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EUROPEAN PROJECT RESPONSE 2
to challenges in forensic drugs analyses
Co-funded by the Internal Security Found of the European Union

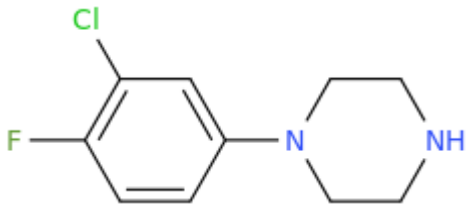
ANALYTICAL REPORT

Kleferein (C10H12ClFN2)

1-(3-chloro-4-fluorophenyl)piperazine

Remark – other compounds: caffeine

Sample ID:	2150-19
Sample description:	powder
Sample type:	seized /KP
Date of entry (DD/MM/YYYY) into NFL database:	11/03/2020
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	1-(3-chloro-4-fluorophenyl)piperazine
Other names	3-Cl-4-FPP
Formula (per base form)	C10H12ClFN2
M_w (g/mol)	214,67
Salt form/anions detected	HCl
StdInChIKey (per base form)	MKXFXPRJCBUTLX-UHFFFAOYSA-N
Other compouns detected	caffeine
Additional info (purity..)	kleferein and caffeine in molar ratio of 1 : 2.37 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 7.1 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 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

