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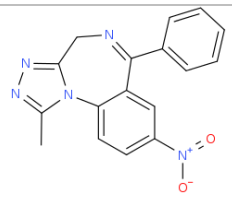
ANALYTICAL REPORT[1,2]

Nitrazolam (C17H13N5O2)

3-methyl-12-nitro-9-phenyl-2,4,5,8-tetraazatricyclo[8.4.0.02,6]tetradeca-1(14),3,5,8,10,12-hexaene

Remark – other NPS detected: none

Sample ID:	1452-16
Sample description:	powder - yellow-bright
Sample type:	collected /Institute of Forensic medicine, University Freiburg, Germany
Date of sample receipt (M/D/Y):	1/14/2016
Date of entry (M/D/Y) into NFL database:	8/10/2016
Report updates (if any) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure[3] (base form)	
Systematic name	3-methyl-12-nitro-9-phenyl-2,4,5,8-tetraazatricyclo[8.4.0.02,6]tetradeca-1(14),3,5,8,10,12-hexaene
Other names	1-methyl-8-nitro-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
Formula (per base form)	C17H13N5O2
M_w (g/mol)	319,32
Salt form/anions detected	base
StdInChIKey	OYRPNABWTHDOFK-UHFFFAOYSA-N
Compound Class	Benzodiazepines
Other NPS detected	none
Add.info (purity..)	pure by GC-MS, HPLC-tof

[1] This report has been produced with the financial support of the Prevention of and fight against crime Programme of the European Union (grant agreement number JUST/2013/ISEC/DRUGS/AG/6413). The contents of this report are the sole responsibility of the National Forensic Laboratory and can in no way be taken to reflect the views of the European Commission.
[2] Acknowledgement: Sample (NMR confirmed) was kindly provided by the Institute of Forensic Medicine, University of Freiburg, Germany (Dr. Verena Angerer). NMR spectra were kindly provided by Dr. Bjoern Moosmann and are enclosed in this report by his permission. Other measurements shown in this report were done in NFL.

[3] Created by OPSIN free tool: http://opsin.ch.cam.ac.uk/ **DOI:** 10.1021/ci100384d

Report updates

date	comments (explanation)

Instrumental methods (if applied) in NFL

1. GC-MS (Agilent): GC-method is RT locked to tetracosane (9.258 min). Injection volume 1 ml and split mode (1:50). Injector temperature: 280 0C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickens 0.25 µm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 ^{0}C for 1 min, followed by heating up to 190 ^{0}C at rate 8 ^{0}C/min, then heating up to 293 0C at a rate of 18 ^{0}C/min, hold for 6.1 min, then heating at 50 ^{0}C/min up to 325 ^{0}C and finally 6.1 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235^{0}C, source and quadropole temperatures 280^{0}C and 180^{0}C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300 until 6 min) amu.

2. HPLC-TOF (Agilent): 6230B TOF with Agilent 1260 Infinity HPLC with binary pump, column: Zorbax Eclipse XDB-C18, 50 x 4.6 mm, 1.8 micron. Mobile phases (A) 0.1% formic acid and 1mM ammonium formate in water; (B) 0.1% formic acid in methanol (B). Gradient: starting at 5% B, changing to 40% B over 4 min, then to 70% over 2 min and in 5 min to 100%, hold 1 min and back to 5%, equilibration for 1.7 min. The flow rate: 1.0 ml/min; Injection volume 1 µl. MS parameters: 2GHz, Extended Dynamic range mode to a maximum of 1700 amu, acquisition rate 1.30 spectra/sec. Sample ionisation: by Agilent Jet Stream technology (Dual AJS ESI). Ion source: positive ion scan mode with mass scanning from 82 to 1000 amu. Other TOF parameters: drying gas (N2) and sheath temperature 325 °C; drying gas flow rate 6 l/min; sheath gas flow rate 8 l/min; nebulizer 25 psig; Vcap. 4000 V; nozzle 2000 V; skimmer 65 V; fragmentor 175 V and Octopole RF 750 V.

