retention time: 27.58-27.64 min – peak 3, at least two matching components in each of PI and PII autoxidation mixtures.

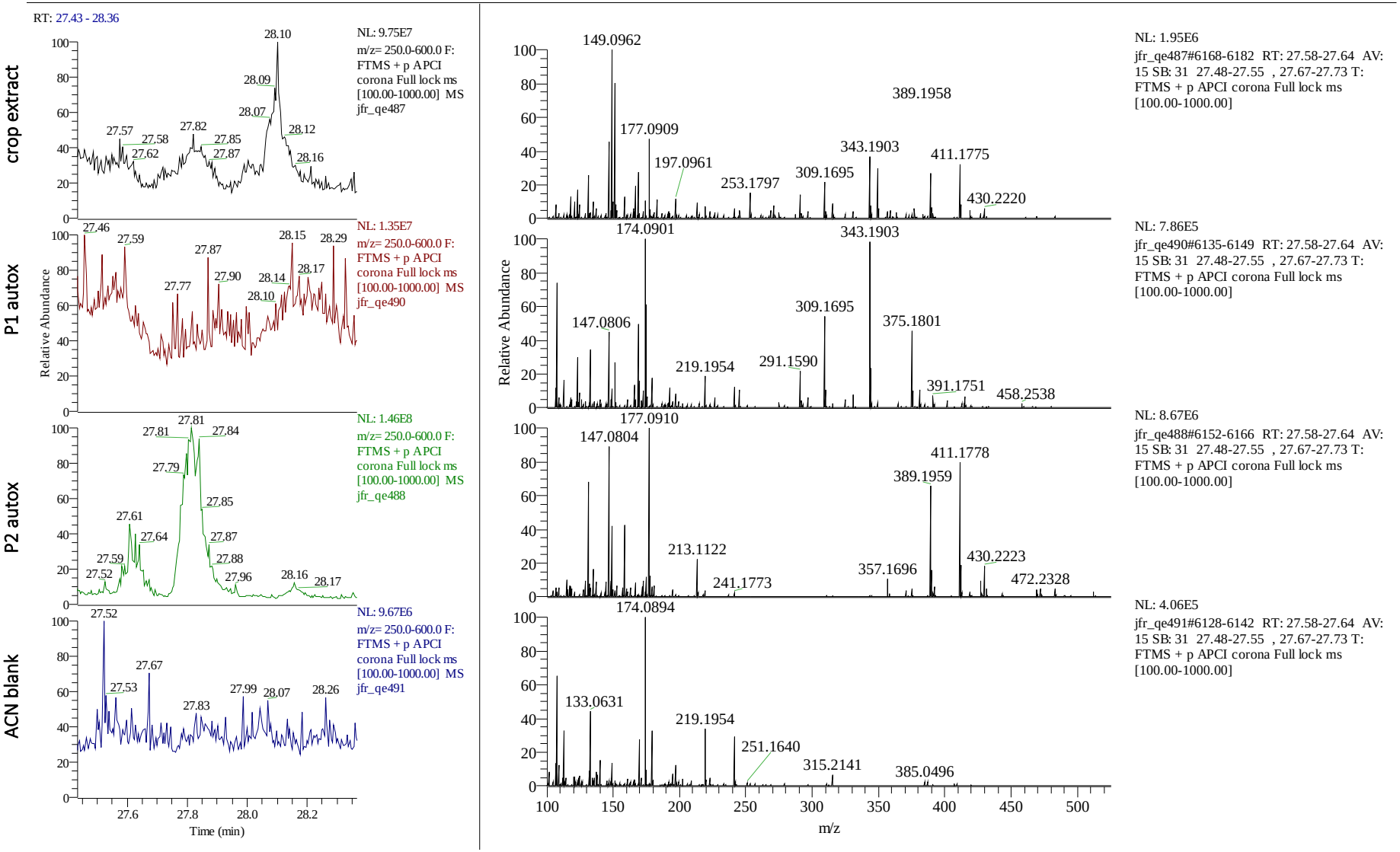


Figure S12. LCMS analysis of peak at 27.6 min

Retention time: 27.26-27.37 min – peak 2, no match.

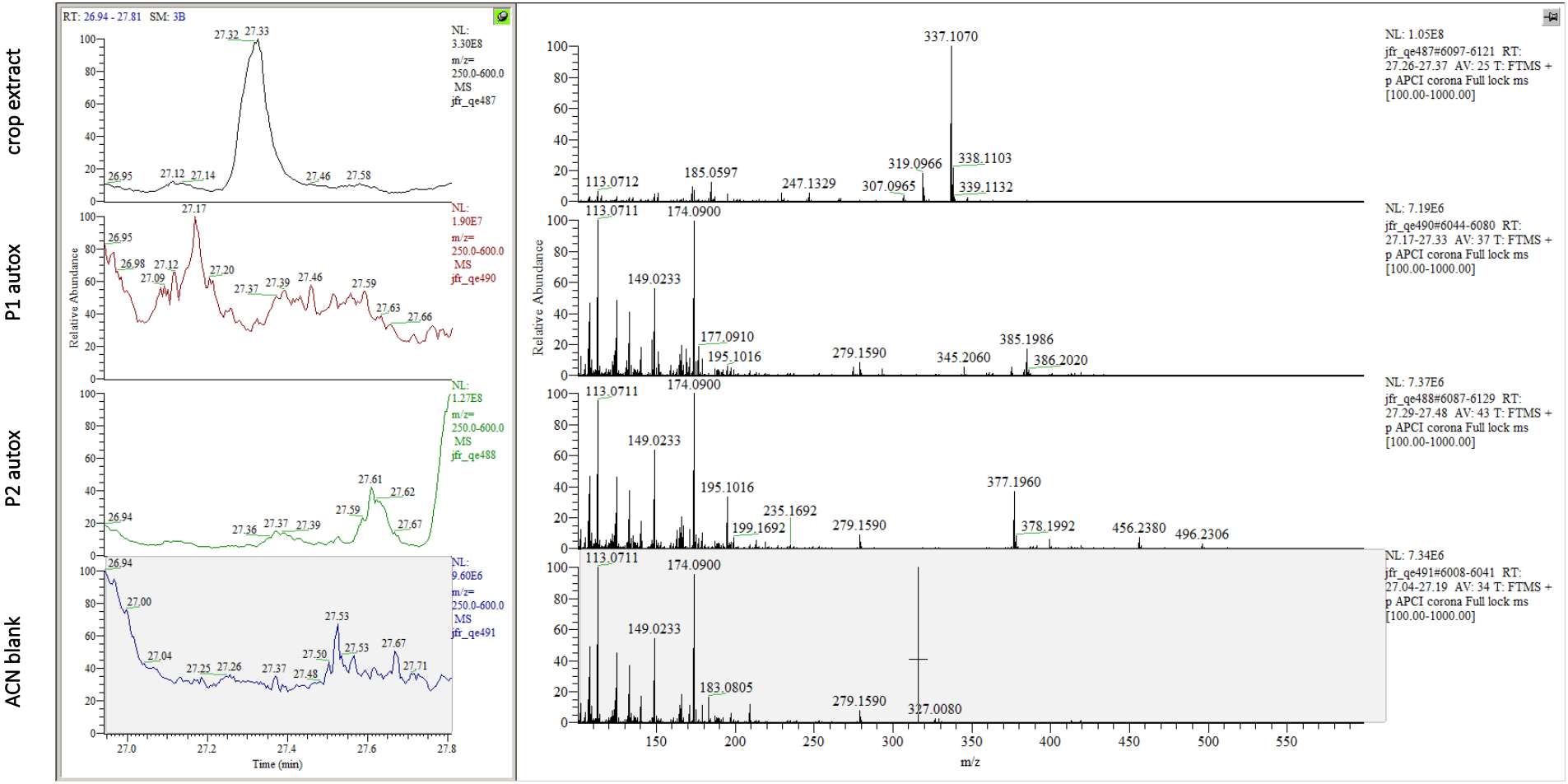


Figure S13. LCMS analysis of peak at 27.3 min

Retention time: 26.80-26.94 min – compound present in blank.

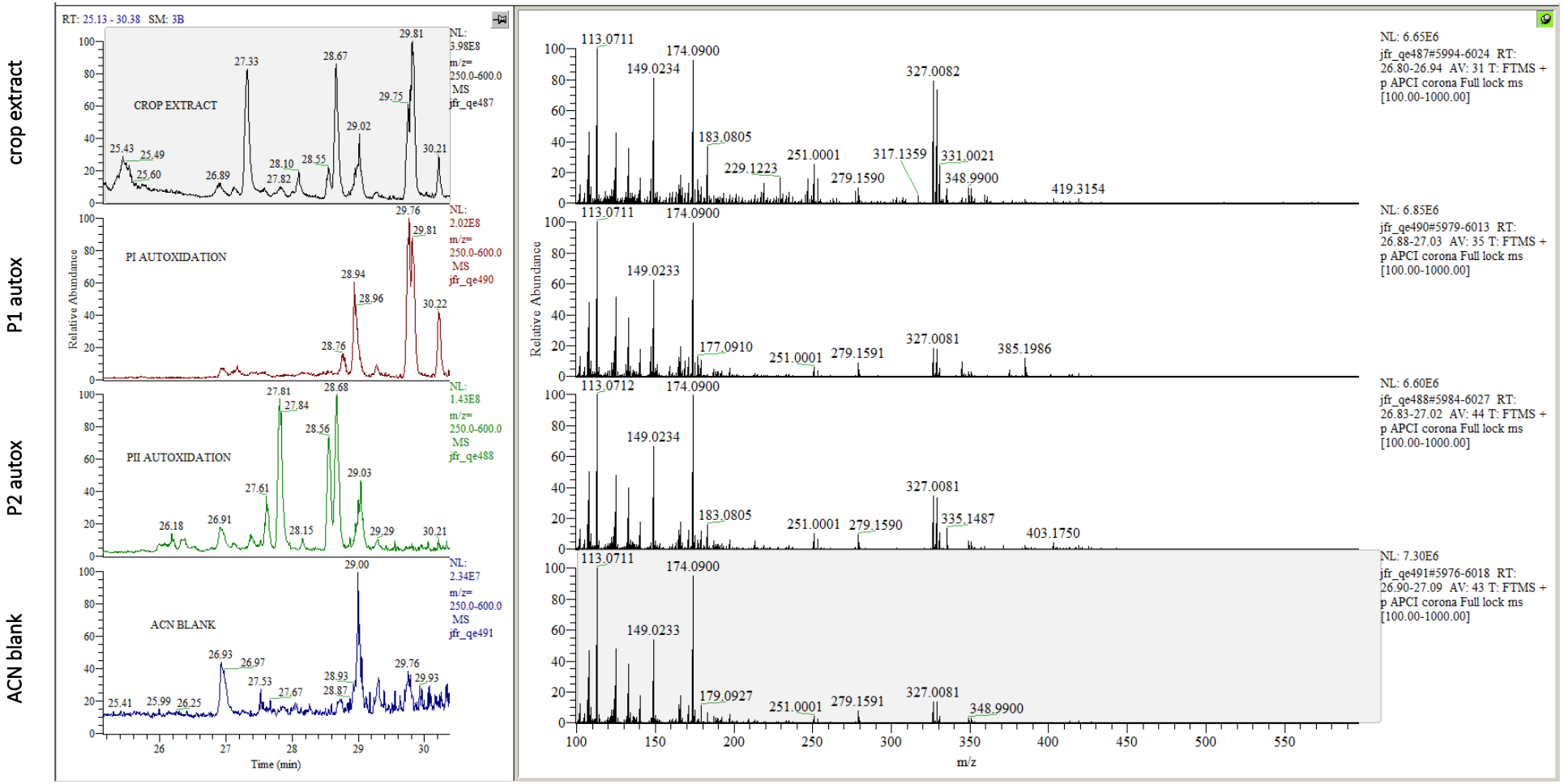


Figure S14. LCMS analysis of peak at 26.9 min

UPLC and LCMS analysis of Compounds A and B, isolated from pyrethrin II autoxidation reaction mixture via preparative HPLC.

