NACIONALNI FORENZIČNI LABORATORIJ
NATIONAL FORENSIC LABORATORY
Vodovodna 95, 1000 LJUBLJANA, SLOVENIA
E: nfl@policija.si

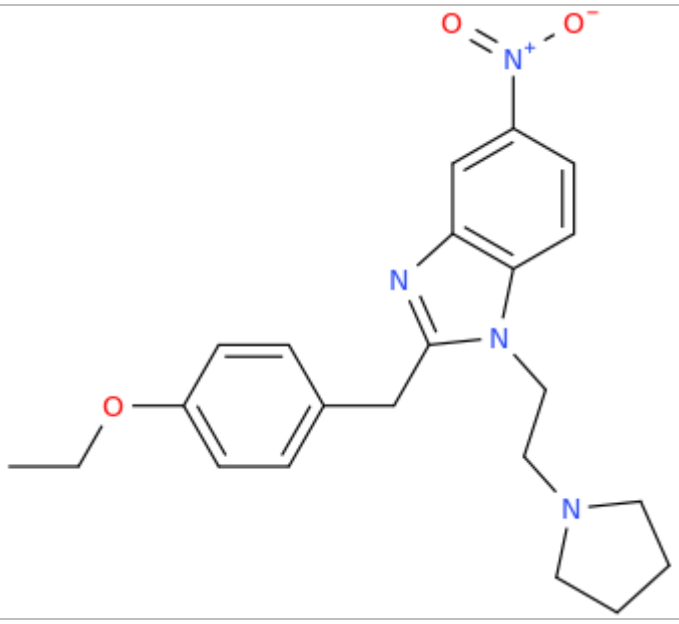
ANALYTICAL REPORT

Etonitazepyne (C22H26N4O3)

2-[(4-ethoxyphenyl)methyl]-5-nitro-1-[2-(pyrrolidin-1-yl)ethyl]-1H-1,3-benzodiazole

Remark – other NPS detected:

Sample ID:	2256-21
Sample description:	powder
Sample type:	test purchase /NFL- purchasing
Date of entry (DD/MM/YYYY) into NFL database:	09/07/2021
Report updates (if any) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure[1] (base form)	
Systematic name	2-[(4-ethoxyphenyl)methyl]-5-nitro-1-[2-(pyrrolidin-1-yl)ethyl]-1H-1,3-benzodiazole
Other names	
Formula (per base form)	C22H26N4O3
M_w (g/mol)	394,48
Salt form/anions detected	HCl
StdInChIKey (per base form)	LQZWZCJCEPUKCJ-UHFFFAOYSA-N
Other NPS detected	
Additional info (purity..)	>95% purity of a sample based on 1H NMR spectrum

[1] 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 µl 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 9.1 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 µl 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 KOH 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