3.FTIR-ATR (Perkin Elmer): scan range 4000-400 cm-1; resolution 4cm-1

4. GC- (MS)-IR condensed phase (GC-MS (Agilent) & IR (Spectra analyses-Danny)
GC-method: Injection volume 1 ml and split mode (1:5). Injector temperature 280 ^{0}C. Chromatographic separation as above **(1).** Split MS : IR = 1: 9.
MSD source EI = 70 eV. GC-MS transfer line T= 235^{0}C, source and quadropole temperatures 280^{0}C and 180^{0}C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300) amu.
IR (condesed (solid) phase): IR scan range 4000 to 650, resolution 4 cm^{-1}.

5. **IC** (anions) (Thermo Scientific, Dionex ICS 2100), Column: IonPac AS19, 2 x 250mm; Eluent: 10mM from 0 to 10 min, 10-58 mM from 10 to 40min; Flow rate: 0.25 ml/min; Temperature: 30˚C; Suppressor: AERS 500 2mm, suppressor current 13mA; Inj. Volume: 25 µl

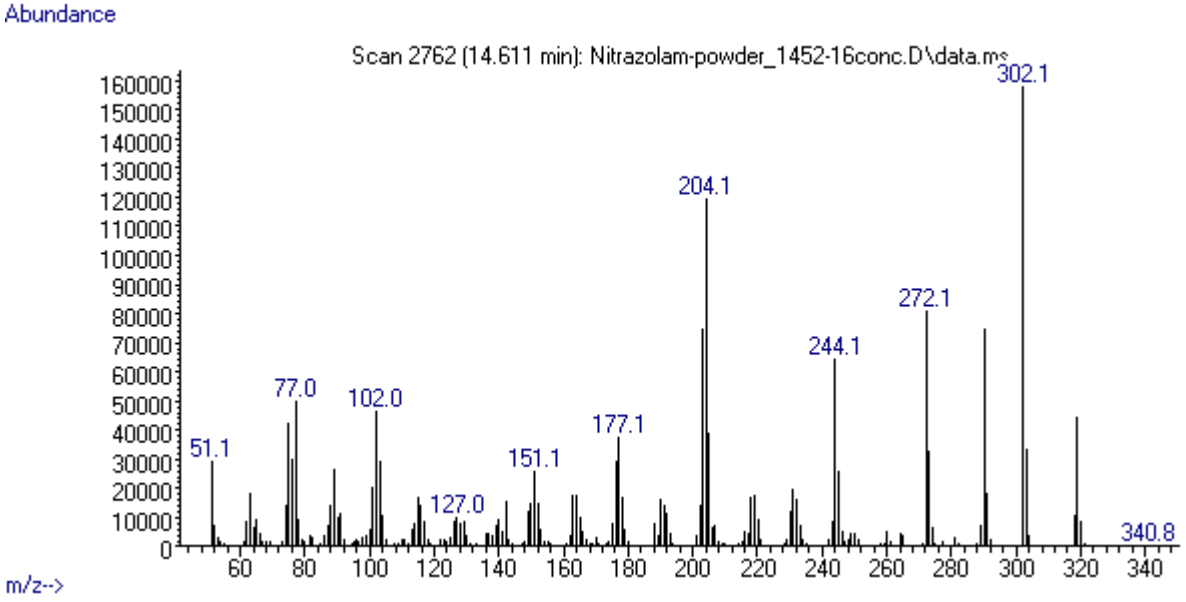
Supporting information

Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	soluble