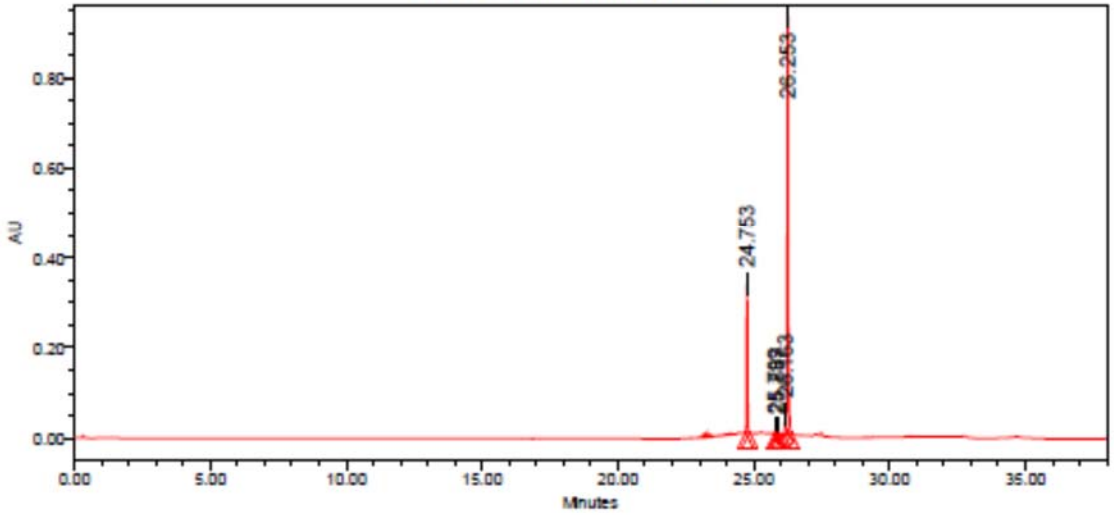
CSIRO Materials Science & Engineering

Run Information

Sample Name: JAF-66_41-46_8 fr 17
Detector: PDA 223.0 nm
HplcColumn: 50x2.1mm BEH C18 50x2.1mm BEH C18
FlowRate: 0.4mL/min 0.4 mL / min
MobilePhase: A1: 5% ACN / H2O B1: 95% ACN / H2O

Date Acquired: 8/11/2012 12:15:48 PM EST
Acq Method Set : 100% A1 to 100% B1 20iso 10gr
Date Processed: 14/11/2012 3:08:23 PM EST
Processing Method: nkh_SL4

HPLC Chromatogram



The Results

	Name	R.Time	Area	% Area	Height	% Height
1		24.753	728178	23.05	329201	25.02
2		25.799	14937	0.47	7627	0.58
3		25.887	21310	0.67	9876	0.75
4		26.163	103161	3.27	42556	3.23
5		26.253	2291199	72.53	926566	70.42

Figure S15. UPLC trace of Compound A.

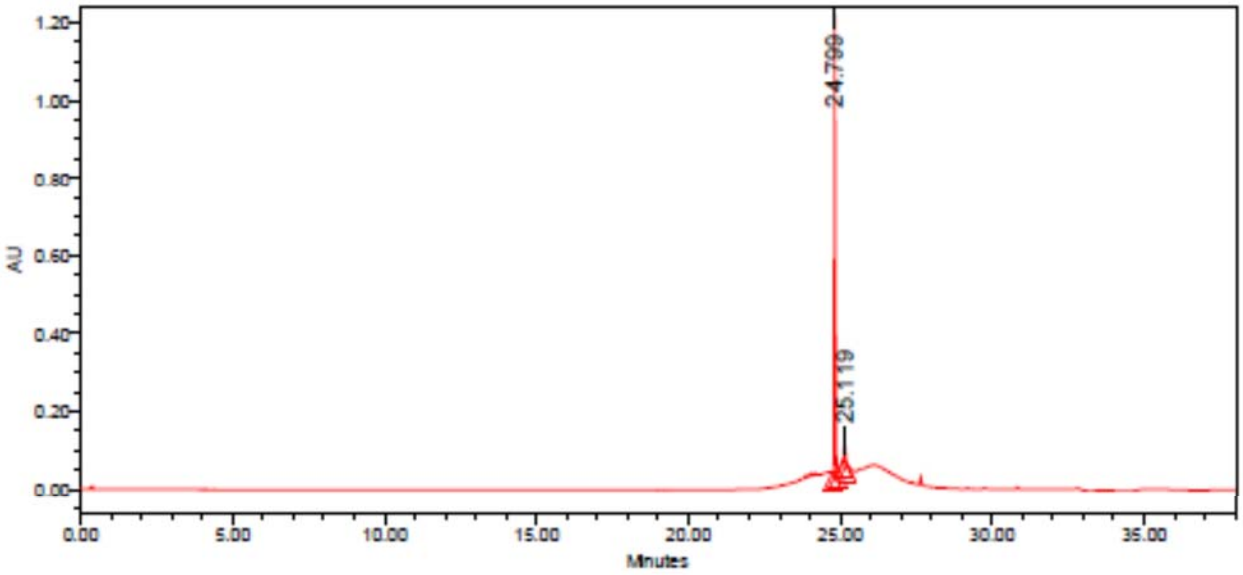
CSIRO Materials Science & Engineering

Run Information

Sample Name: JAF-65_41-46_8 fr 1
Detector: PDA 223.0 nm
HplcColumn: 50x2.1mm BEH C18 50x2.1mm BEH C18
FlowRate: 0.4mL/min 0.4 mL / min
MobilePhase: A1: 5% ACN / H2O B1: 95% ACN / H2O

Date Acquired: 7/11/2012 12:49:41 PM EST
Acq Method Set : 100% A1 to 100% B1 20iso 10gr
Date Processed: 14/11/2012 3:07:12 PM EST
Processing Method: nkh_SL4

HPLC Chromatogram

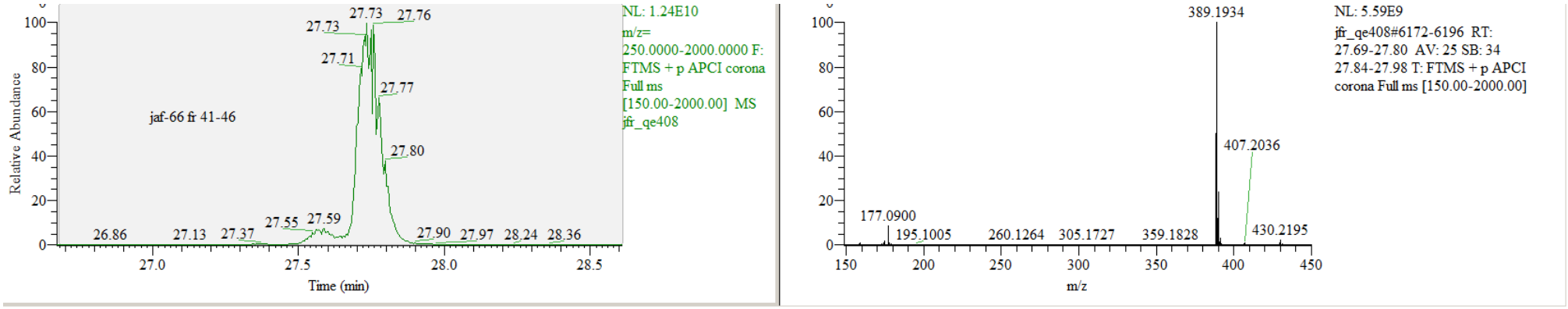


The Results

	Name	R.Time	Area	% Area	Height	% Height
1		24.799	2680101	95.12	1175459	95.87
2		25.119	137388	4.88	50691	4.13

Figure S16. UPLC trace of Compound B

LCMS analysis of Compound A:



Flow injection MS Analysis of Compound B:

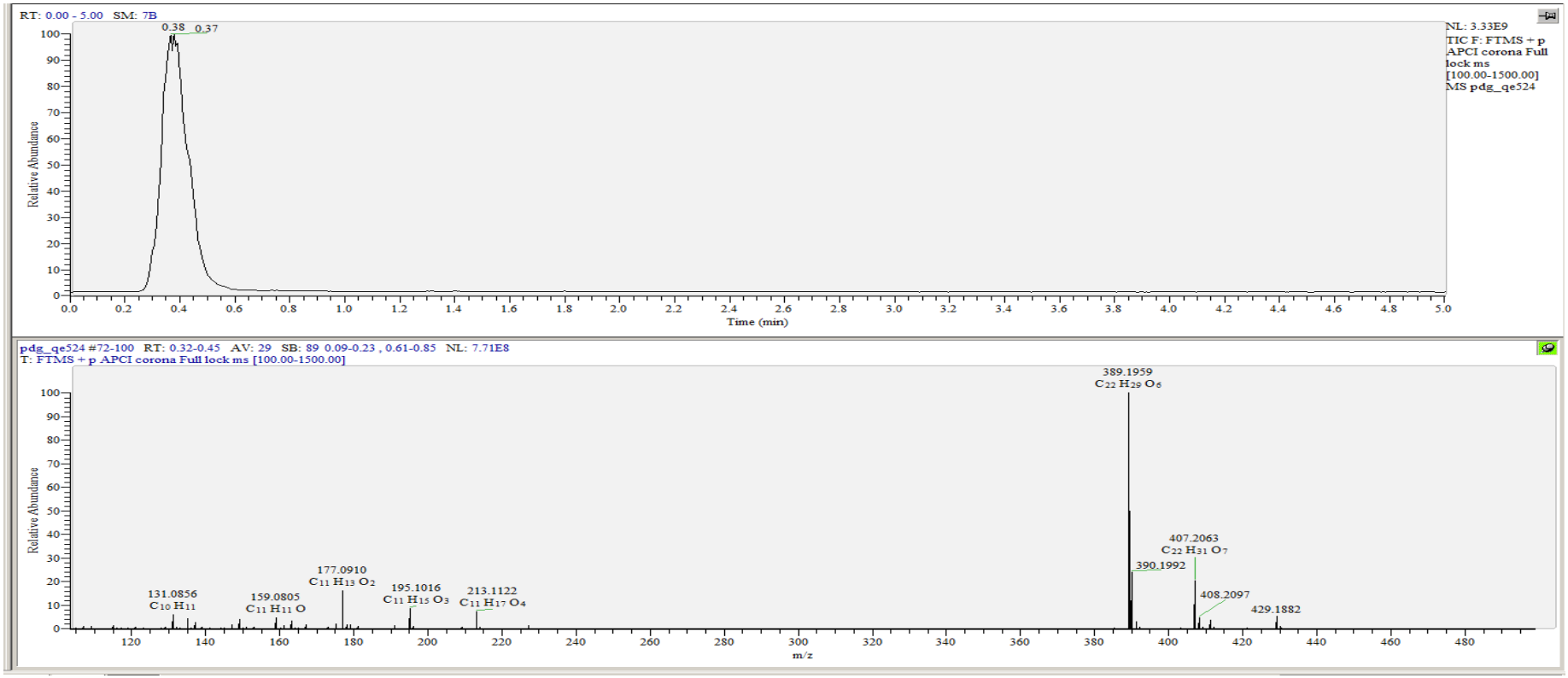


Figure S17. LCMS analyses of Compounds A and B

LCMS evidence for the presence of Compounds A and B in bulk stored crop extract.