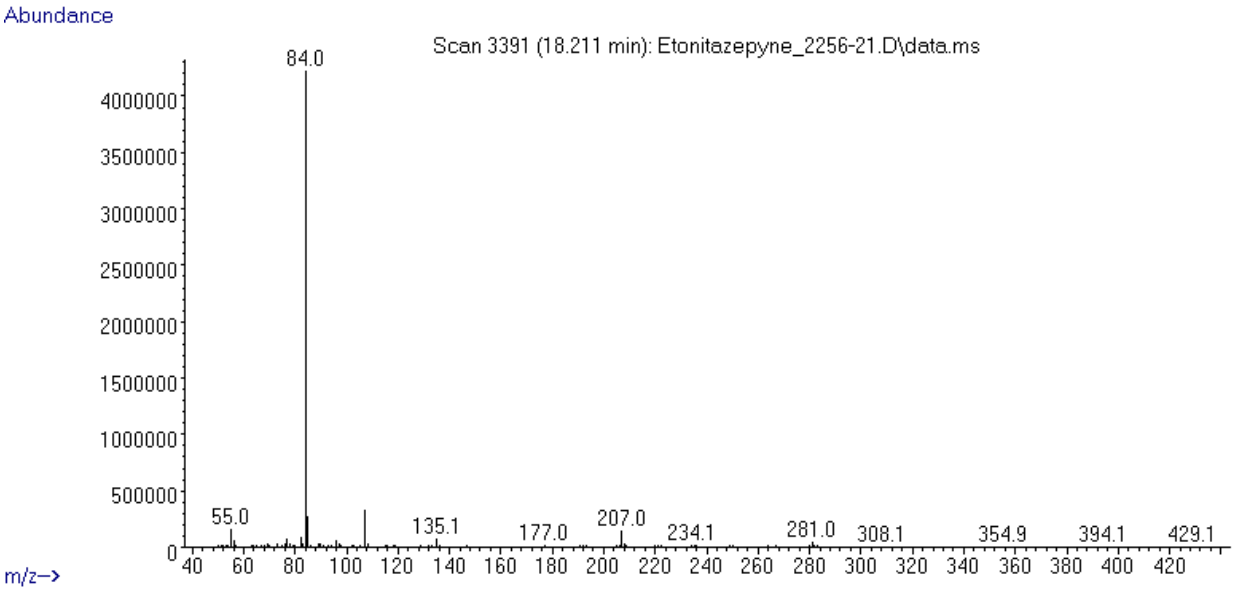
Supporting information

Solubility in	result/remark
CH_2Cl_2	not soluble
MeOH	soluble
H_2O	partially

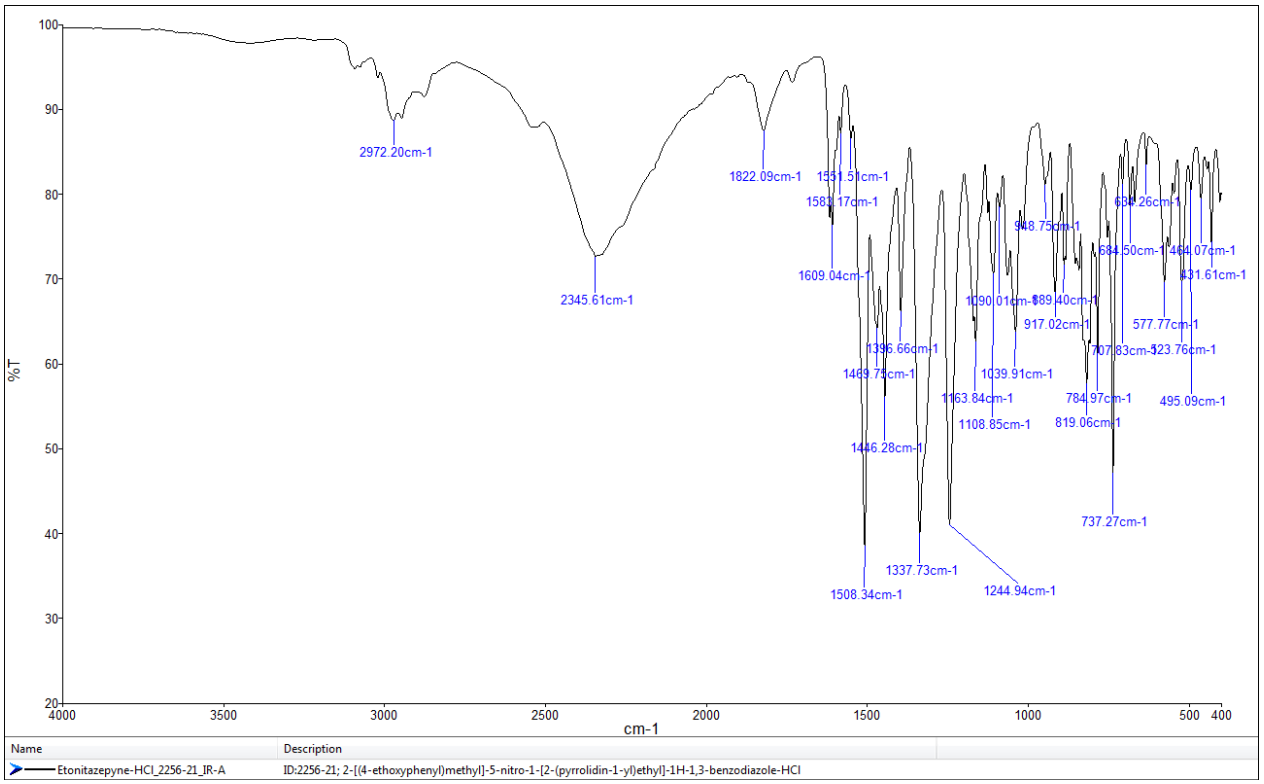
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 18,21 BP(1): 84; BP(2): 107,BP(3) :85,
HPLC-TOF	+	Exact mass (theoretical): 394,2005; measured value Δppm:-1,26; formula:C22H26N4O3
FTIR-ATR	+	direct measurement (sample as received)
FTIR (solid phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	+	
validation		
other		