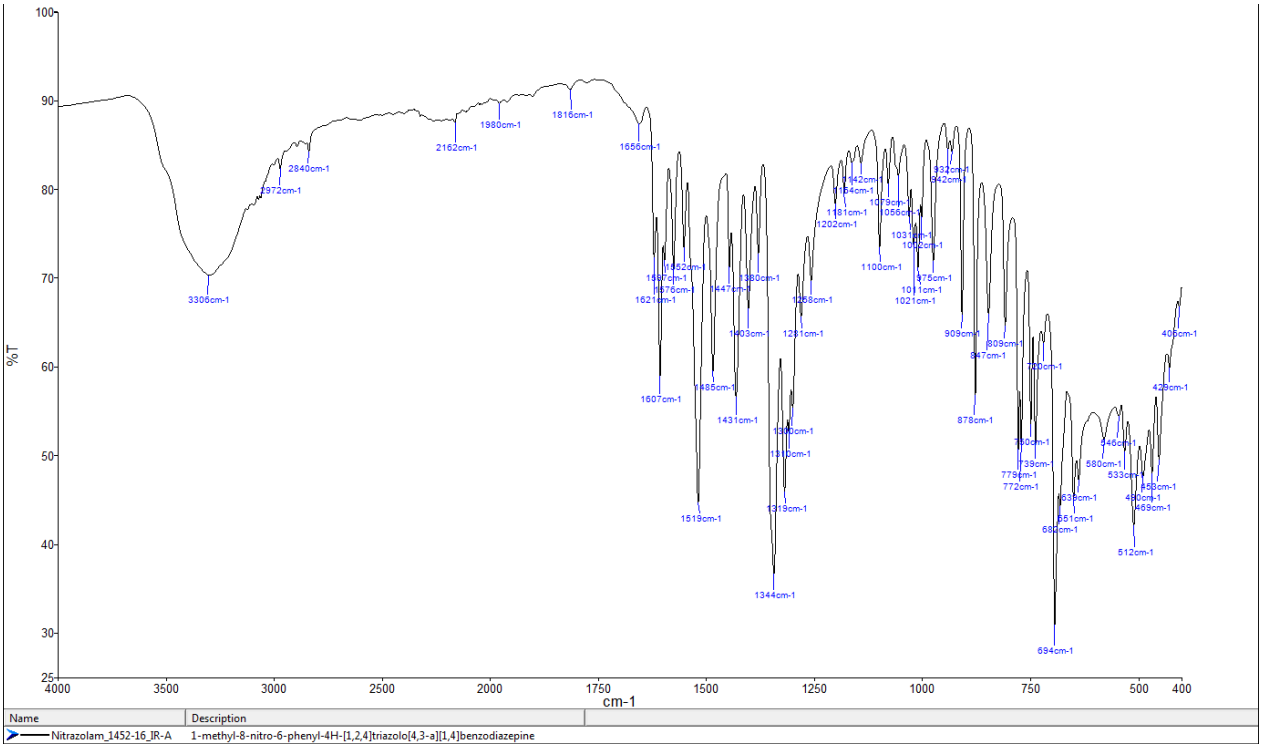
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 14,61 BP(1): 302; BP(2): 204,BP(3) :272,
HPLC-TOF	+	Exact mass (theoretical): 319,1069; measured value Δppm:-0,88; formula:C17H13N5O2
FTIR-ATR	+	direct measurement (sample as received)
FTIR (condensed phase) always as base form	-	
IC (anions)	+	
NMR	+	Kindly provided by Dr. Bjoern Moosmann, Institute of Forensic Medicine, University of Freiburg, Germany
validation		
other		

ANALYTICAL RESULTS

MS (EI)



FTIR-ATR - direct measurement (sample as received)



TOF REPORT

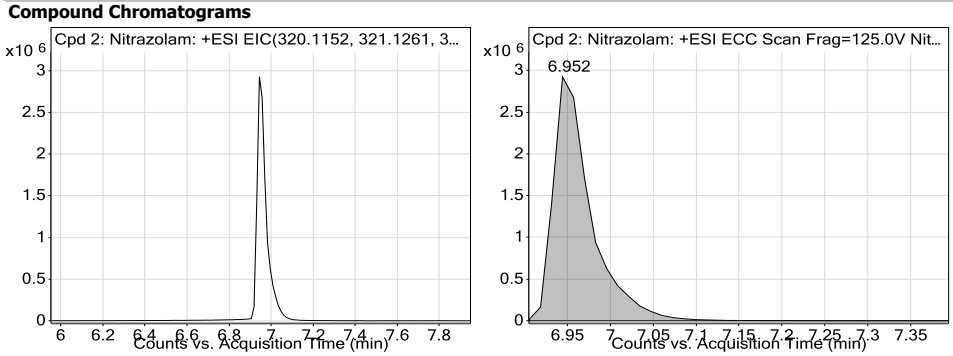
Data File	Nitrazolam_1452-16_TOF.d	**Sample Name**	ID_1452-16
Sample Type	Sample	**Position**	P1-F6
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-1512015-XDB-C18-ESI-poz-pod.m	**Acquired Time**	2/23/2016 12:02:20 PM
IRM Calibration Status	Success	**DA Method**	Drugs_NFL.m
Comment	extract in MeOH		