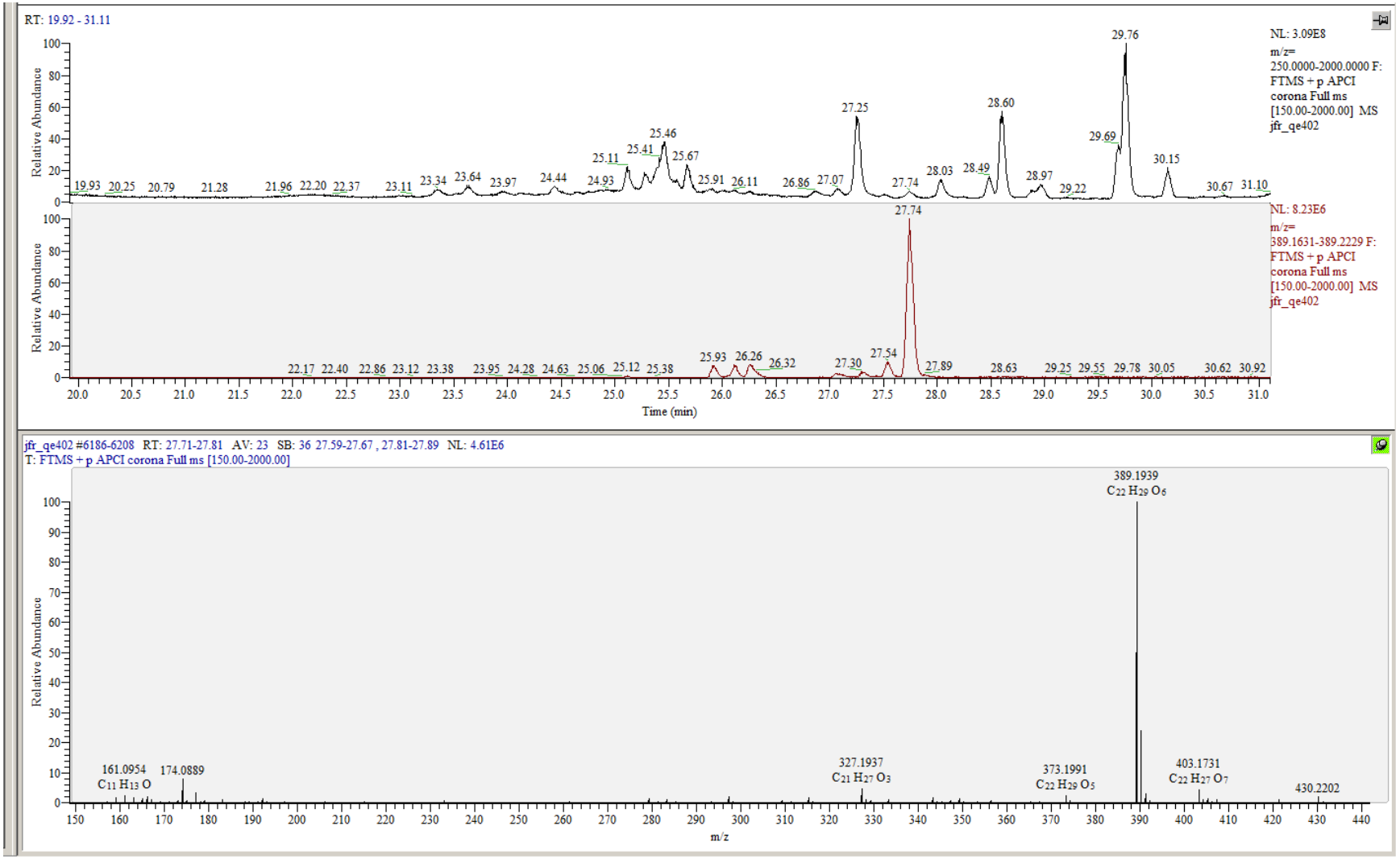


Figure S18. LCMS identification of Compound A in stored crop extract.

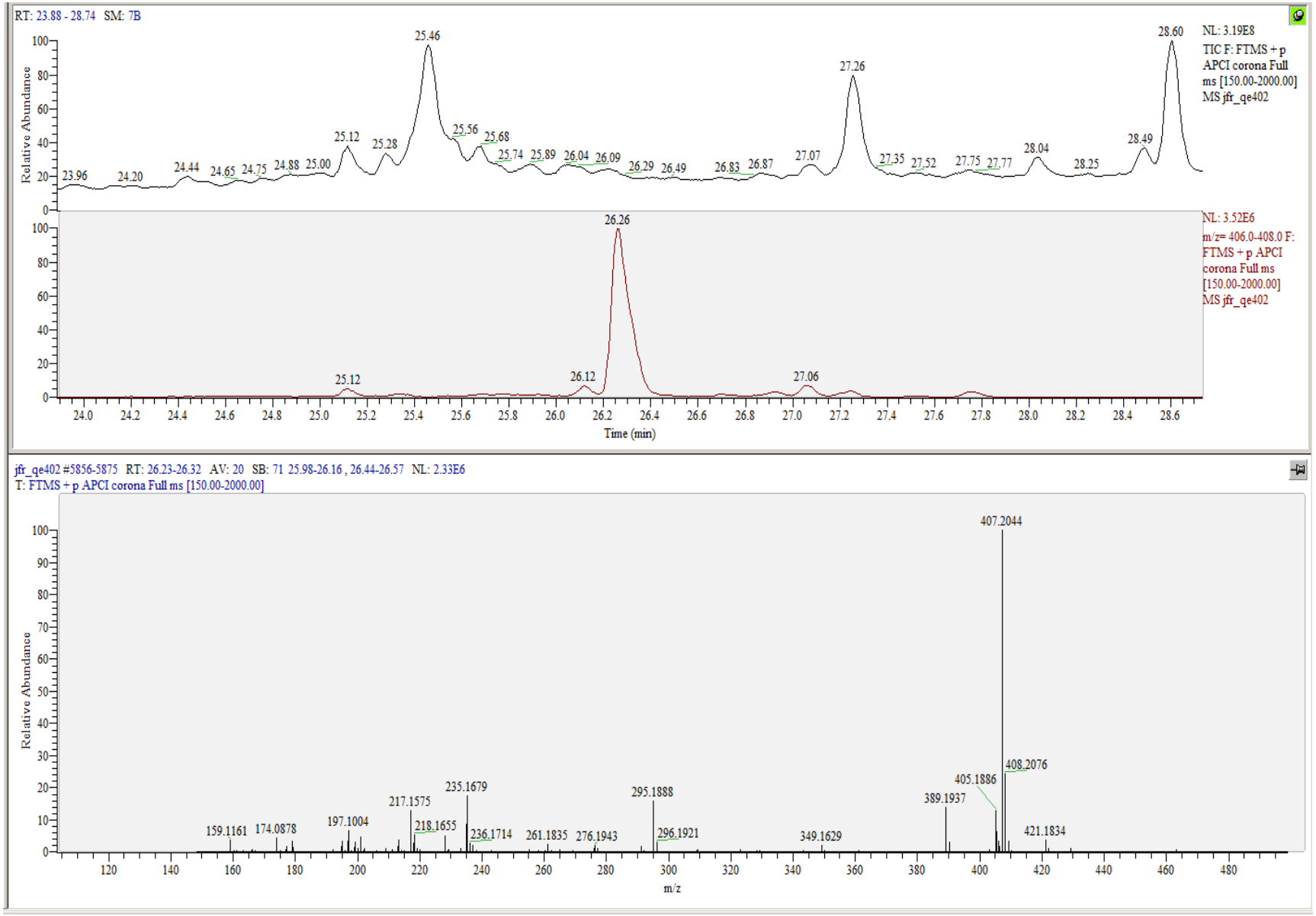


Figure S19. LCMS identification of Compound B in stored crop extract

Infrared analysis of compounds A and B.

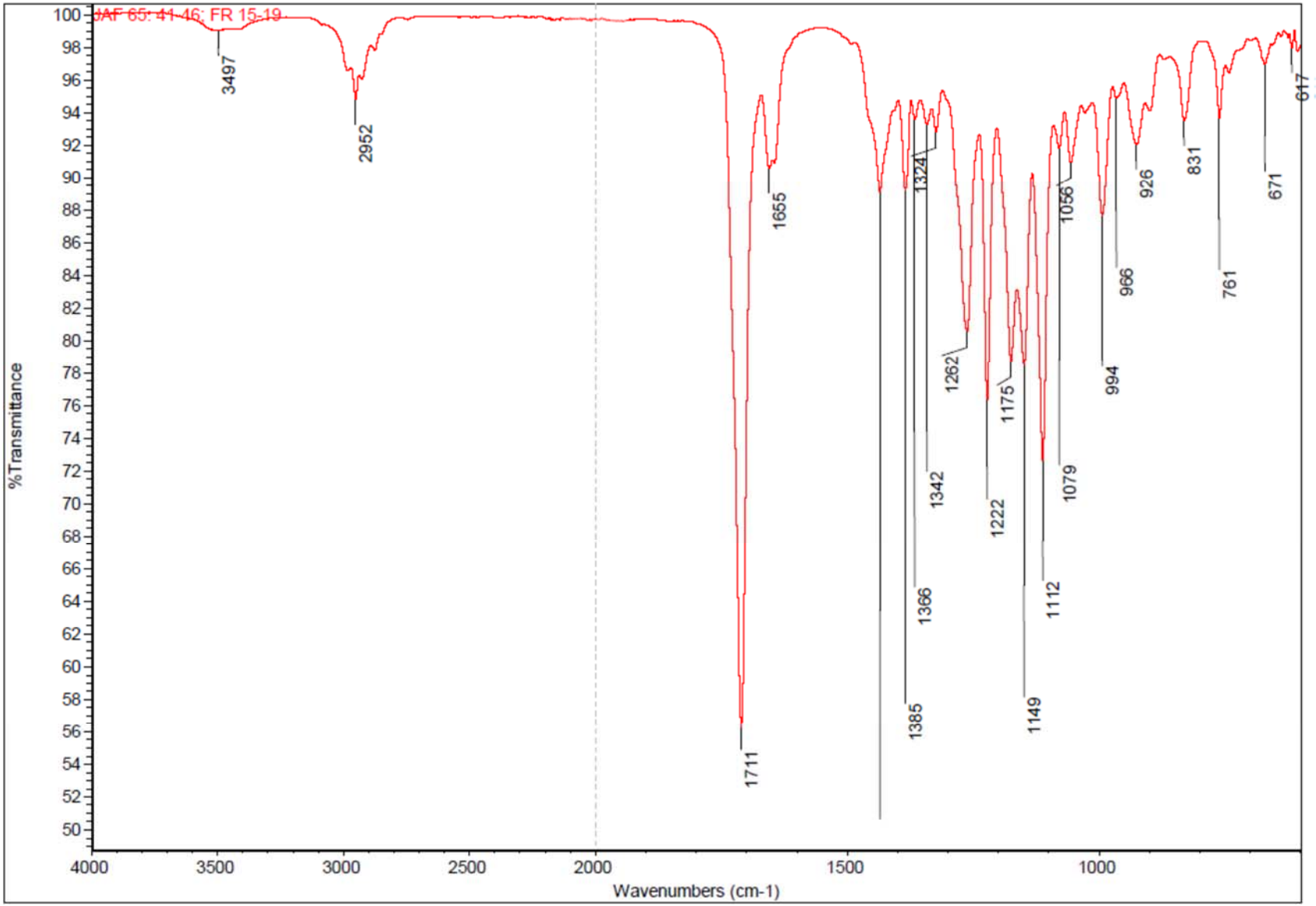


Figure S20. IR spectrum of Compound A.