ANALYTICAL RESULTS

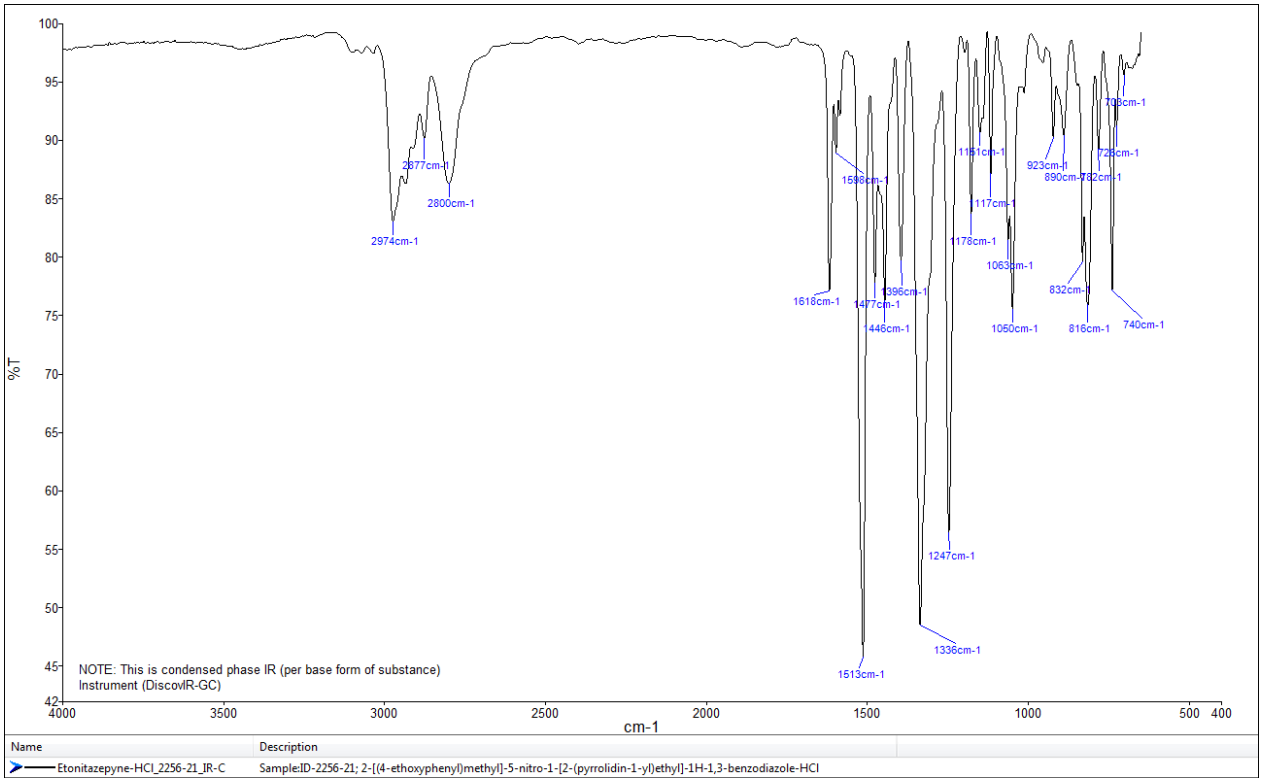
MS (EI)