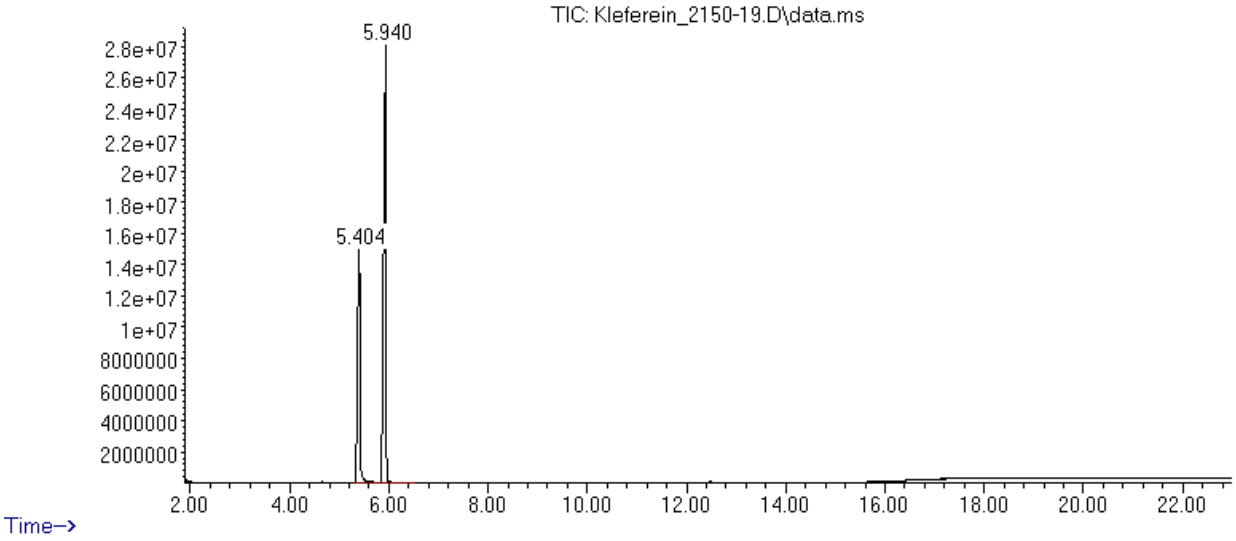
Supporting information

Solubility in	result/remark
CH_2Cl_2	partially
MeOH	partially
H_2O	partially

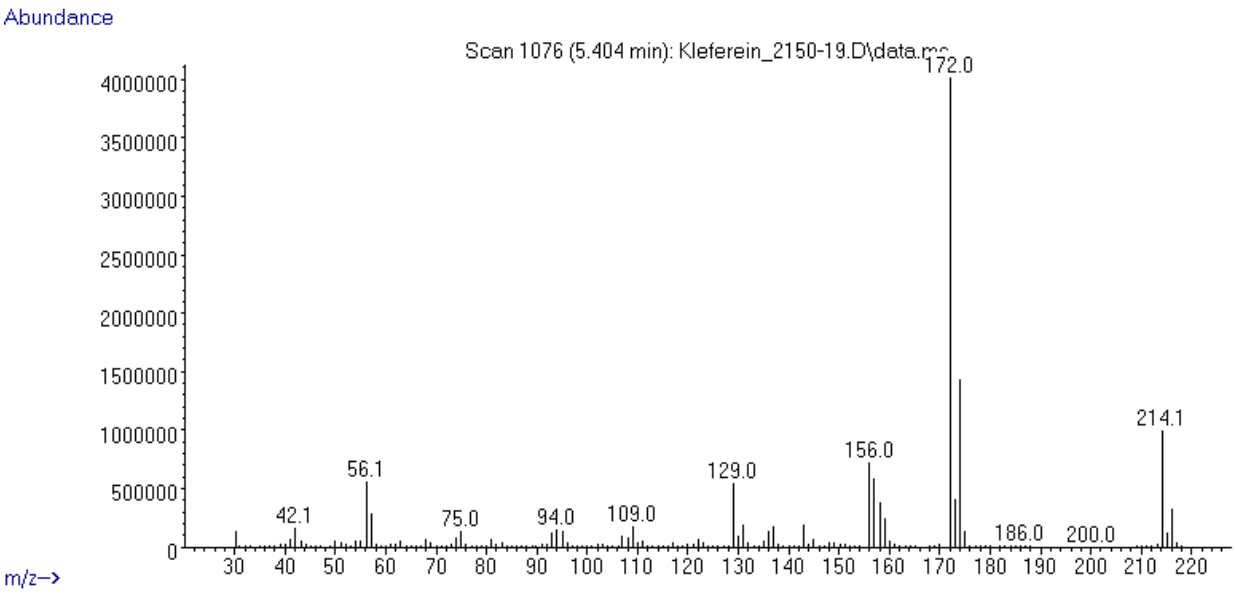
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 5,4 BP(1): 172; BP(2): 174,BP(3) :214,
HPLC-TOF	+	Exact mass (theoretical): 214,0673; measured value Δppm:0,69; formula:C10H12ClFN2
FTIR-ATR	-	direct measurement (sample as received)
FTIR (solid phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	+	
validation		
other		