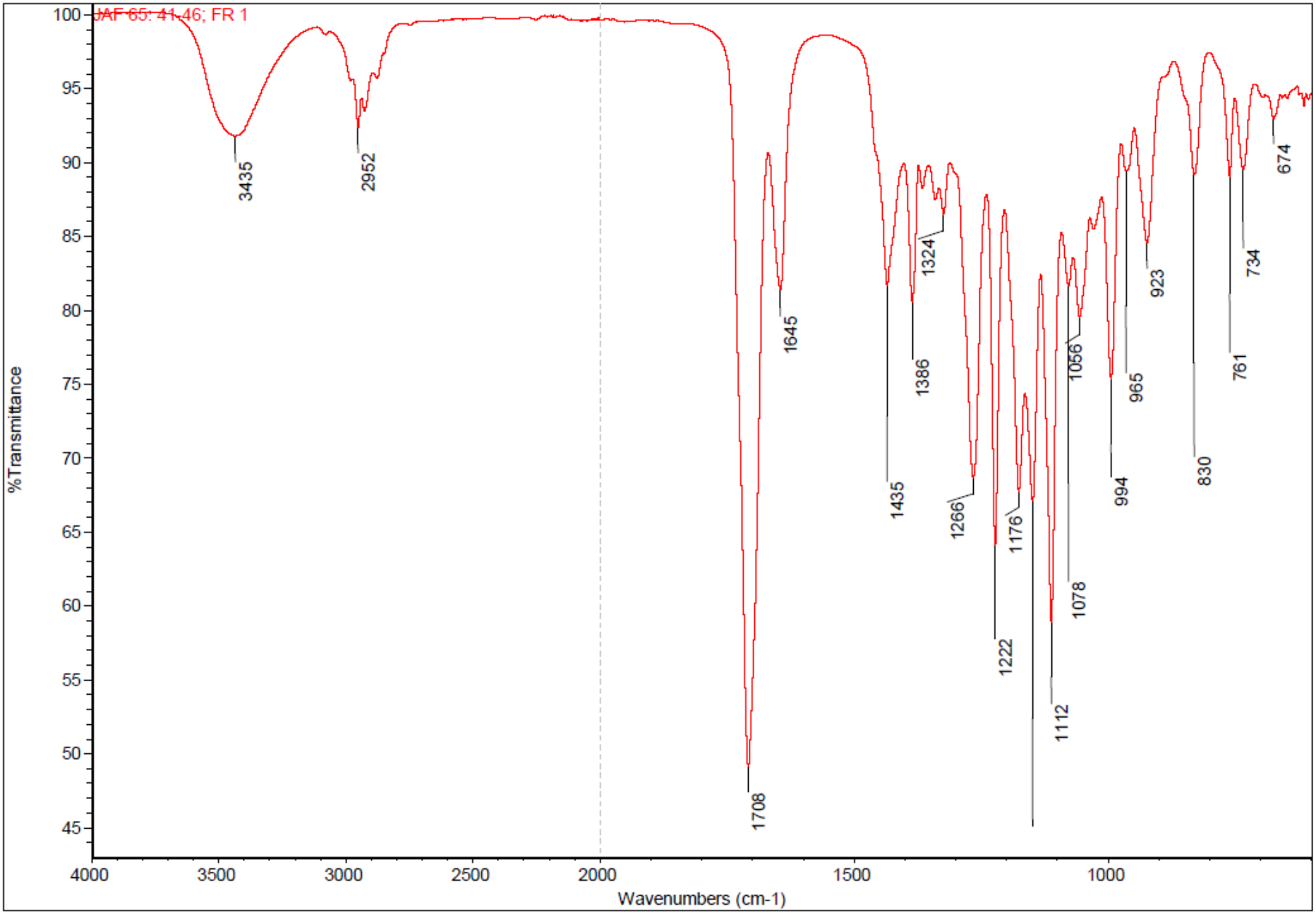
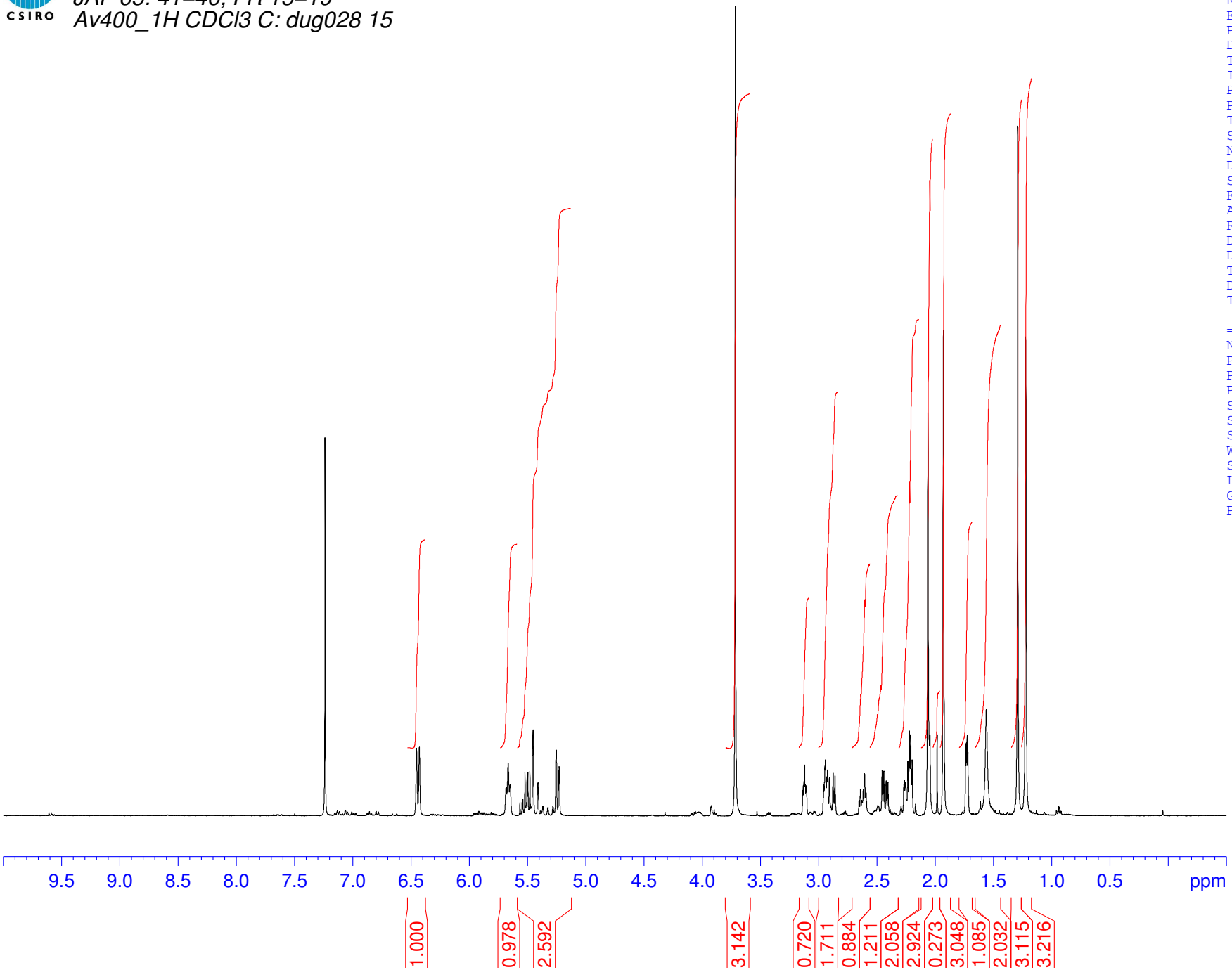


Figure S21. IR analysis of Compound B

Username P. Duggan
WBS R–108–1–1
JAF 65: 41–46; FR 15–19
Av400_1H CDCl3 C: dug028 15

NMR characterisation of Compound A

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TD                   32768
SOLVENT              CDCl3
NS                       32
DS                        2
SWH              5995.204 Hz
FIDRES           0.182959 Hz
AQ              2.7329011 sec
RG                     181
DW                  83.400 usec
DE                   12.00 usec
TE                   298.2 K
D1             1.00000000 sec
TD0                       1

