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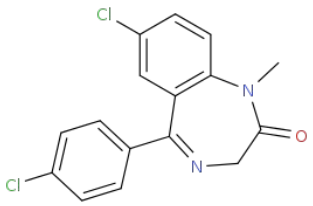
ANALYTICAL REPORT[1]

Ro5-4864 (C16H12Cl2N2O)

7-Chloro-5-(4-chlorophenyl)-1-methyl-3H-1,4-benzodiazepin-2-one

Remark – other NPS detected: none

Sample ID:	1567-16
Sample description:	powder - white-off
Sample type:	test purchase /RESPONSE -purchasing
Date of sample receipt (M/D/Y):	5/10/2016
Date of entry (M/D/Y) into NFL database:	5/24/2016
Report updates (if any) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure[2] (base form)	
Systematic name	7-Chloro-5-(4-chlorophenyl)-1-methyl-3H-1,4-benzodiazepin-2-one
Other names	4'-Chlorodiazepam; Ro 5-4864; 4-Chlorodiazepam; RO5-4864; Chlorodiazepam
Formula (per base form)	C16H12Cl2N2O
M_w (g/mol)	319,19
Salt form/anions detected	base
StdInChIKey	PUMYFTJOWAJIKF-UHFFFAOYSA-N
Compound Class	Benzodiazepines
Other NPS detected	none
Add.info (purity..)	pure by HPLC-TOF, GC-MS

[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] 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