ANALYTICAL RESULTS

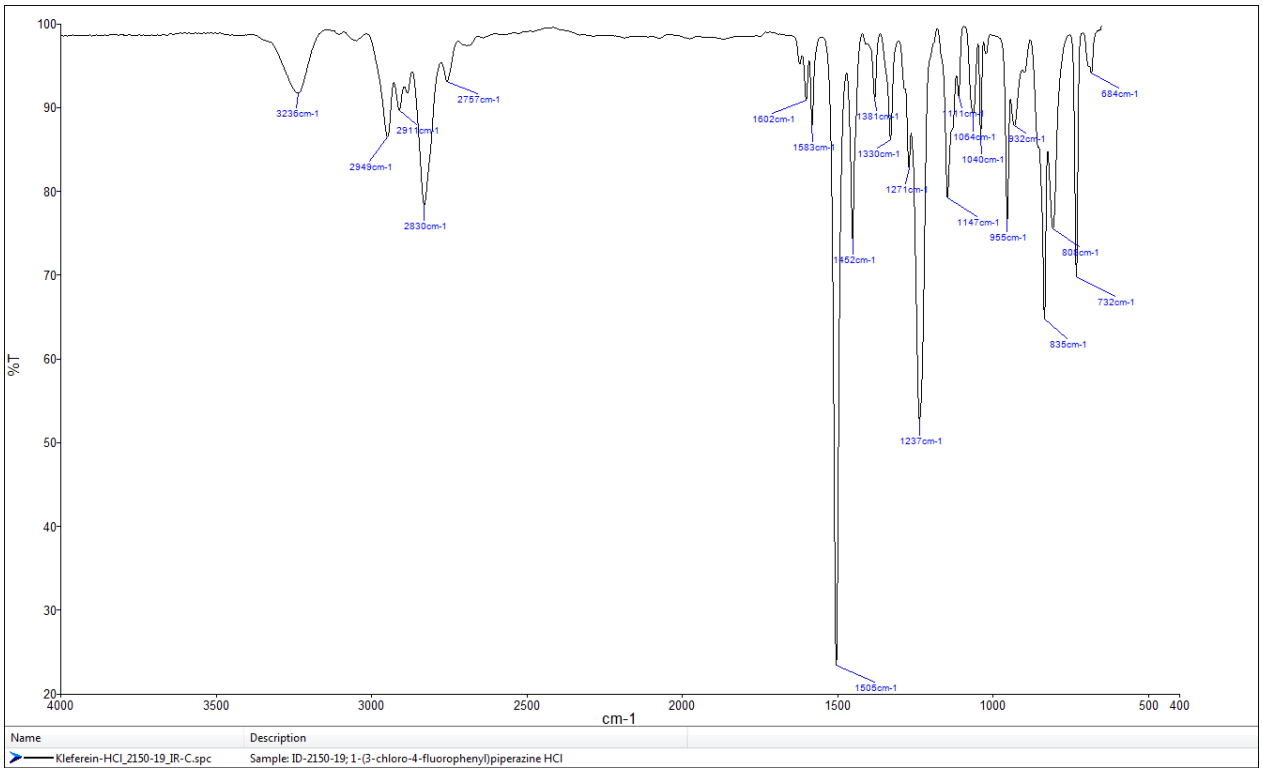
GC-MS: RT=5.4 min kleferein and RT 5.9min caffeine



MS (EI) – kleferein



IR (solid phase – after chromatographic separation)



TOF REPORT

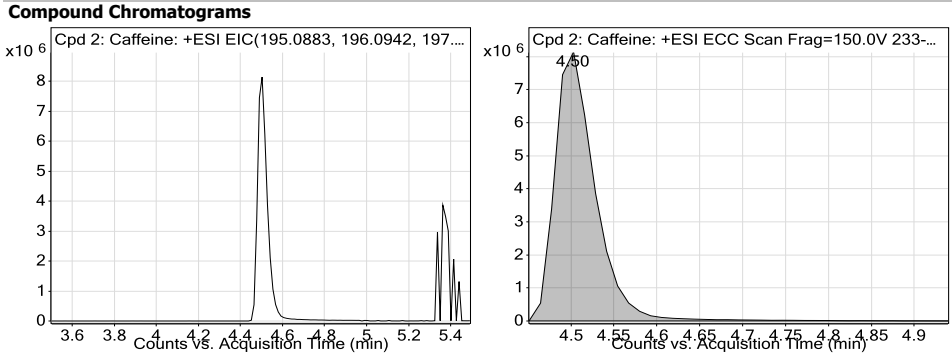
Data File	233-4893-2019_14.d	**Sample Name**	vz-14
Sample Type	Sample	**Position**	P1-D2
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-19_10_2019-XDB-C18-ESI+.m	**Acquired Time**	1/14/2020 1:29:50 PM
IRM Calibration Status	Success	**DA Method**	a-Drugs_NFL.m
Comment	MeOH		