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P1                    9.80 usec
PL1                  -1.00 dB
PL1W          28.77672577 W
SFO1          400.1326104 MHz
SI                   65536
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WDW                      EM
SSB                       0
LB                     0.10 Hz
GB                        0
PC                     1.00
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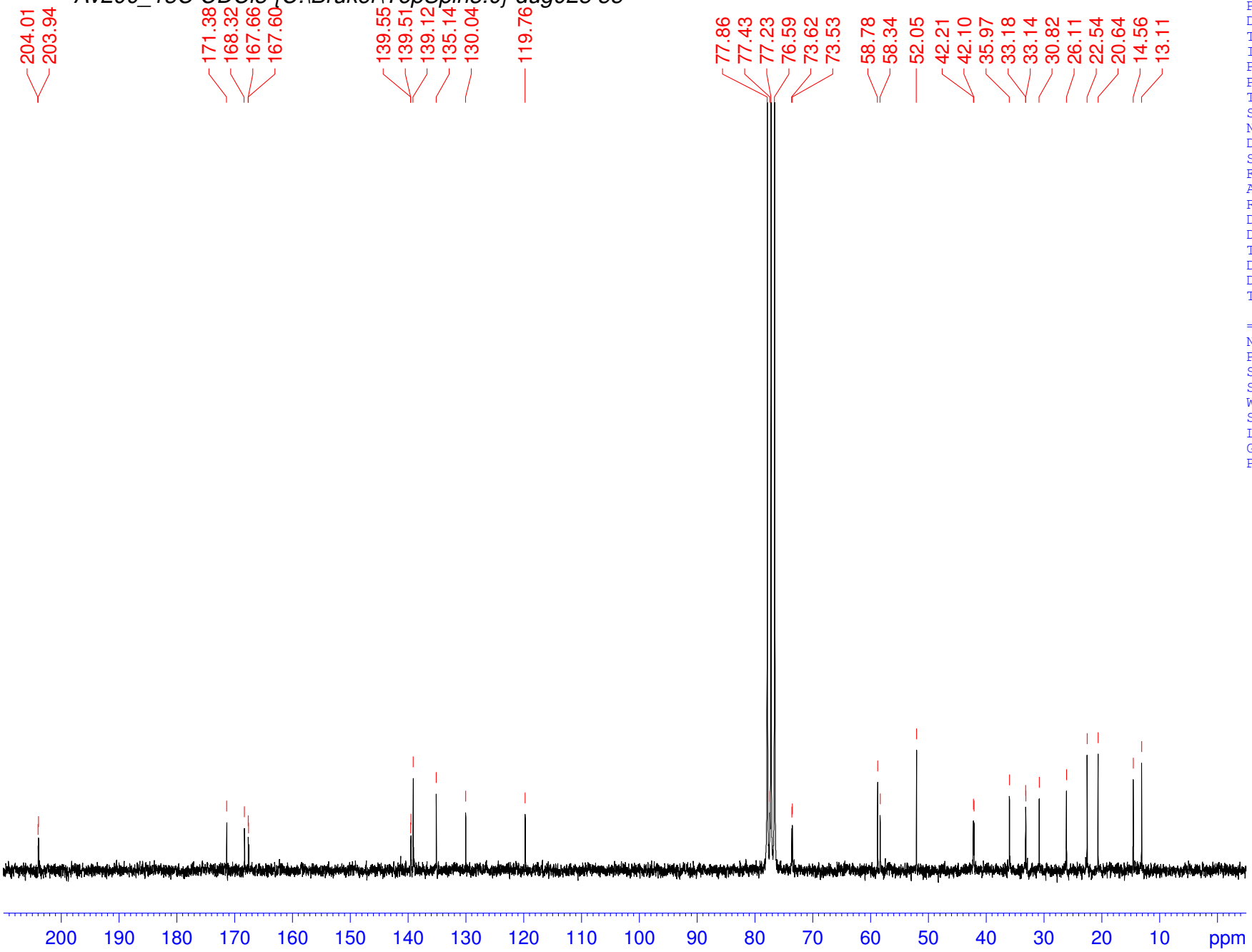
P. Duggan
R–108–1–1
JAF 65: 41–46; FR 15–19
Av200_13C CDCl3 {C:\Bruker\TopSpin3.0} dug028 55

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TD                      32768
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RG                       90.5
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DE                       6.50 usec
TE                      298.2 K
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D11                0.03000000 sec
TD0                         1

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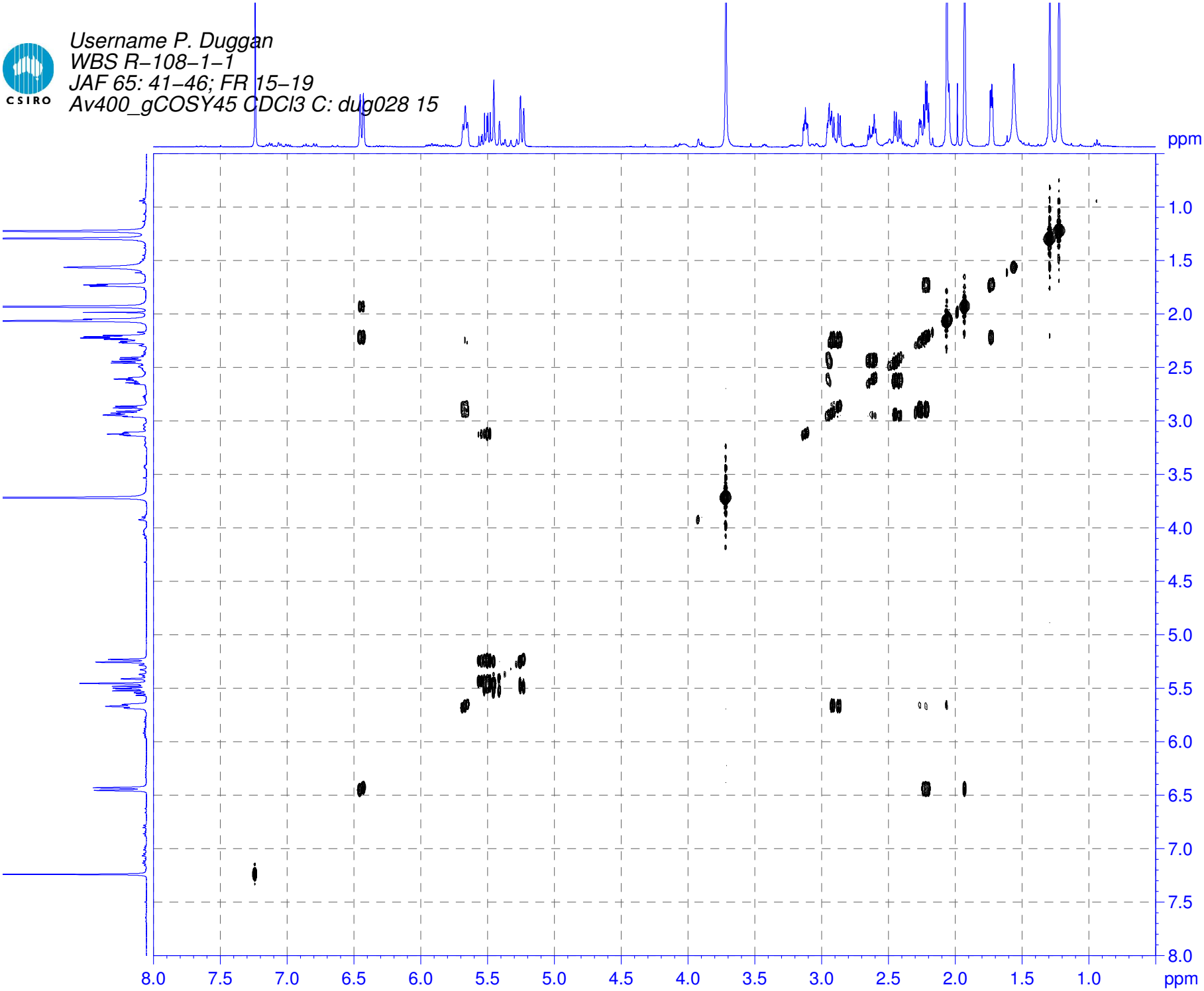
Username P. Duggan
WBS R–108–1–1
JAF 65: 41–46; FR 15–19
Av400_gCOSY45 CDCl3 C: dug028 15



BRUKER

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TD                          4096
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NS                            1
DS                           16
SWH                    4789.272 Hz
FIDRES                 1.169256 Hz
AQ                    0.4276724 sec
RG                       1290.2
DW                       104.400 usec
DE                         12.00 usec
TE                         298.2 K
D0                   0.00000300 sec
D1                   1.00000000 sec
D13                  0.00000400 sec
D16                  0.00020000 sec
IN0                  0.00020880 sec

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NUC1                         1H
P1                         9.80 usec
PL1                       -1.00 dB
PL1W                 28.77672577 W
SFO1                400.1320007 MHz

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GPNAM2                  sine.100
GPZ1                       10.00 %
GPZ2                       10.00 %
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ND0                           1
TD                          256
SFO1                    400.132 MHz
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SW                        11.969 ppm
FnMODE                        QF
SI                          2048
SF                  400.1300175 MHz
WDW                         SINE
SSB                            0
LB                          0.00 Hz
GB                             0
PC                          1.00
SI                          2048
MC2                           QF
SF                  400.1300175 MHz
WDW                         SINE
SSB                            0
LB                          0.00 Hz
GB                             0
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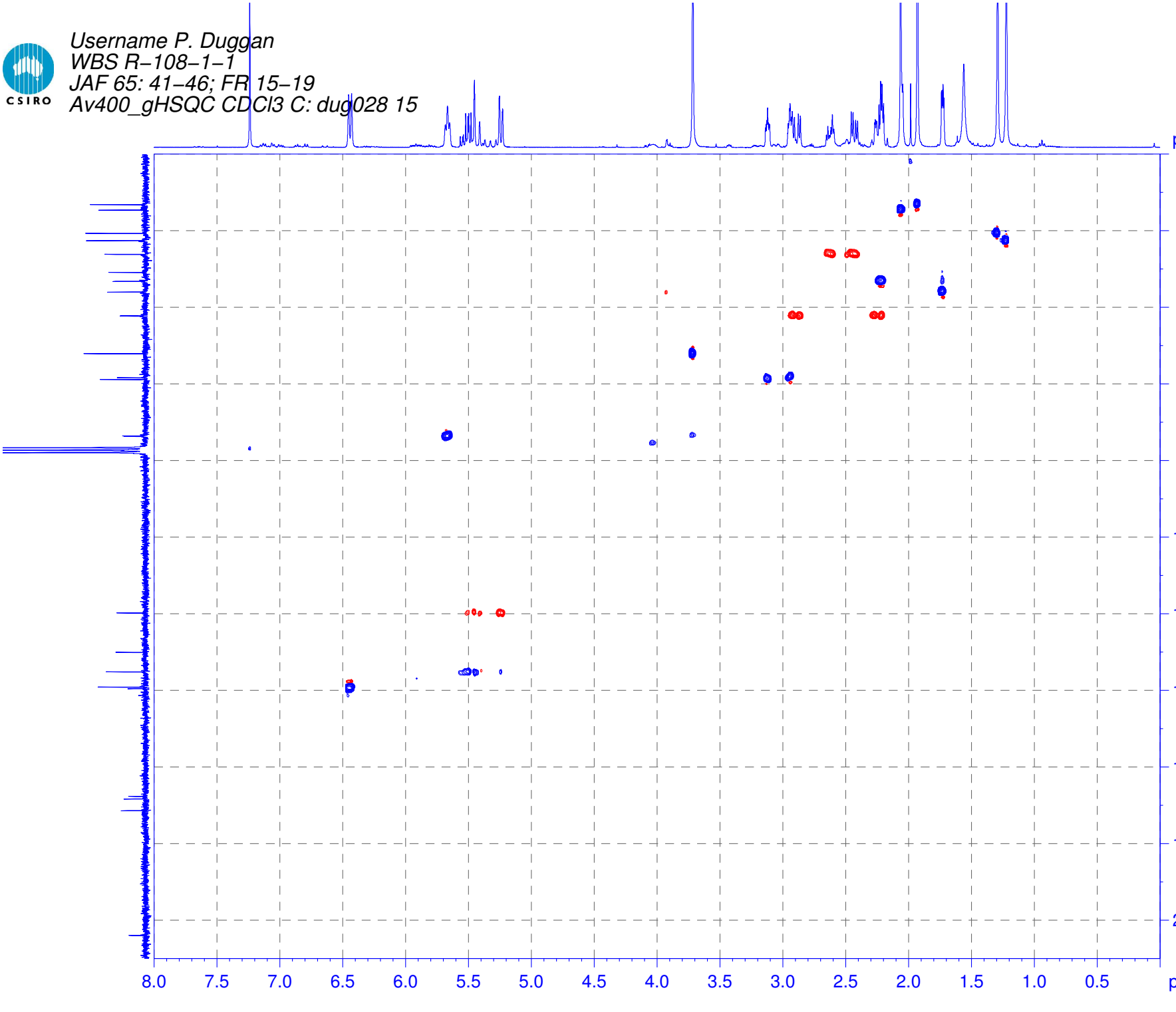
Username P. Duggan
WBS R–108–1–1
JAF 65: 41–46; FR 15–19
Av400_gHSQC CDCl3 C: dug028 15