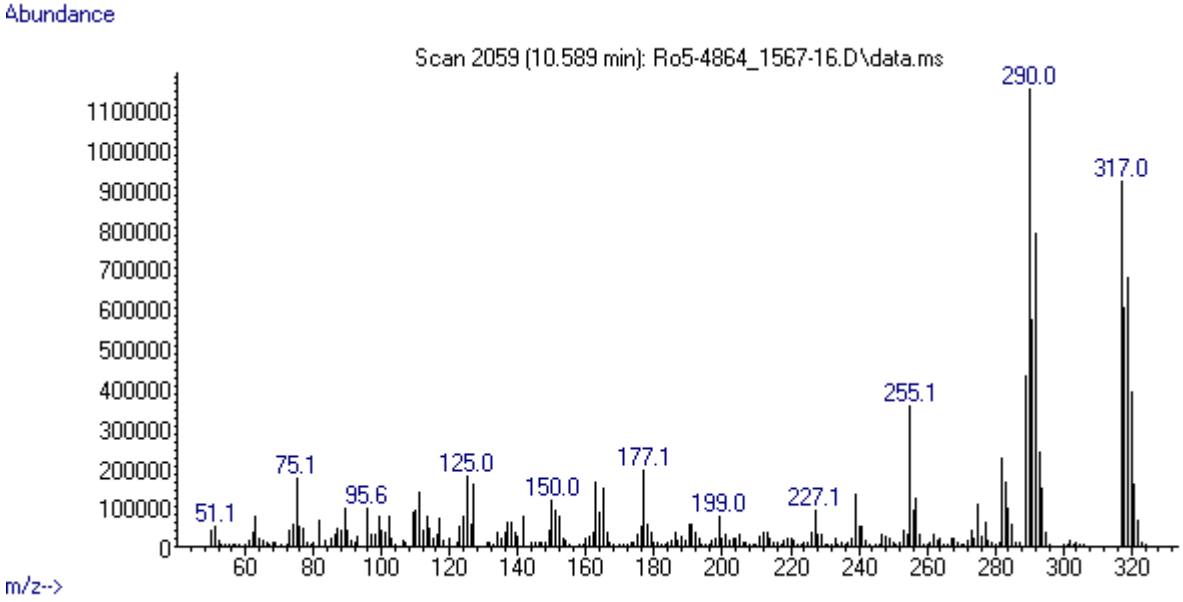
Supporting information

Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	partially

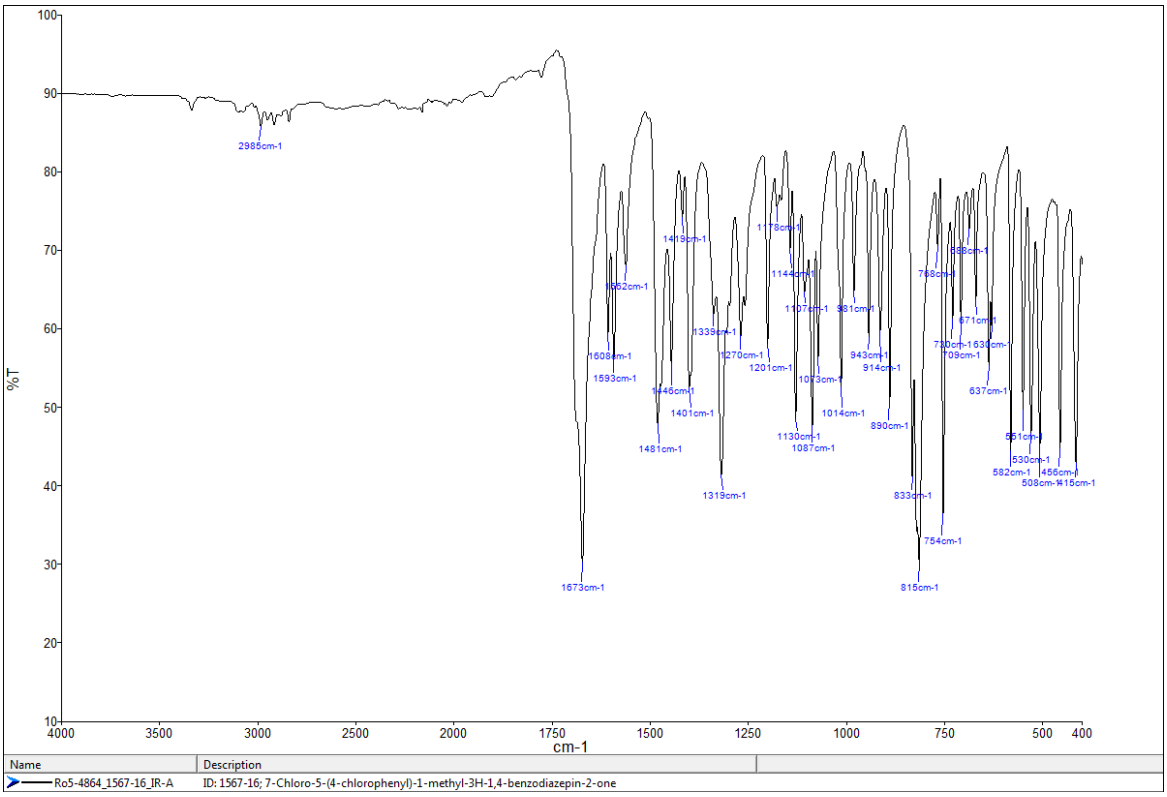
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 10,59 BP(1): 290; BP(2): 317,BP(3) :292,
HPLC-TOF	+	Exact mass (theoretical): 318,0327; measured value Δppm:-0,33; formula:C16H12Cl2N2O
FTIR-ATR	+	direct measurement (sample as received)
FTIR (condensed phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	-	
validation		
other		Substance was identified by MS spectrum from NIST library (QM=99)

ANALYTICAL RESULTS

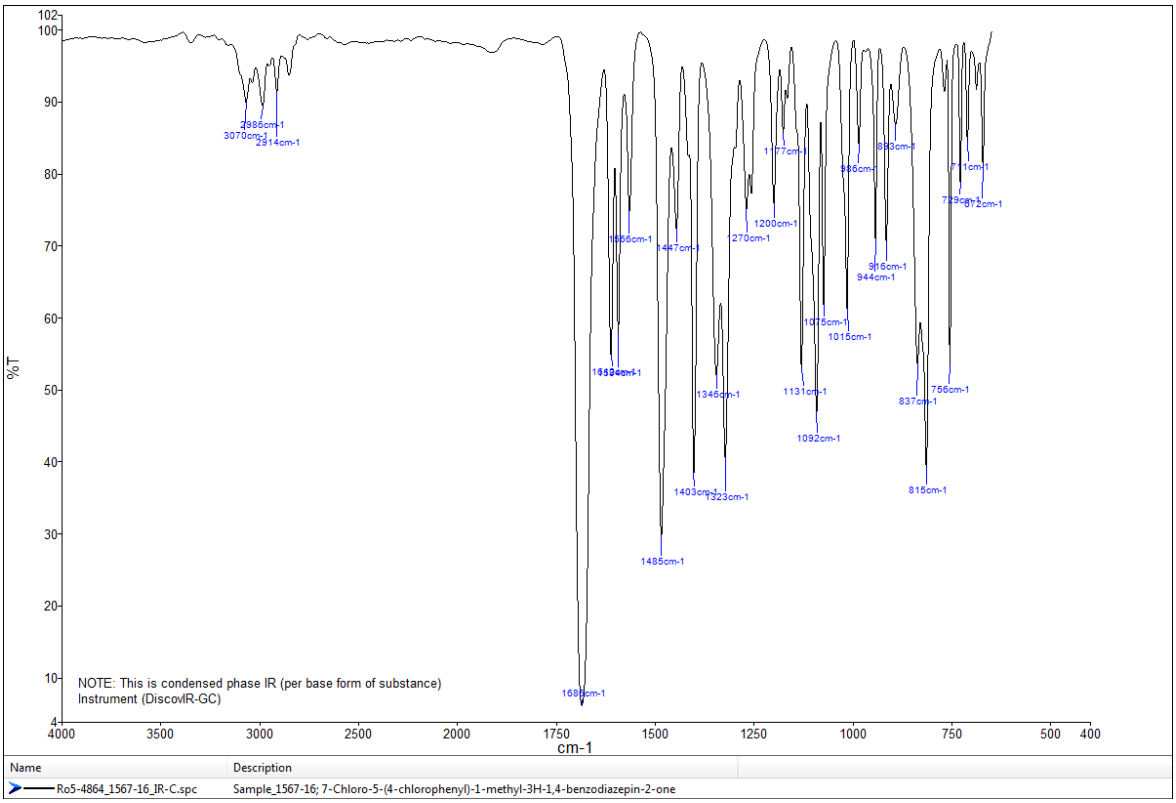
MS (EI)