Compound Table

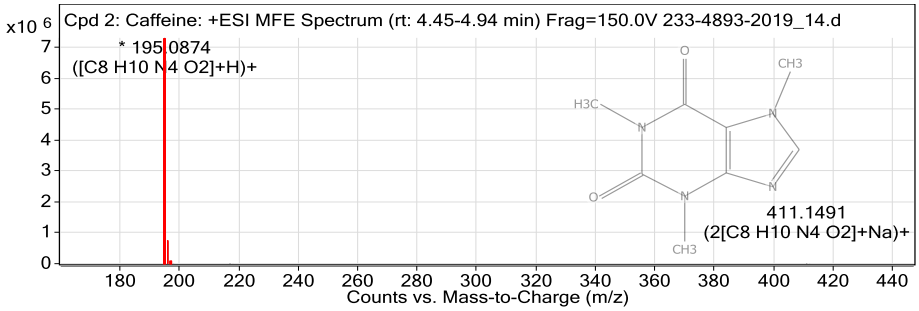
Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 2: Caffeine	Caffeine	C8 H10 N4 O2	4.5	194.0802
Cpd 4: Kleferein	Kleferein	C10 H12 Cl F N2	5.37	214.0672

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
Caffeine	195.0874	4.5	194.0802	4.5	C8 H10 N4 O2	194.0804	0.82

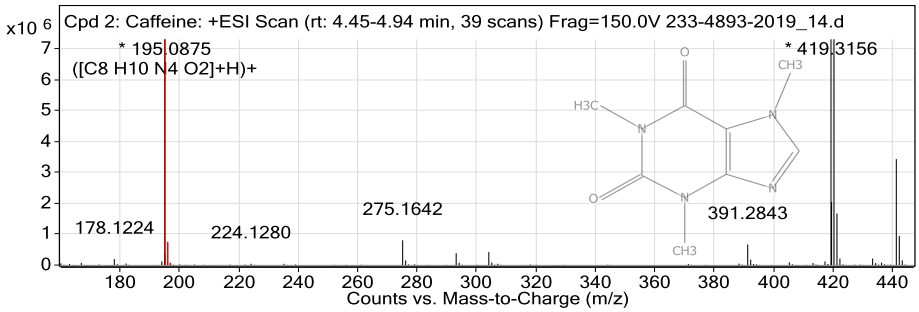
Compound Chromatograms



MFE MS Zoomed Spectrum



MS Zoomed Spectrum



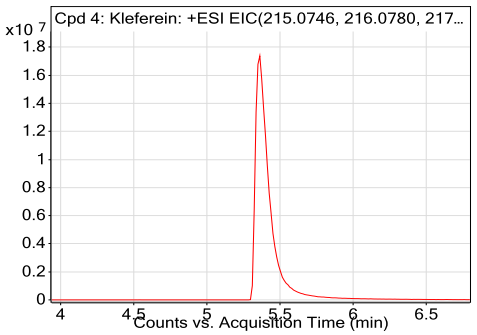
MS Spectrum Peak List

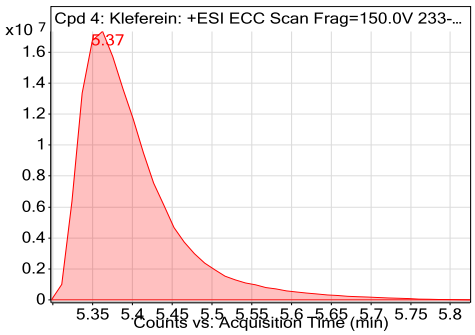
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
195.0874	1	7311211	C8 H10 N4 O2	(M+H)+
196.0905	1	721934.04	C8 H10 N4 O2	(M+H)+
197.0922	1	59832	C8 H10 N4 O2	(M+H)+
198.0966	1	3067.53	C8 H10 N4 O2	(M+H)+
217.0699	1	11259.86	C8 H10 N4 O2	(M+Na)+
218.0732	1	1441.33	C8 H10 N4 O2	(M+Na)+
411.1491	1	9572.04	C8 H10 N4 O2	(2M+Na)+
412.1514	1	2179.01	C8 H10 N4 O2	(2M+Na)+

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
Kleferein	215.0743	5.37	214.0672	5.42	C10 H12 Cl F N2	214.0673	0.69