FTIR-ATR - direct measurement (sample as received)



IR (solid phase – after chromatographic separation)



TOF REPORT

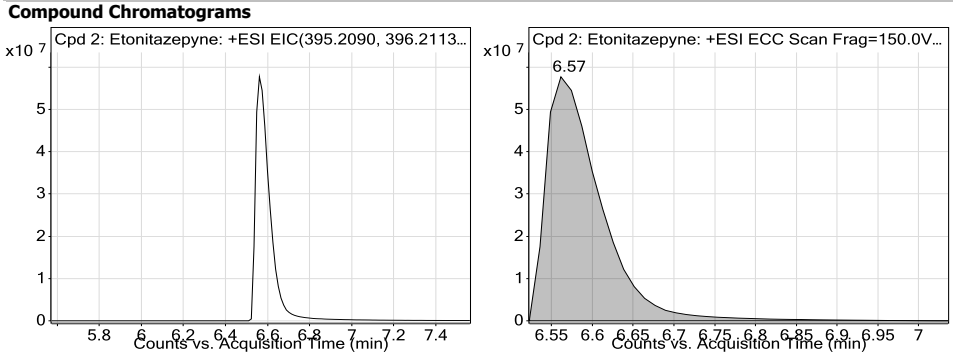
Data File	Etonitazepyne_2256_21.d	**Sample Name**	ID-2256-21
Sample Type	Sample	**Position**	P1-C2
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-15_01_2020-XDB-C18-ESI+.m	**Acquired Time**	3/16/2021 1:44:41 PM
IRM Calibration Status	Success	**DA Method**	a-Drugs_NFL.m
Comment	MeOH		

Compound Table

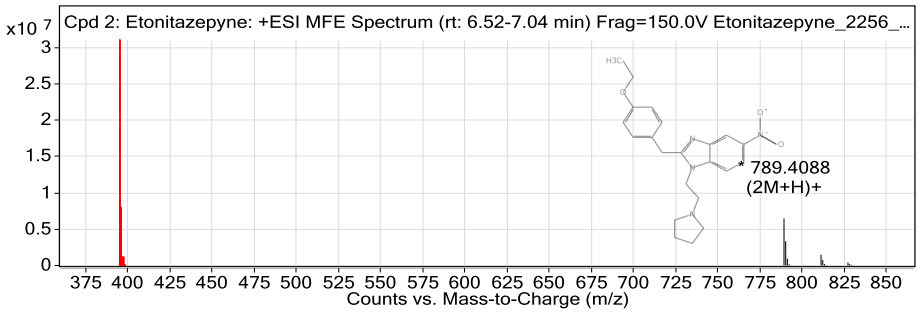
Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 2: Etonitazepyne	Etonitazepyne	C22 H26 N4 O3	6.57	394.201

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
Etonitazepyne	395.2082	6.57	394.201	6.57	C22 H26 N4 O3	394.2005	-1.26