BRUKER

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TD            2048
SOLVENT       CDCl3
NS               2
DS              16
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FIDRES     1.956255 Hz
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RG           2580.3
DW          124.800 usec
DE           12.00 usec
TE           298.2 K
CNST2    145.0000000
CNST17    -0.5000000
D0       0.00000300 sec
D1       1.50000000 sec
D4       0.00172414 sec
D11      0.03000000 sec
D16      0.00020000 sec
D21      0.00344828 sec
D24      0.00086207 sec
IN0      0.00002925 sec

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P2            19.60 usec
P28            0.10 usec
PL1           -1.00 dB
PL1W    28.77672577 W
SFO1    400.1320007 MHz

======== CHANNEL f2 ========
CPDPRG2       garp4
NUC2            13C
P3            13.00 usec
P14          500.00 usec
P24         2000.00 usec
PCPD2         70.00 usec
PL0          120.00 dB
PL2           -6.00 dB
PL12           8.62 dB
PL0W     0.00000000 W
PL2W   163.00000000 W
PL12W    5.62584305 W
SFO2    100.6208180 MHz
SP3           -0.12 dB
SP7           -0.12 dB
SPNAM3   Crp60,0.5,20.1
SPNAM7     Crp60comp.4
SPOAL3        0.500
SPOAL7        0.500
SPOFFS3        0.00 Hz
SPOFFS7        0.00 Hz

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GPNAM2      sine.100
GPNAM3      sine.100
GPNAM4      sine.100
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GPZ2          20.10 %
GPZ3          11.00 %
GPZ4          -5.00 %
P16         1000.00 usec
P19          600.00 usec
ND0              2
TD             256
SFO1       100.6208 MHz
FIDRES    66.818512 Hz
SW          170.000 ppm
FnMODE    Echo-Antiecho
SI            2048
SF     400.1300175 MHz
WDW          QSINE
SSB              2
LB            0.00 Hz
GB               0
PC            1.00
SI           1024
MC2     echo-antiecho
SF     100.6127588 MHz
WDW          QSINE
SSB              2
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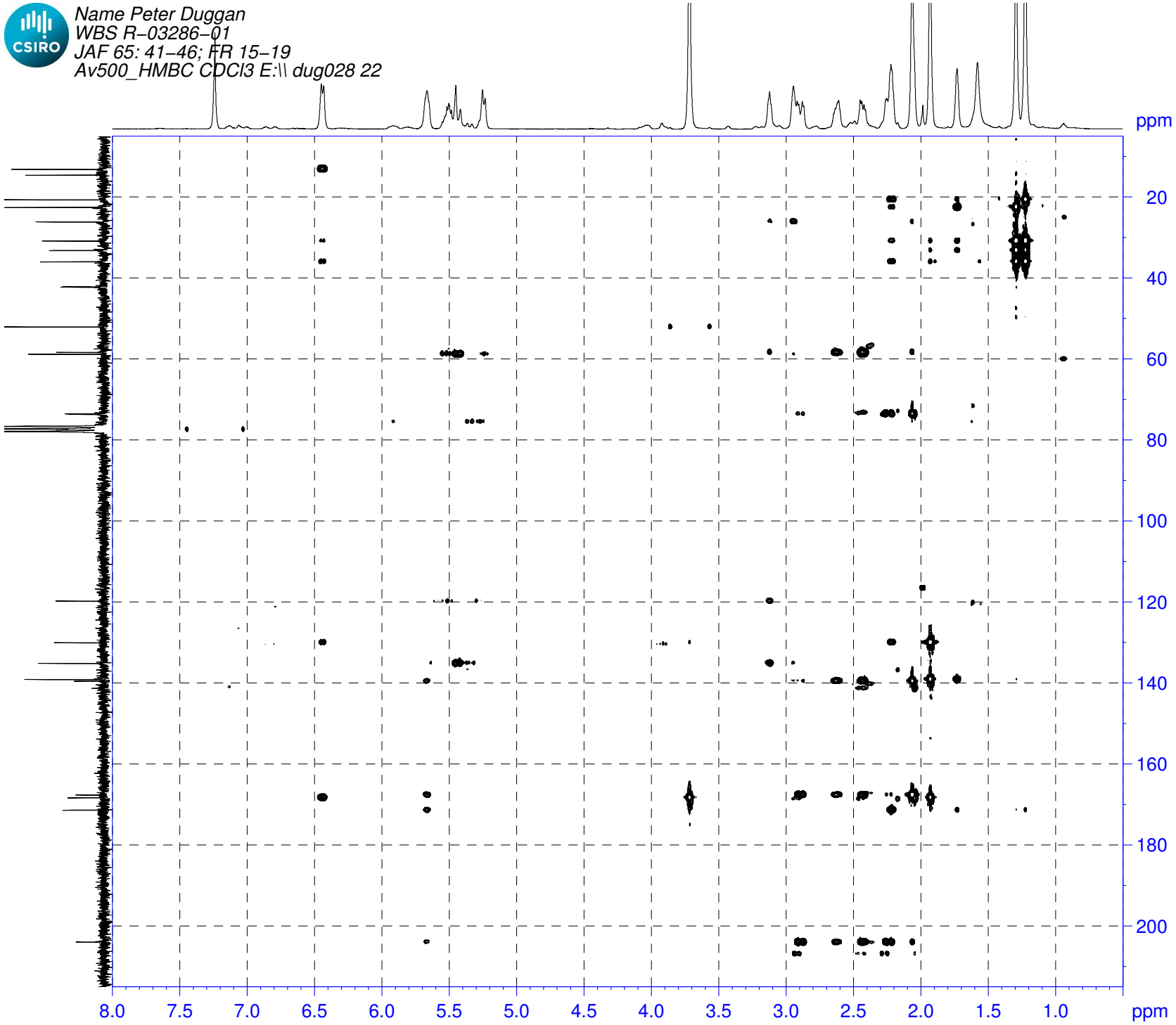
Name Peter Duggan
WBS R–03286–01
JAF 65: 41–46; FR 15–19
Av500_HMBC CDCl3 E:\\ dug028 22

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DS                     16
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FIDRES          1.467191 Hz
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RG                   203
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DE                  6.50 usec
TE                298.2 K
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CNST7        165.0000000
CNST13         8.0000000
CNST30         0.5981140
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D1           1.50000000 sec
D6           0.06250000 sec
D16          0.00020000 sec
IN0          0.00001810 sec

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SFO1         500.1327507 MHz
NUC1                  1H
P1                  7.90 usec
P2                 15.80 usec
PLW1         13.43999958 W

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NUC2                 13C
P3                 11.80 usec
P24              2000.00 usec
PLW2         197.96000671 W
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SPOFFS7   0 Hz
SPW7         42.11399841 W

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GPNAM[4]       SMSQ10.100
GPNAM[5]       SMSQ10.100
GPZ1                80.00 %
GPZ3                15.00 %
GPZ4               -10.00 %
GPZ5                -5.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    512
SFO1              125.771 MHz
FIDRES          53.953728 Hz
SW                219.640 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                   2048
SF           500.1300219 MHz
WDW                 QSINE
SSB                     2
LB        0 Hz
GB        0
PC                   1.00

F1 - Processing parameters
SI                   2048
MC2        echo-antiecho
SF           125.7577787 MHz
WDW                 QSINE
SSB                     2
LB        0 Hz
GB        0
```

Name Peter Duggan
WBS R–03286–01
JAF 65: 41–46; FR 1
Av500_1H CDCl3 E:\\ dug028 21

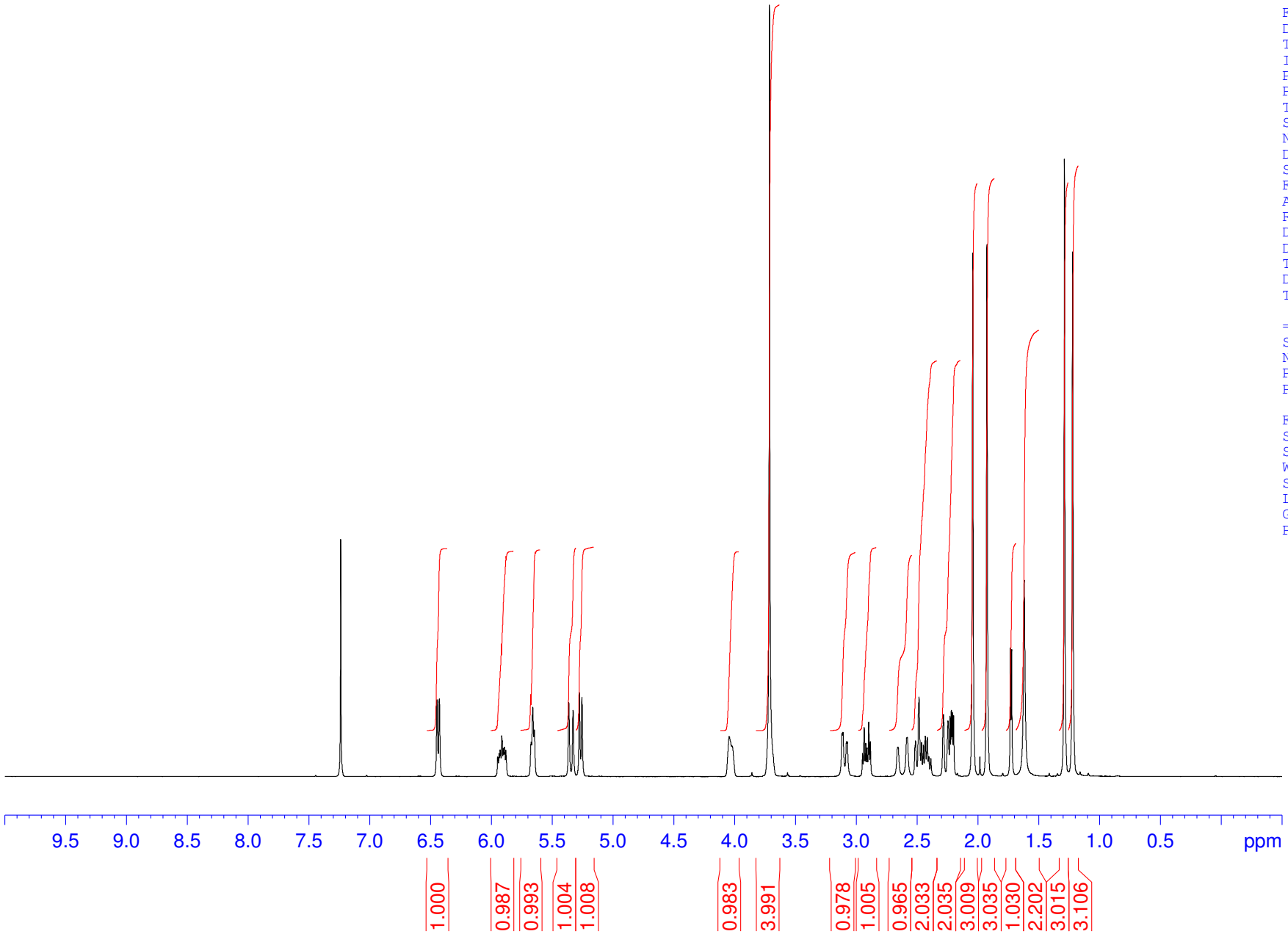
NMR Characterisation of Compound B