Compound Table

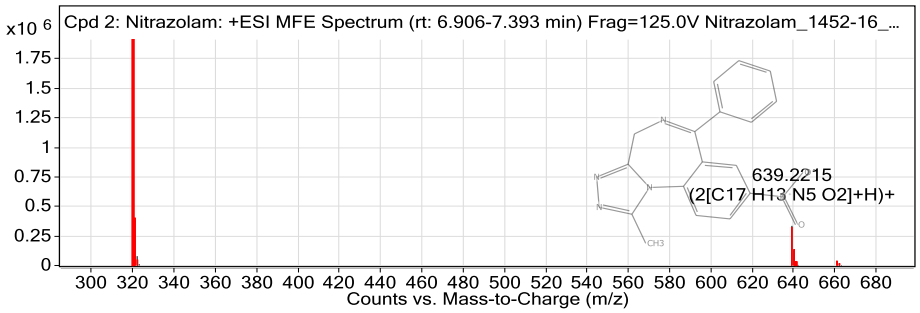
Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 2: Nitrazolam	Nitrazolam	C17 H13 N5 O2	6.952	319.1072

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
Nitrazolam	320.1143	6.952	319.1072	6.95	C17 H13 N5 O2	319.1069	-0.88

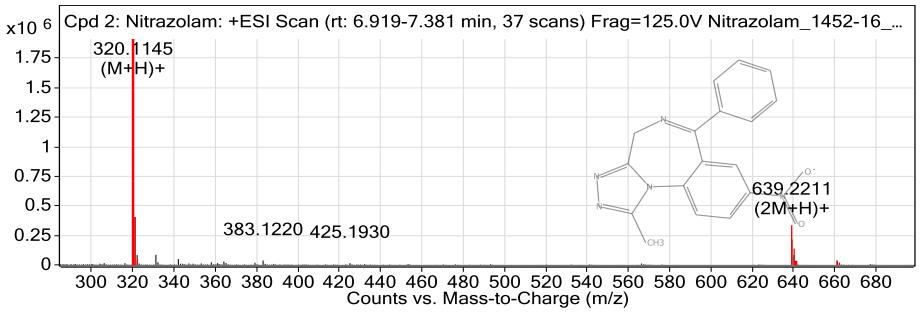
Compound Chromatograms



MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

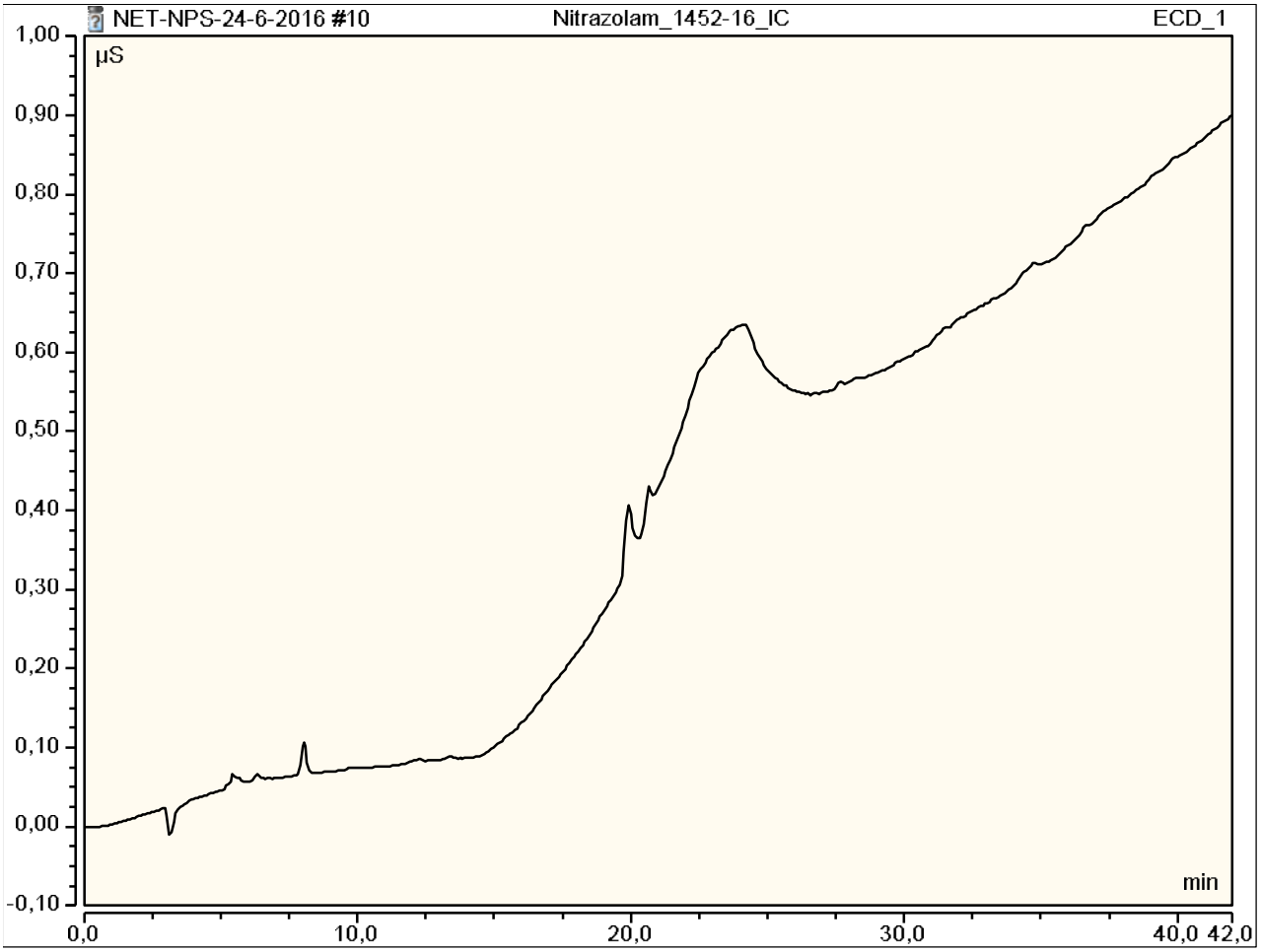
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
320.1143	1	1912323.25	C17 H13 N5 O2	(M+H)+
321.1179	1	400376.43	C17 H13 N5 O2	(M+H)+
322.124	1	79021.76	C17 H13 N5 O2	(M+H)+
323.1288	1	10668.9	C17 H13 N5 O2	(M+H)+
639.2215	1	335495.69	C17 H13 N5 O2	(2M+H)+
640.224	1	127864.77	C17 H13 N5 O2	(2M+H)+
641.2267	1	27965.17	C17 H13 N5 O2	(2M+H)+
642.2303	1	4548.14	C17 H13 N5 O2	(2M+H)+
661.2027	1	37709.03	C17 H13 N5 O2	(2M+Na)+
662.2052	1	15094.34	C17 H13 N5 O2	(2M+Na)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**Nitrazolam_1452-16_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**24-jun-2016 / 18:30**	*Run Time:*	**42,00**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount n.a.
		TOTAL:		0,00	0,00	0,00