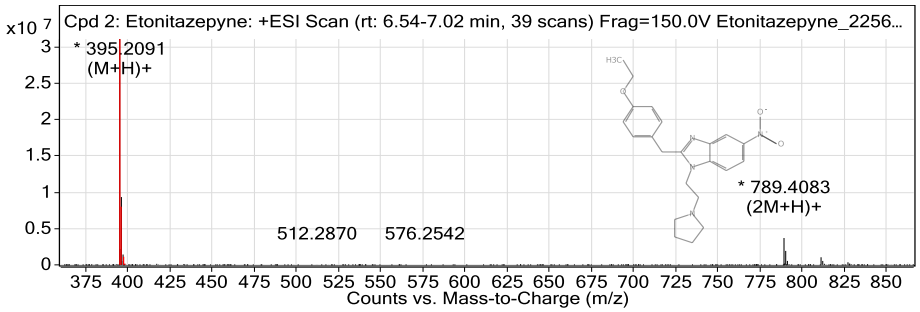
Compound Chromatograms



MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

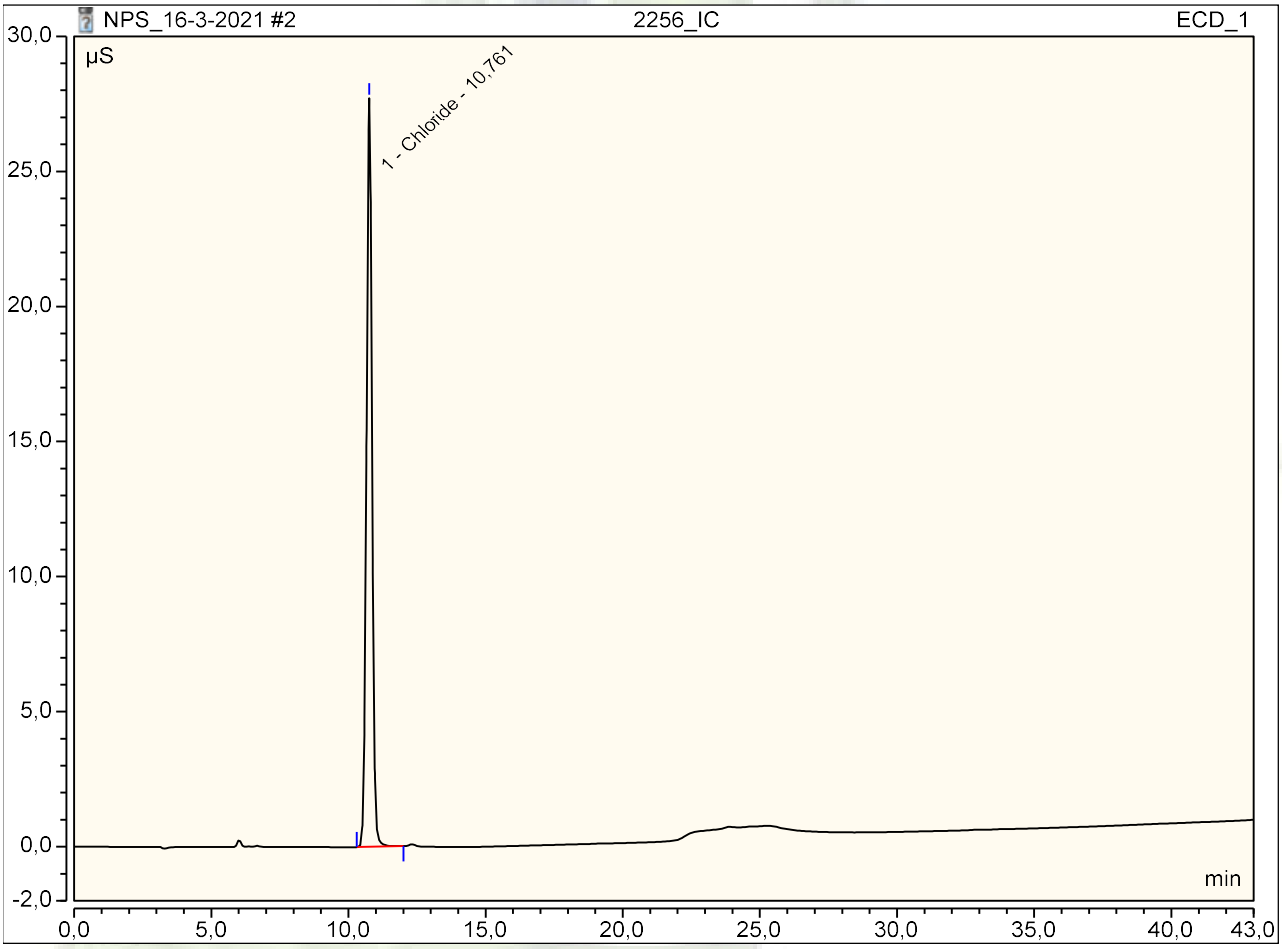
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
395.2082	1	31046504	C22 H26 N4 O3	(M+H)+
396.2116	1	7949682.13	C22 H26 N4 O3	(M+H)+
397.2144	1	1125119.1	C22 H26 N4 O3	(M+H)+
789.4088	1	6471315		(2M+H)+
790.4117	1	3343165.9		(2M+H)+
791.4154	1	930164.88		(2M+H)+
811.391	1	1462540		(2M+Na)+
812.3944	1	759774.8		(2M+Na)+
827.3649	1	409200.38		(2M+K)+
828.3676	1	206608.63		(2M+K)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**2256_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Instrument Method:	**ANIONI**	*Operator:*	**Admin**
Inj. Date / Time:	**16-Mar-2021 / 15:36**	*Run Time:*	**43,00**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount mg/L
1	10,76	**Chloride**	BMB	6,525	27,711	n.a.
		TOTAL:		6,52	27,71	0,00