```
Current Data Parameters
NAME          duggan50002
EXPNO                   1
PROCNO                  1

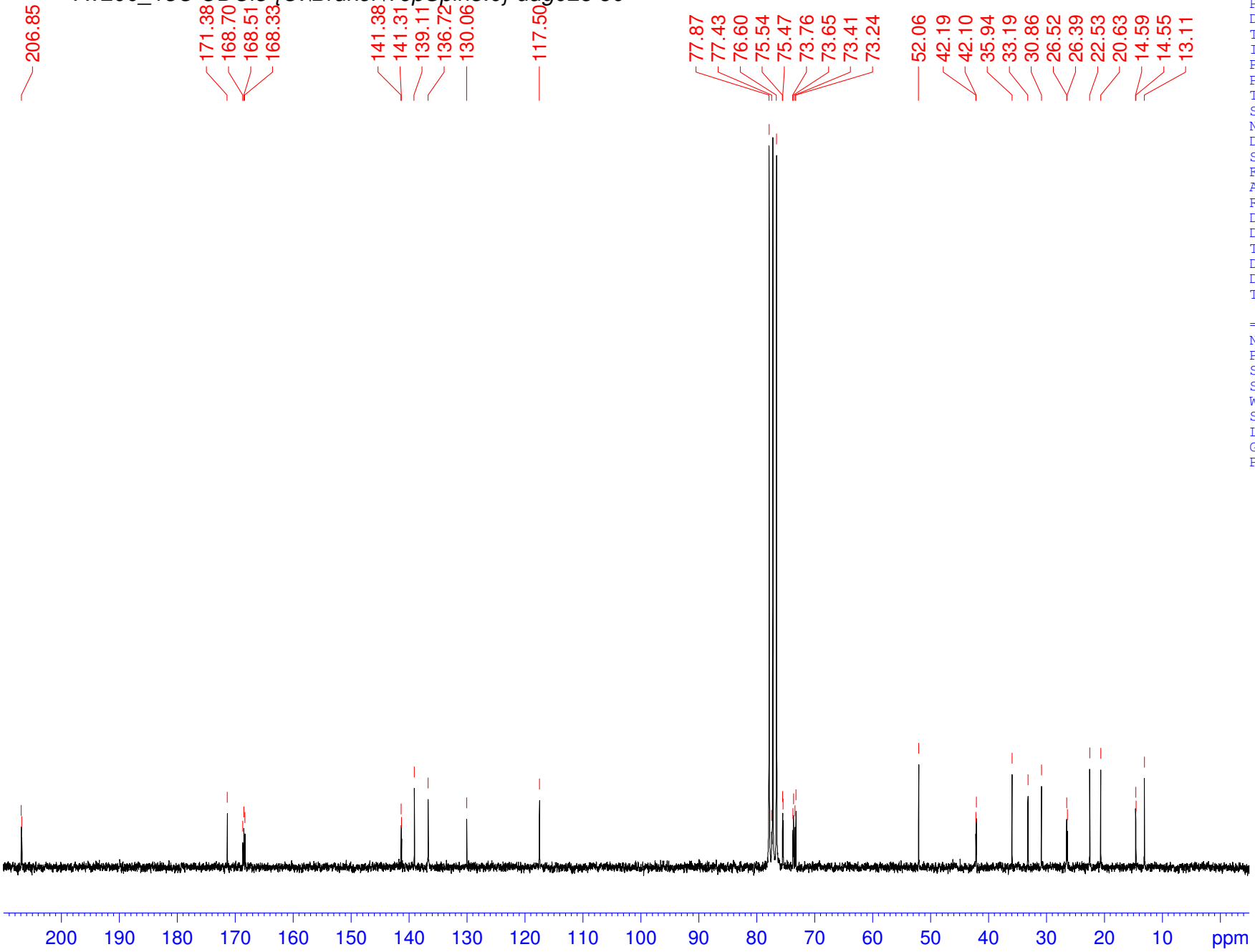
F2 - Acquisition Parameters
Date_            20121113
Time               15.16
INSTRUM            Av500
PROBHD   5 mm PATXI 1H/
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     2
SWH             7500.000 Hz
FIDRES          0.228882 Hz
AQ             2.1845334 sec
RG                   203
DW                66.667 usec
DE                 6.50 usec
TE                298.3 K
D1            2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.1330008 MHz
NUC1                  1H
P1                  7.90 usec
PLW1         13.43999958 W

F2 - Processing parameters
SI                 65536
SF           500.1300231 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



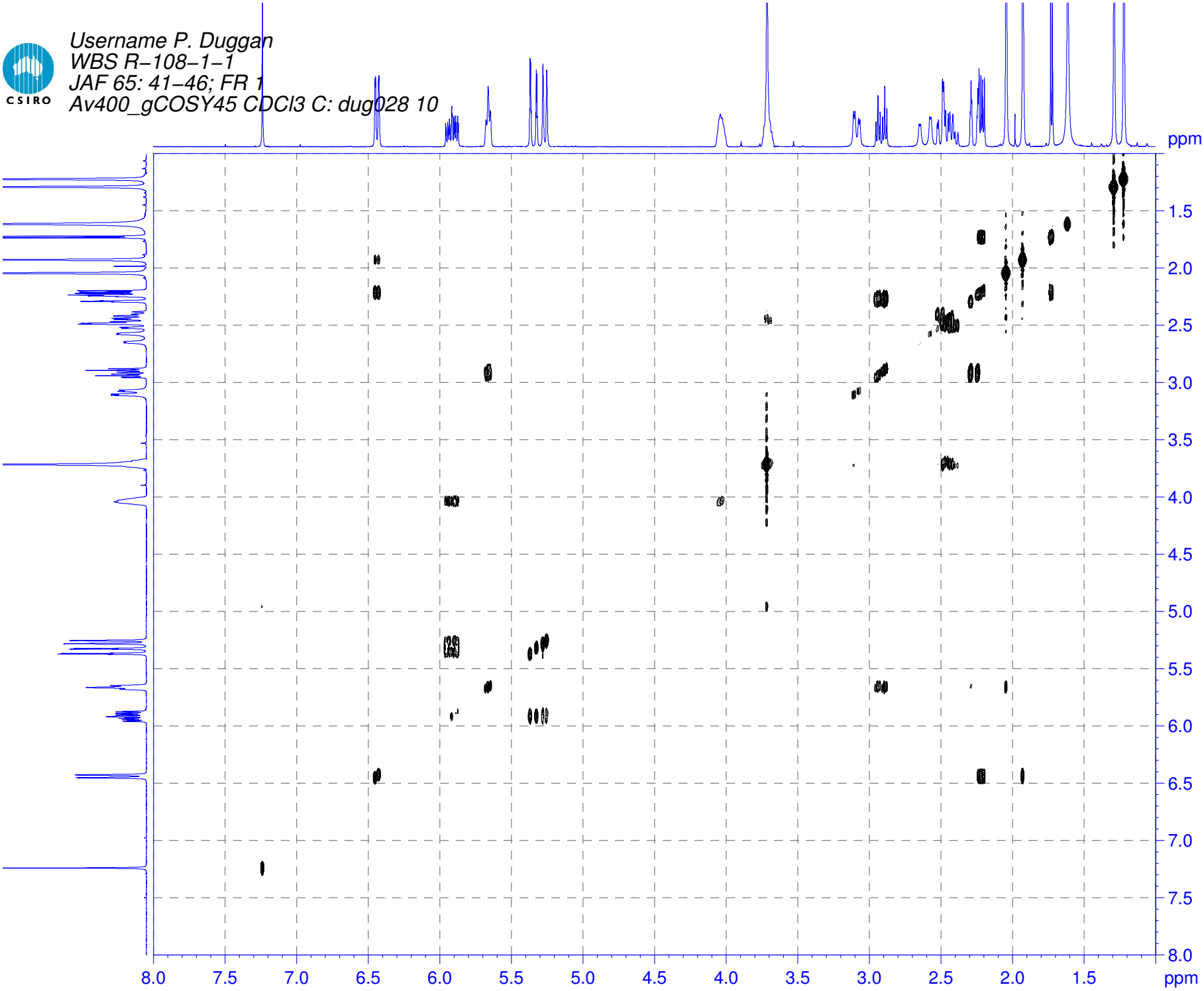
P. Duggan
R–108–1–1
JAF 65: 41–46; FR 1
Av200_13C CDCl3 {C:\Bruker\TopSpin3.0} dug028 50




```
NAME                duggan20030
EXPNO                        10
PROCNO                        1
Date_                  20121113
Time                       1.31
INSTRUM                   Av200
PROBHD          5 mm QNP 1H/13
PULPROG                  zgdc30
TD                        32768
SOLVENT                   CDCl3
NS                        10000
DS                            4
SWH                   13111.888 Hz
FIDRES                 0.400143 Hz
AQ                    1.2496030 sec
RG                         90.5
DW                       38.133 usec
DE                         6.50 usec
TE                        298.2 K
D1                   1.00000000 sec
D11                  0.03000000 sec
TD0                           1

======== CHANNEL f1 ========
NUC1                         13C
P1                         6.80 usec
SI                        32768
SF                   50.3227188 MHz
WDW                          EM
SSB                           0
LB                         1.00 Hz
GB                            0
PC                         1.00
```

Username P. Duggan
WBS R–108–1–1
JAF 65: 41–46; FR 1
Av400_gCOSY45 CDCl3 C: dug028 10