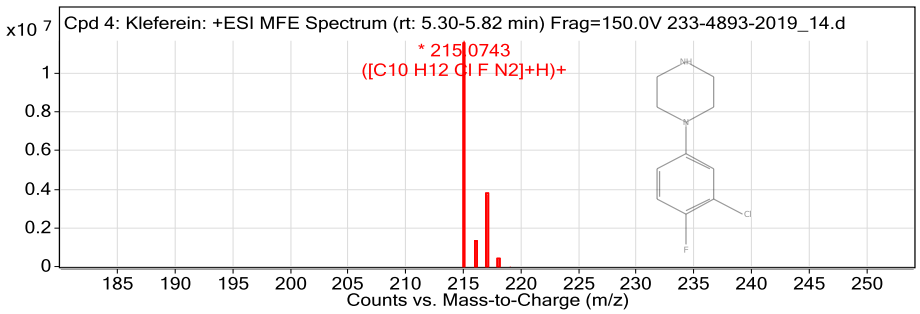
Compound Chromatograms

TOF REPORT

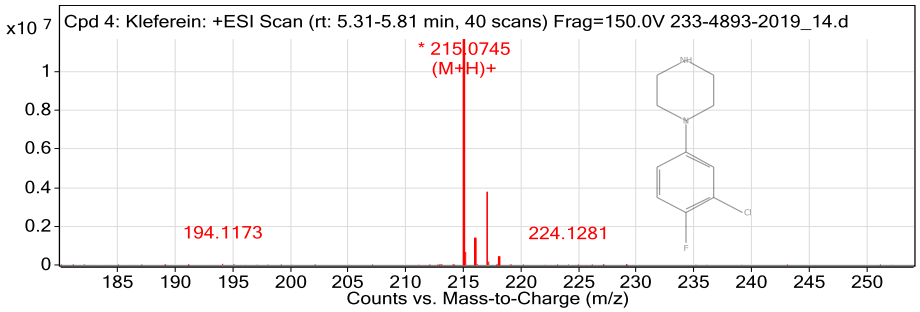




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

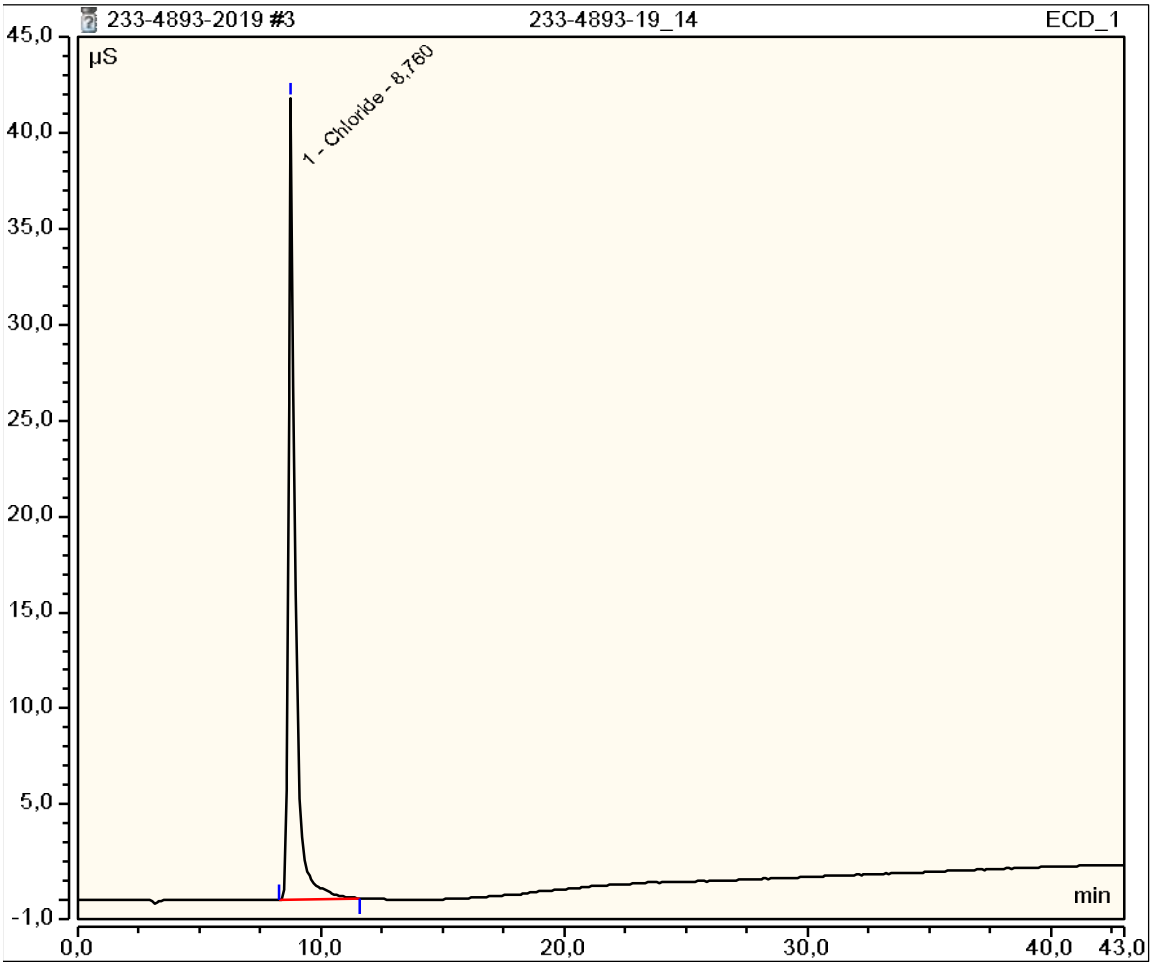
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
215.0743	1	11678844	C10 H12 Cl F N2	(M+H)+
216.0779	1	1310032.98	C10 H12 Cl F N2	(M+H)+
217.0717	1	3718136.8	C10 H12 Cl F N2	(M+H)+
218.075	1	417308.28	C10 H12 Cl F N2	(M+H)+
219.0779	1	23750.46	C10 H12 Cl F N2	(M+H)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**233-4893-19_14**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**15-jan-2020 / 09:54**	*Run Time:*	**43,00**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount mg/L
1	8,76	**Chloride**	BMB	13,706	41,785	n.a.
		TOTAL:		13,71	41,79	0,0