FTIR-ATR - direct measurement (sample as received)



IR (condensed phase – after chromatographic separation)



TOF REPORT

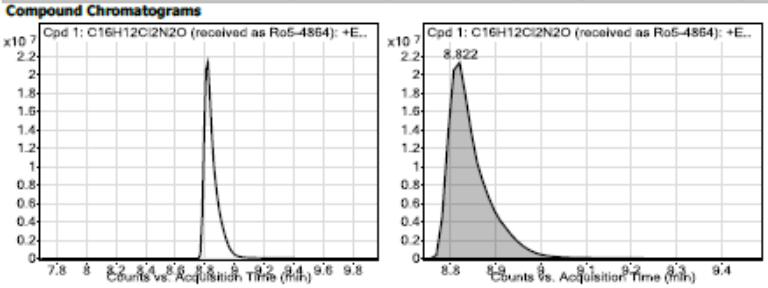
Data File	Ro5-4864_1567-16_TOF.d	**Sample Name**	ID_1567-16
Sample Type	Sample	**Position**	P1-C4
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-1512015-XDB-C18-ESI-poz-pod.m	**Acquired Time**	5/18/2016 11:03:32 AM
IRM Calibration Status	Success	**DA Method**	Drugs_NFL.m
Comment	extract in MeOH		

Compound Table

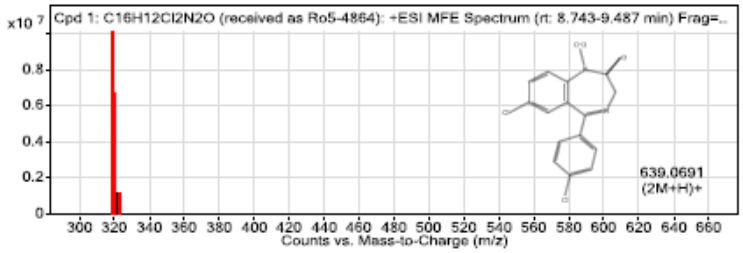
Label	Compound Name	Obs. RT	Obs. Mass
Cpd 1: C16H12Cl2N2O (received as Ro5-4864)	C16H12Cl2N2O (received as Ro5-4864)	8.822	318.0328

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
C16H12Cl2N2O (received as Ro5-4864)	319.04	8.822	318.0328	8.82	C16 H12 Cl2 N2 O	318.0327	-0.33

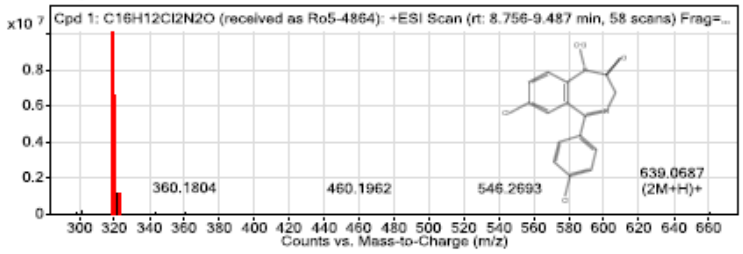
Compound Chromatograms



MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Charge	Abund	Formula	Ion/Isotope
319.04	1	10048108	C16 H12 Cl2 N2 O	(M+H)+
320.0434	1	1827601.99	C16 H12 Cl2 N2 O	(M+H)+
321.0373	1	6561848.62	C16 H12 Cl2 N2 O	(M+H)+
322.0407	1	1127866.24	C16 H12 Cl2 N2 O	(M+H)+
323.0355	1	1066900.86	C16 H12 Cl2 N2 O	(M+H)+
324.0379	1	165150.39	C16 H12 Cl2 N2 O	(M+H)+
325.0404	1	15847.62	C16 H12 Cl2 N2 O	(M+H)+
341.0217	1	14457.4		(M+Na)+
637.0716	1	12833.32		(2M+H)+
639.0691	1	17257.25		(2M+H)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**Ro5-4864_1567-16_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**17-maj-2016 / 15:11**	*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