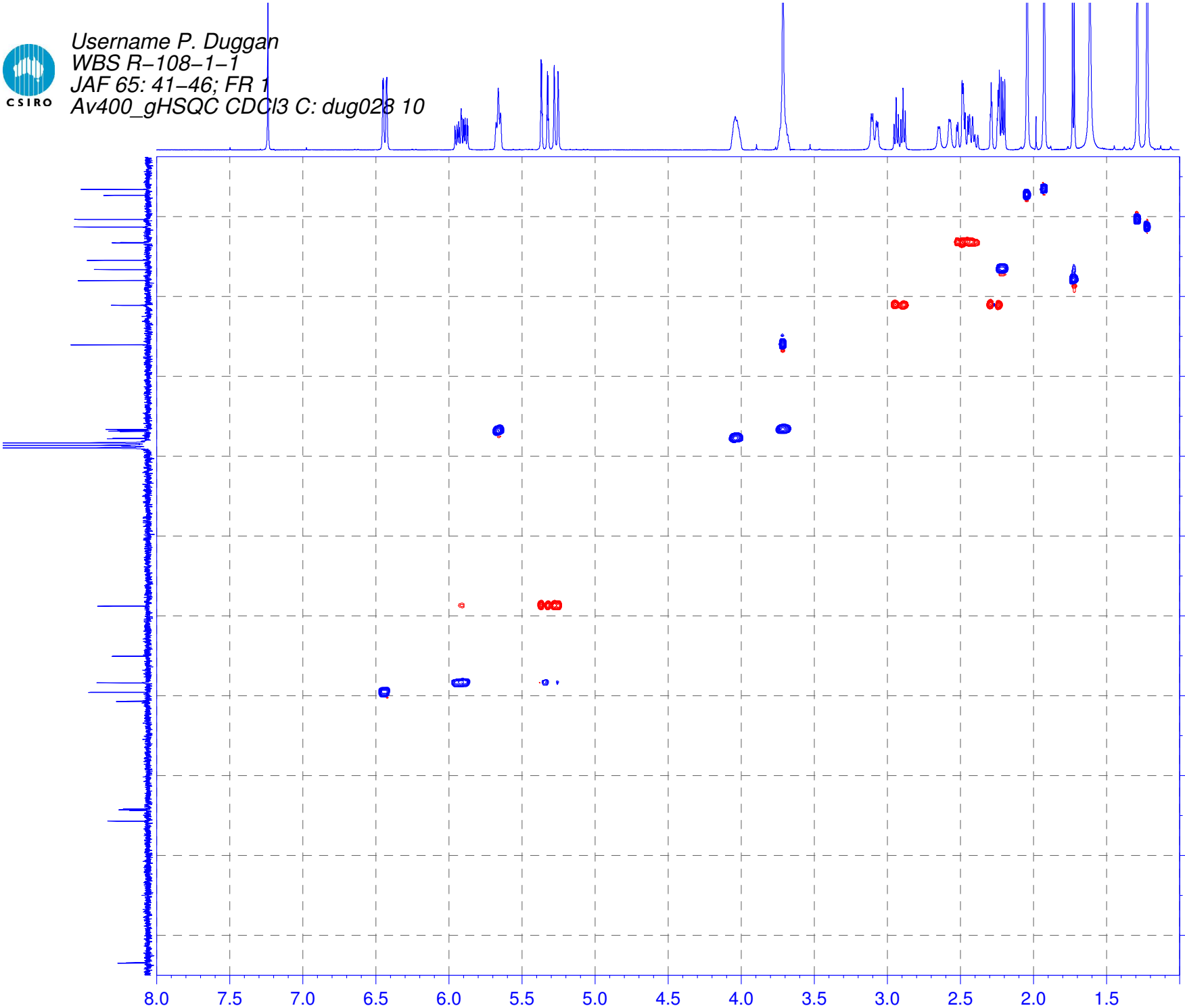



```
NAME          duggan40159
EXPNO                  11
PROCNO                  1
Date_            20121112
Time                15.46
INSTRUM             Av400
PROBHD    5 mm PABBI 1H/
PULPROG   cosygpqf45_CSIRO
TD                    4096
SOLVENT             CDCl3
NS                      1
DS                     16
SWH             4789.272 Hz
FIDRES          1.169256 Hz
AQ            0.4276724 sec
RG               1149.4
DW               104.400 usec
DE                 12.00 usec
TE                 298.2 K
D0            0.00000300 sec
D1            1.00000000 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00020880 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                   9.80 usec
PL1                 -1.00 dB
PL1W         28.77672577 W
SFO1         400.1320007 MHz

====== GRADIENT CHANNEL =====
GPNAM1          sine.100
GPNAM2          sine.100
GPZ1               10.00 %
GPZ2               10.00 %
P16              1000.00 usec
ND0                     1
TD                    256
SFO1              400.132 MHz
FIDRES         18.708094 Hz
SW                11.969 ppm
FnMODE                 QF
SI                   2048
SF          400.1300174 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00
SI                   2048
MC2                    QF
SF          400.1300174 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
```

Username P. Duggan
WBS R–108–1–1
JAF 65: 41–46; FR 1
Av400_gHSQC CDCl3 C: dug023 10



BRUKER

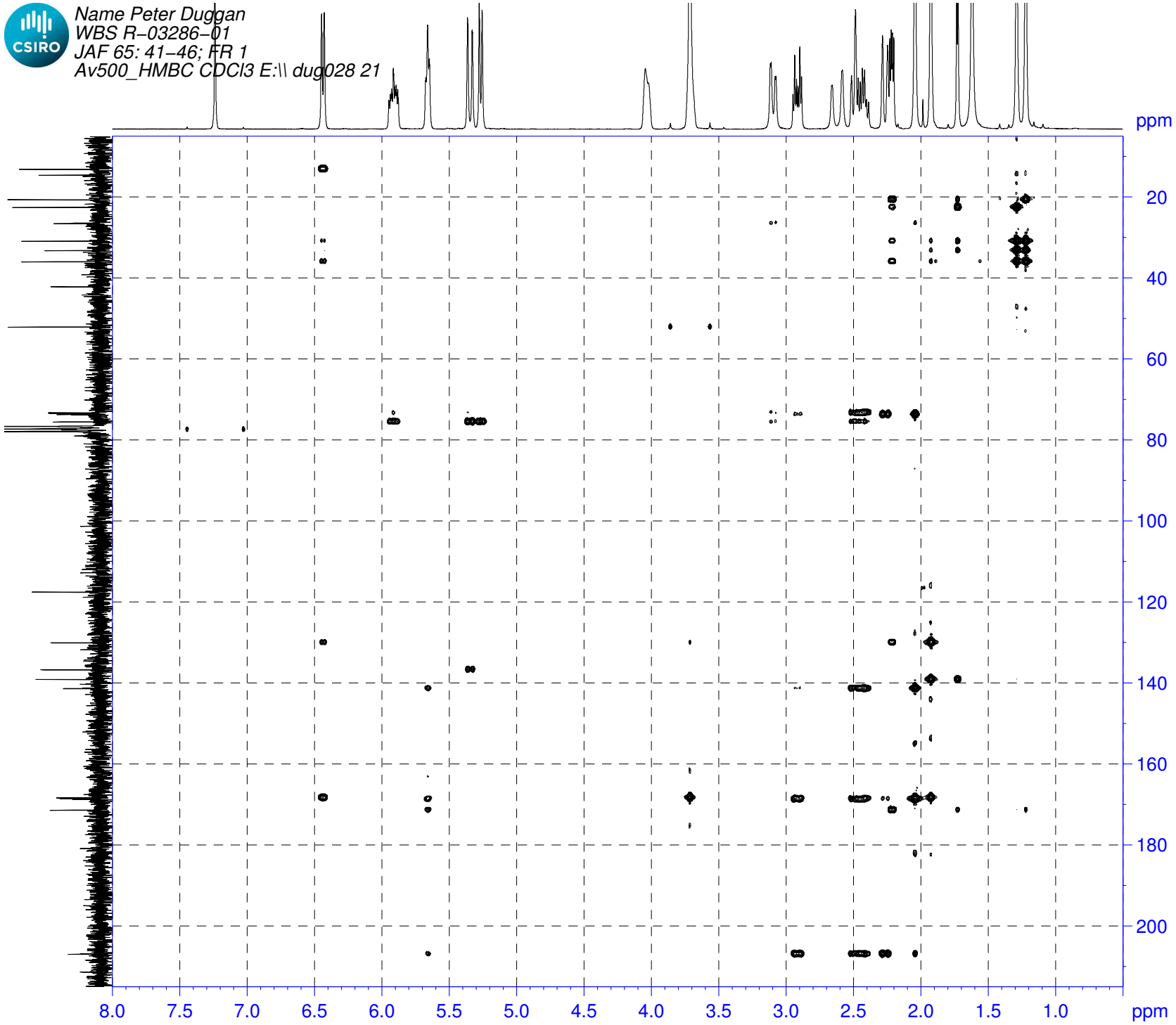
```
ggan40159
                12
PROCNO           1
Date_        20121112
Time            15.55
INSTRUM         Av400
PROBHD   5 mm PABBI 1H/
PULPROG  hsqcedetgpsisp2.2_CSIRO
TD            2048
SOLVENT       CDCl3
NS               2
DS              16
SWH        4006.410 Hz
FIDRES     1.956255 Hz
AQ       0.2556404 sec
RG          1625.5
DW         124.800 usec
DE          12.00 usec
TE          298.2 K
CNST2    145.0000000
CNST17     -0.5000000
D0       0.00000300 sec
D1       1.50000000 sec
D4       0.00172414 sec
D11      0.03000000 sec
D16      0.00020000 sec
D21      0.00344828 sec
D24      0.00086207 sec
IN0      0.00002925 sec

======== CHANNEL f1 ========
NUC1             1H
P1             9.80 usec
P2            19.60 usec
P28            0.10 usec
PL1           -1.00 dB
PL1W     28.77672577 W
SFO1     400.1320007 MHz

======== CHANNEL f2 ========
CPDPRG2       garp4
NUC2            13C
P3            13.00 usec
P14          500.00 usec
P24         2000.00 usec
PCPD2         70.00 usec
PL0          120.00 dB
PL2           -6.00 dB
PL12           8.62 dB
PL0W     0.00000000 W
PL2W   163.00000000 W
PL12W    5.62584305 W
SFO2     100.6208180 MHz
SP3           -0.12 dB
SP7           -0.12 dB
SPNAM3   Crp60,0.5,20.1
SPNAM7     Crp60comp.4
SPOAL3         0.500
SPOAL7         0.500
SPOFFS3         0.00 Hz
SPOFFS7         0.00 Hz

====== GRADIENT CHANNEL =====
GPNAM1      sine.100
GPNAM2      sine.100
GPNAM3      sine.100
GPNAM4      sine.100
GPZ1           80.00 %
GPZ2           20.10 %
GPZ3           11.00 %
GPZ4           -5.00 %
P16         1000.00 usec
P19          600.00 usec
ND0              2
TD             256
SFO1       100.6208 MHz
FIDRES    66.818512 Hz
SW          170.000 ppm
FnMODE   Echo-Antiecho
SI            2048
SF     400.1300174 MHz
WDW          QSINE
SSB              2
LB            0.00 Hz
GB               0
PC            1.00
SI            1024
MC2     echo-antiecho
SF     100.6127588 MHz
WDW          QSINE
SSB              2
```

Name Peter Duggan
WBS R–03286–01
JAF 65: 41–46; FR 1
Av500_HMBC CDCl3 E:\\ dug028 21




```
Current Data Parameters
NAME          duggan50002
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20121113
Time                15.18
INSTRUM             Av500
PROBHD   5 mm PATXI 1H/
PULPROG     hmbcetgpl2nd
TD                   4096
SOLVENT             CDCl3
NS                     16
DS                     16
SWH             6009.615 Hz
FIDRES          1.467191 Hz
AQ             0.3407872 sec
RG                   203
DW                83.200 usec
DE                  6.50 usec
TE                298.3 K
CNST6        120.0000000
CNST7        165.0000000
CNST13         8.0000000
CNST30         0.5981140
D0           0.00000300 sec
D1           1.50000000 sec
D6           0.06250000 sec
D16          0.00020000 sec
IN0          0.00001810 sec

======== CHANNEL f1 ========
SFO1         500.1327507 MHz
NUC1                  1H
P1                  7.90 usec
P2                 15.80 usec
PLW1         13.43999958 W

======== CHANNEL f2 ========
SFO2         125.7709921 MHz
NUC2                 13C
P3                 11.80 usec
P24              2000.00 usec
PLW2         197.96000671 W
SPNAM[7]     Crp60comp.4
SPOAL7             0.500
SPOFFS7   0 Hz
SPW7         42.11399841 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPNAM[5]      SMSQ10.100
GPZ1               80.00 %
GPZ3               15.00 %
GPZ4              -10.00 %
GPZ5               -5.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                    512
SFO1              125.771 MHz
FIDRES          53.953728 Hz
SW                219.640 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                   2048
SF          500.1300230 MHz
WDW                 QSINE
SSB                     2
LB        0 Hz
GB        0
PC                   1.00

F1 - Processing parameters
SI                   2048
MC2        echo-antiecho
SF          125.7577787 MHz
WDW                 QSINE
SSB                     2
LB        0 Hz
GB        0
```