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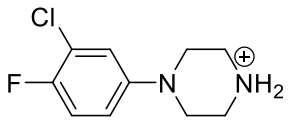
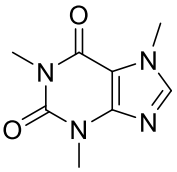
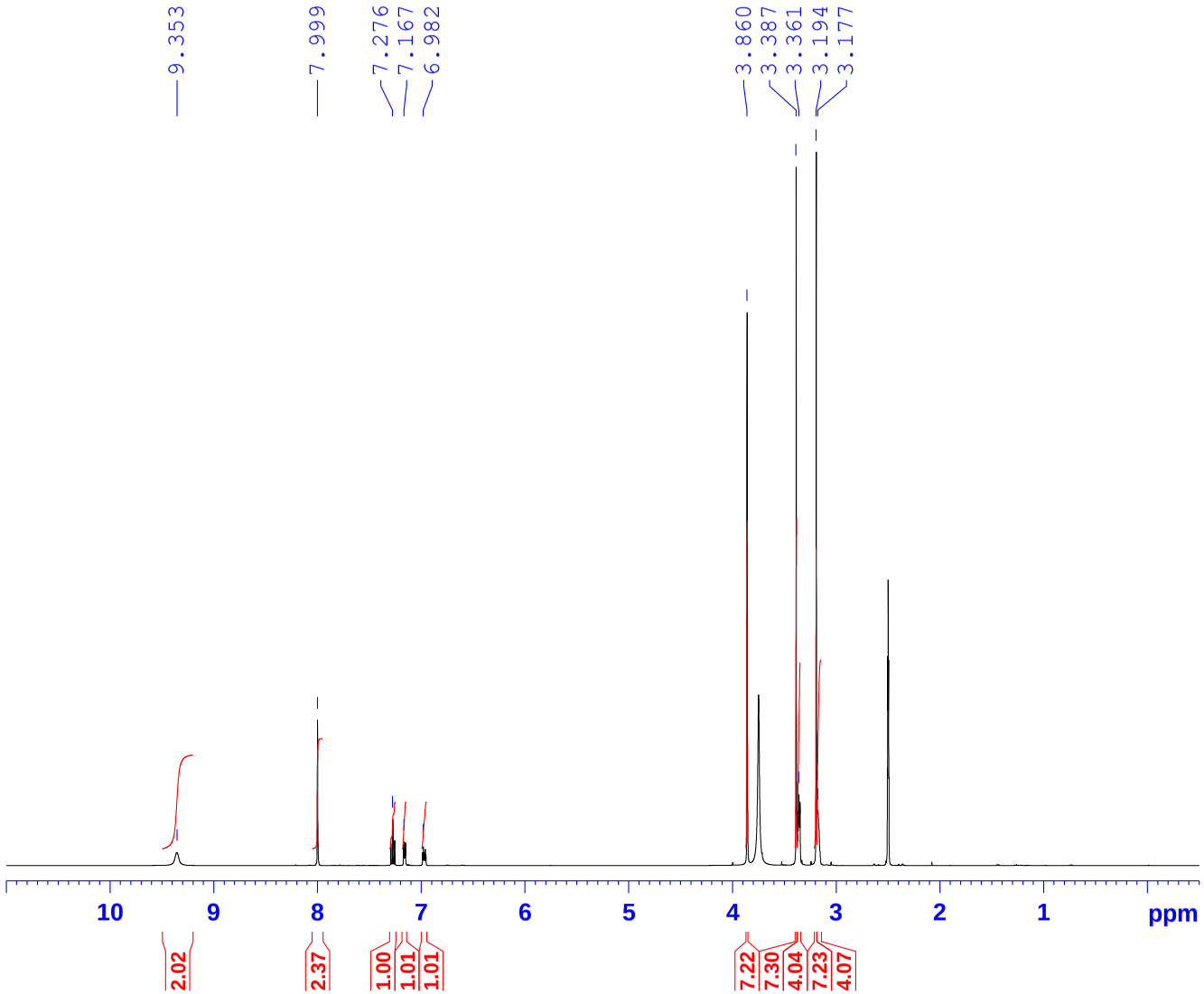
of Ljubljana

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:	**2150-19**
Received date:	January 23, 2020
Our notebook code:	NFL-2150-19
NMR sample preparation:	19.0 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, ^{19}F
Proposed structure with formula, exact mass, molecular weight:	**A**  Chemical Formula: $C_{10}H_{13}ClFN_2^+$ Exact Mass: 215,0746 Molecular Weight: 215,6759 **B**  Chemical Formula: $C_8H_{10}N_4O_2$ Exact Mass: 194,0804 Molecular Weight: 194,1940
Chemical name:	*N*-protonated 1-(3-chloro-4-fluorophenyl)piperazine (**A**), caffeine (**B**)
Comments:	- Structure elucidation based on 1D and 2D NMR spectra and HRMS. - The sample consists of herein identified compound **A** and caffeine (**B**) in molar ration 1 : 2.37, based on ^{1}H NMR spectrum. - >97% 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:	February 17, 2020