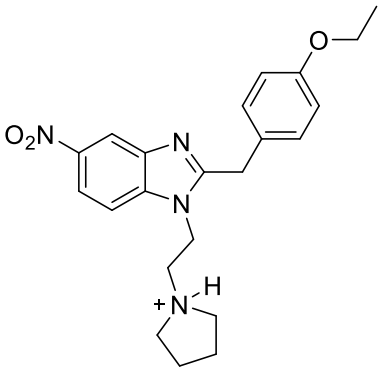


Večna pot 113
P. O. Box 537
SI-1001 Ljubljana
Slovenia
Phone: +386 1 479 8558
janez.kosmrlj@fkkt.uni-lj.si

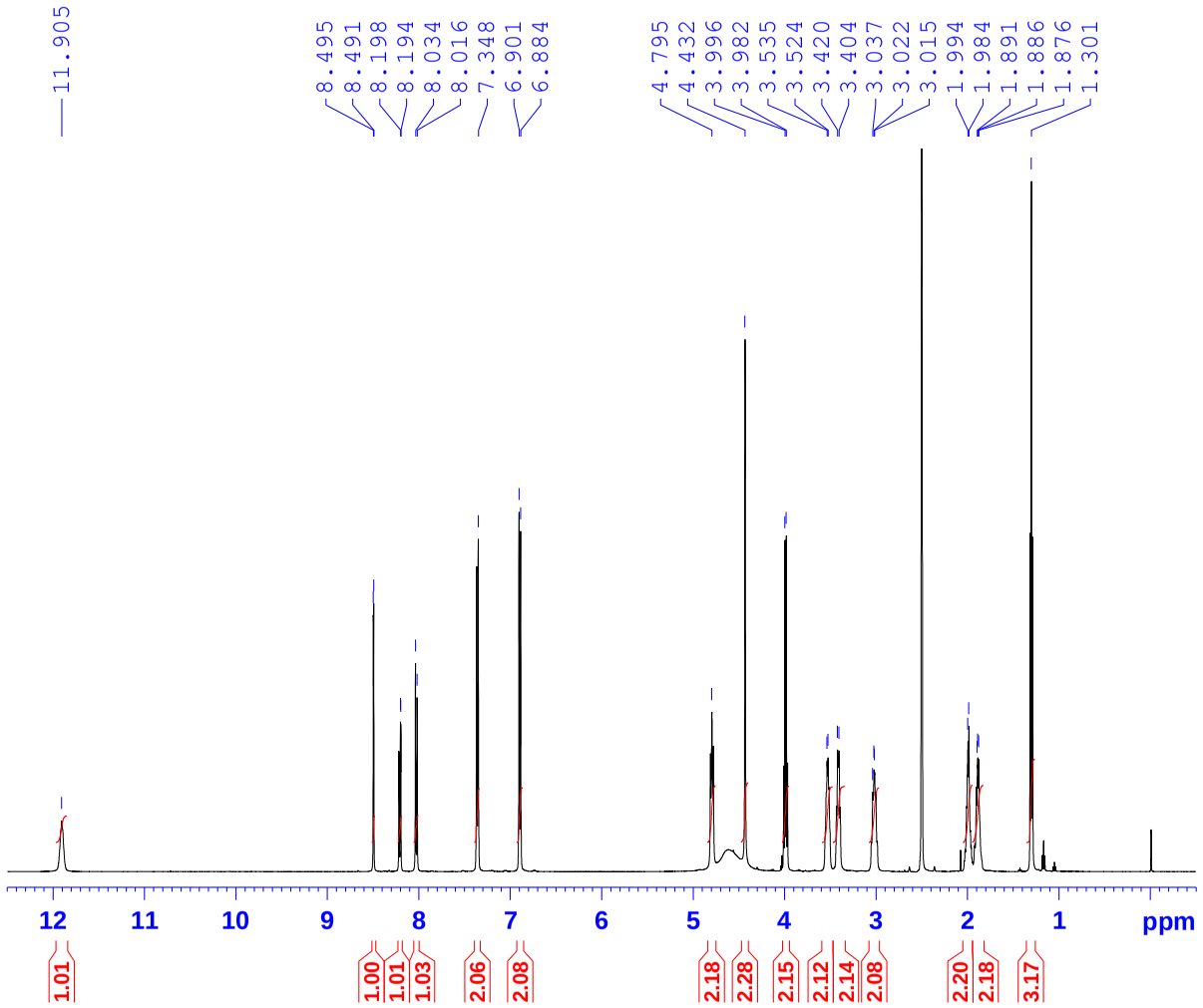
University
of Ljubljana

Faculty *of Chemistry*
and Chemical Technology

R E P O R T

Contract No.	C1714-19-460155 (Republic of Slovenia, Ministry of the Interior, POLICE)
Sample ID:	**2256-21**
Received date:	May 7, 2021
Our notebook code:	NFL-2256-21
NMR sample preparation:	20.0 mg dissolved in 0.7 mL DMSO-d_6
NMR experiments:	^{1}H, ^{13}C, ^{1}H–^{1}H *gs*-COSY, ^{1}H–^{13}C *gs*-HSQC, ^{1}H–^{13}C *gs*-HMBC, ^{1}H–^{15}N *gs*-HMBC
Proposed structure with formula, exact mass, molecular weight:	 Chemical Formula: $C_{22}H_{27}N_4O_3^+$ Exact Mass: 395,21 Molecular Weight: 395,48
Chemical name:	*N*-protonated 2-(4-ethoxybenzyl)-5-nitro-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-benzo[d]imidazole
Comments:	- Structure elucidation based on 1D and 2D NMR spectra and HRMS. ->95% purity of a sample based on ^{1}H NMR spectrum.
Supporting information:	Copies of ^{1}H and ^{13}C NMR spectra, ^{1}H and ^{13}C FIDs.
Principal investigator:	Prof. Dr. Janez Košmrlj
Date of report:	May 31, 2021