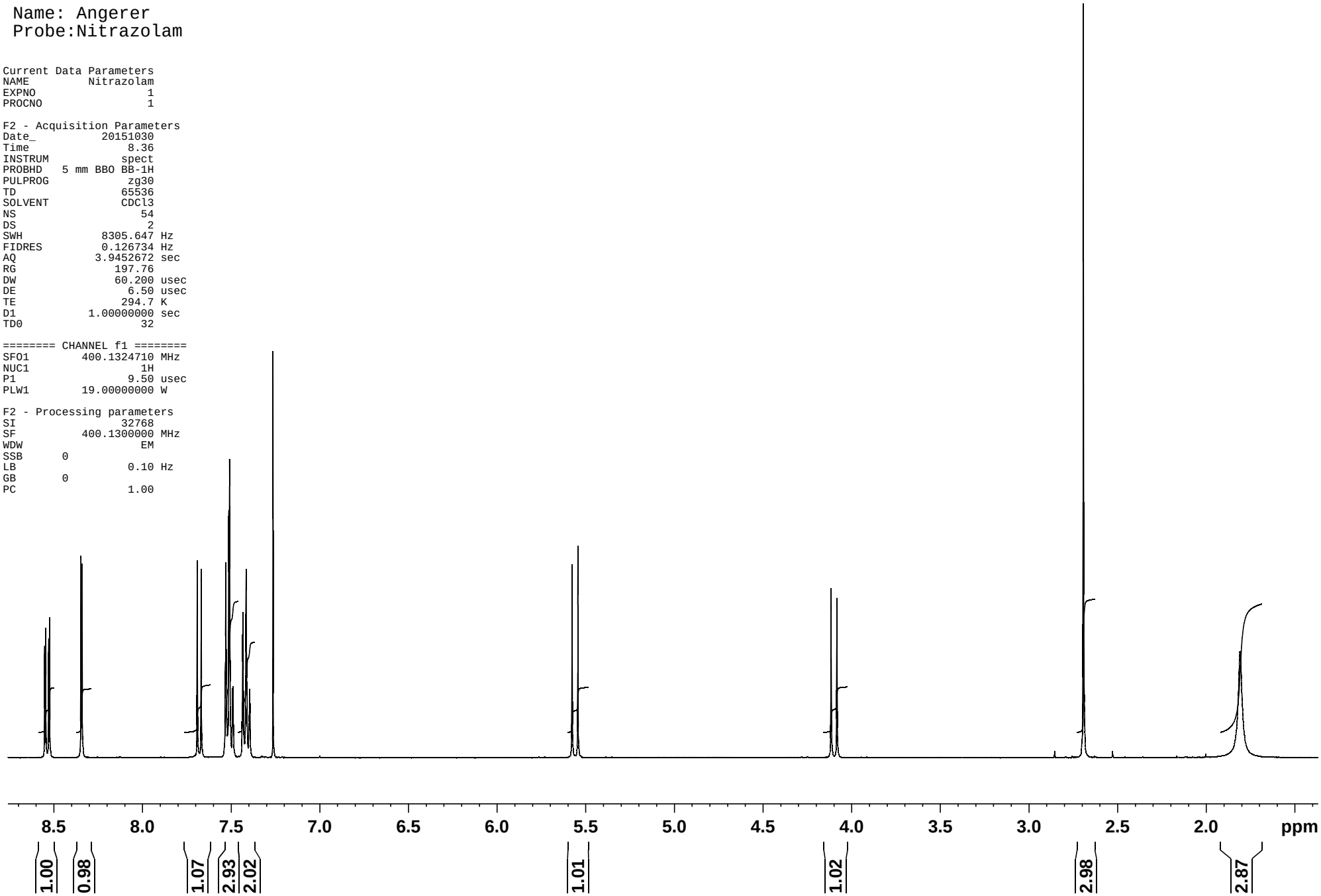
Name: Angerer
Probe:Nitrazolam

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

F2 - Acquisition Parameters
Date_          20151030
Time               8.36
INSTRUM           spect
PROBHD   5 mm BBO BB-1H
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   54
DS                    2
SWH            8305.647 Hz
FIDRES         0.126734 Hz
AQ            3.9452672 sec
RG               197.76
DW               60.200 usec
DE                6.50 usec
TE               294.7 K
D1           1.00000000 sec
TD0                  32

======== CHANNEL f1 ========
SFO1        400.1324710 MHz
NUC1                1H
P1                9.50 usec
PLW1        19.00000000 W

F2 - Processing parameters
SI                32768
SF          400.1300000 MHz
WDW                EM
SSB      0
LB                0.10 Hz
GB       0
PC                1.00
```



Name: Angerer
Probe:Nitrazolam

```
Current Data Parameters
NAME         Nitrazolam
EXPNO                 5
PROCNO                1

F2 - Acquisition Parameters
Date_          20151030
Time              11.05
INSTRUM           spect
PROBHD   5 mm BBO BB-1H
PULPROG          zgpg30
TD                50248
SOLVENT           CDCl3
NS                 1452
DS                    4
SWH           25000.000 Hz
FIDRES         0.497532 Hz
AQ            1.0049599 sec
RG               197.76
DW               20.000 usec
DE                 6.50 usec
TE                295.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                2000

======== CHANNEL f1 ========
SFO1        100.6228298 MHz
NUC1                13C
P1                 9.00 usec
PLW1        45.60400009 W

======== CHANNEL f2 ========
SFO2        400.1316005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        19.00000000 W
PLW12        0.28382999 W
PLW13        0.22990000 W

F2 - Processing parameters
SI                 32768
SF          100.6127743 MHz
WDW                   EM
SSB       0
LB                 2.00 Hz
GB        0
PC                 1.40
```