NFL-2150-19
1H

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Current Data Parameters
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EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200127
Time              16.44
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT             DMSO
NS                    32
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           500.1300041 MHz
WDW                  EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
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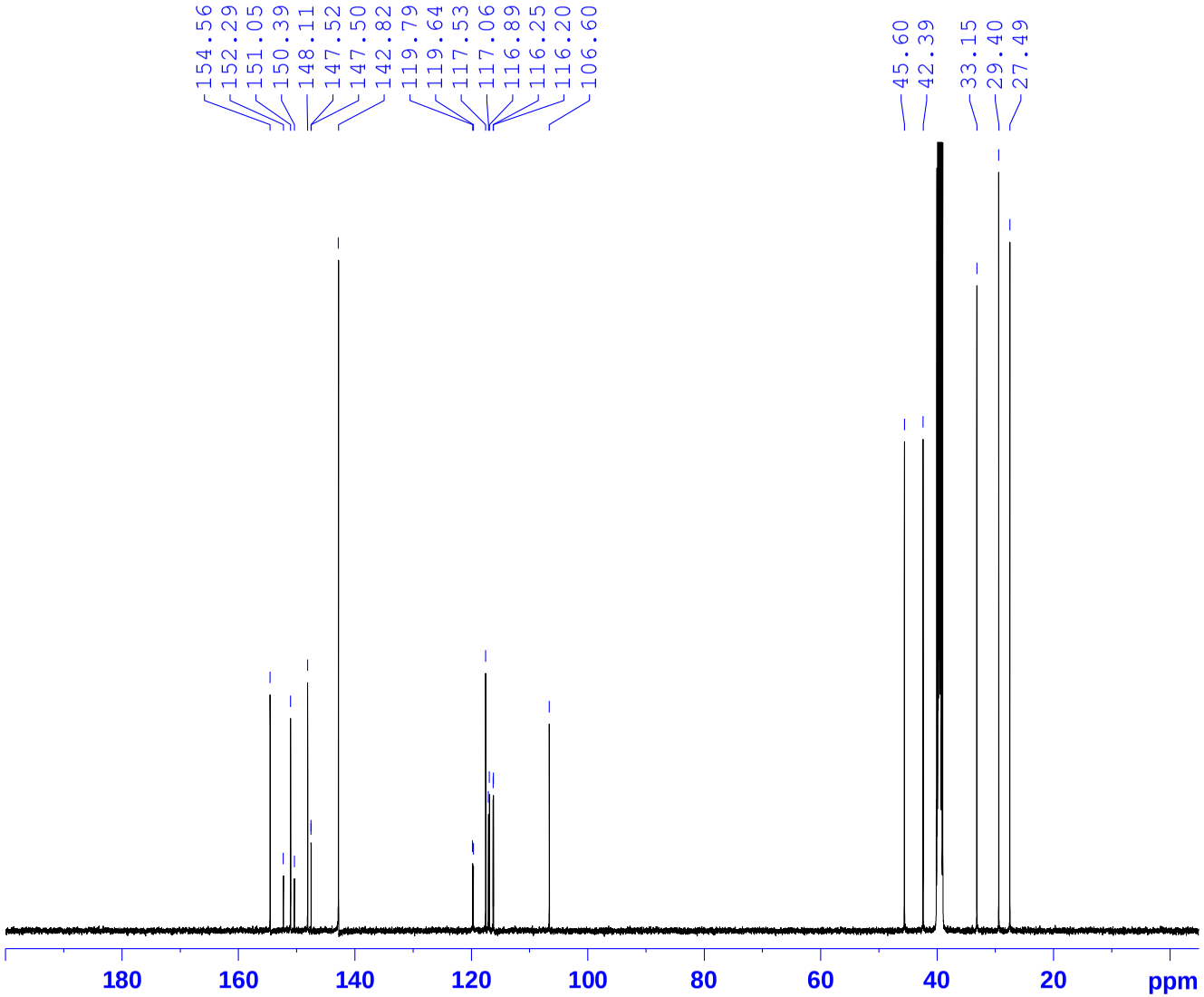
NFL-2150-19
13C

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EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
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Time              22.10
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                65536
SOLVENT             DMSO
NS                 6144
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           2.00000000 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          125.7578443 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```