NFL-2256-21
1H



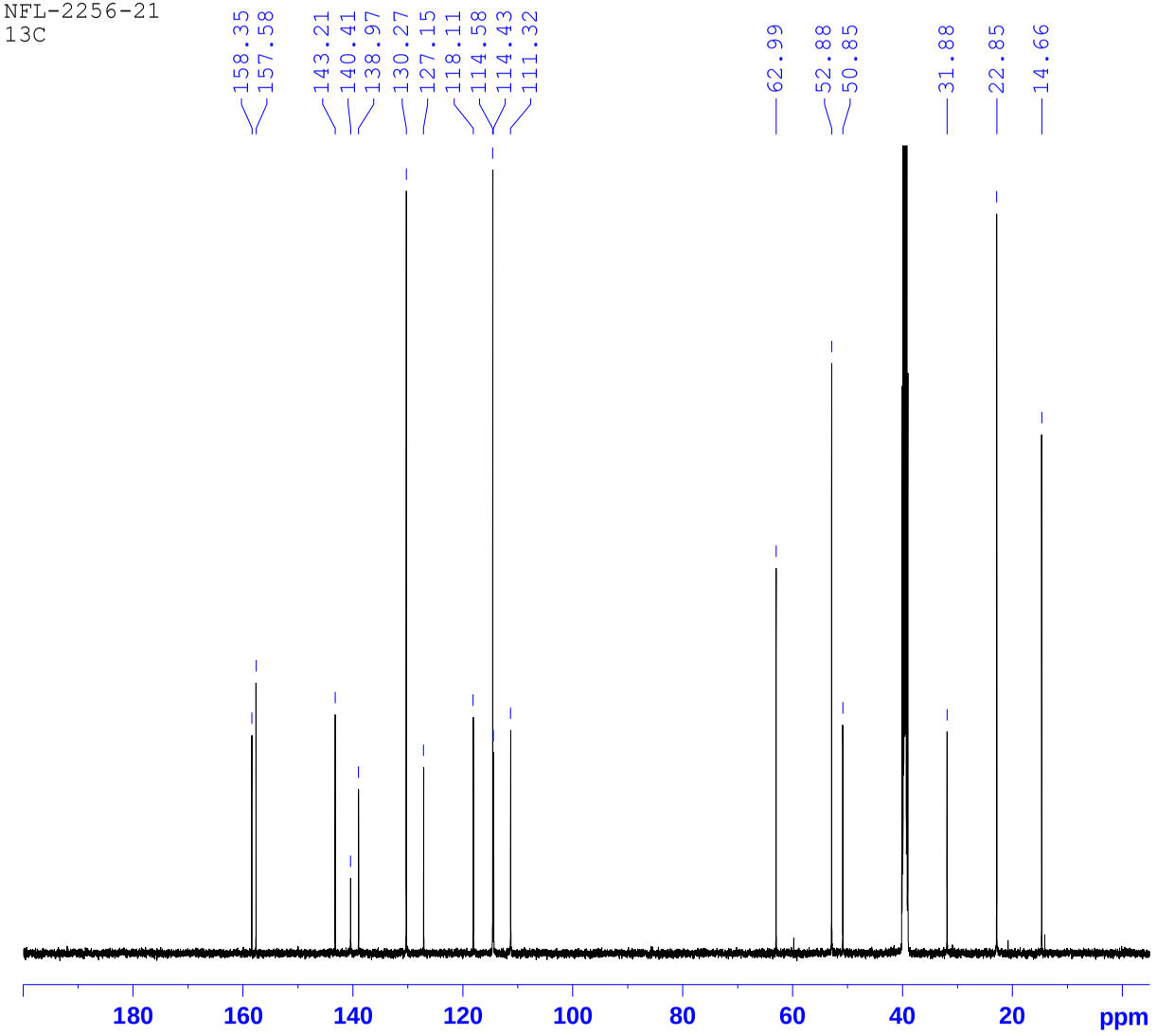

```
Current Data Parameters
NAME         NFL-2256-21
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210509
Time                6.43
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH            10000.000 Hz
FIDRES          0.152588 Hz
AQ             3.2767999 sec
RG                  80.6
DW                50.000 usec
DE                 6.50 usec
TE                296.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.1330885 MHz
NUC1                   1H
P1                  8.70 usec
PLW1        26.00000000 W

F2 - Processing parameters
SI                 65536
SF           500.1300043 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

NFL-2256-21
13C




```
Current Data Parameters
NAME         NFL-2256-21
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210509
Time               8.35
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD                65536
SOLVENT             DMSO
NS                 3072
DS                    4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010048 sec
RG                 2050
DW               16.800 usec
DE                6.50 usec
TE                296.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.7703637 MHz
NUC1                 13C
P1                  8.70 usec
PLW1       122.00000000 W

======== CHANNEL f2 ========
SFO2        500.1320005 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2              80.00 usec
PLW2         26.00000000 W
PLW12         0.30046001 W
PLW13         0.15113001 W

F2 - Processing parameters
SI                 32768
SF          125.7578462 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```