



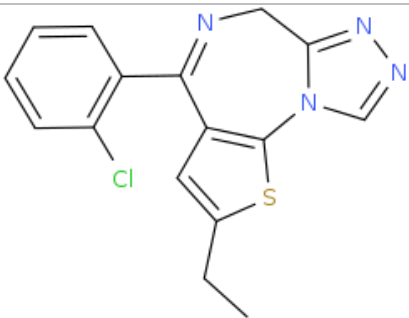
ANALYTICAL REPORT¹

Metizolam (C₁₆H₁₃ClN₄S)

7-(2-chlorophenyl)-4-ethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0.2,6]trideca-2(6),4,7,10,12-pentaene

Remark – other NPS detected: **none**

Sample ID:	1562-16
Sample description:	tablet - light blue
Sample type:	test purchase /RESPONSE -purchasing
Date of sample receipt (M/D/Y):	4/14/2016
Date of entry (M/D/Y) into NFL database:	5/31/2016
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-(2-chlorophenyl)-4-ethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0.2,6]trideca-2(6),4,7,10,12-pentaene
Other names	Desmethyletizolam, 4-(2-Chlorophenyl)-2-ethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine
Formula (per base form)	C ₁₆ H ₁₃ ClN ₄ S
M _w (g/mol)	328,82
Salt form/anions detected	chloride
StdInChIKey	NQSSWDKQLVBUQN-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 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 °C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 °C for 1 min, followed by heating up to 190 °C at rate 8 °C/min, then heating up to 293 °C at a rate of 18 °C/min, hold for 6.1 min, then heating at 50 °C/min up to 325 °C and finally 6.1 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235 °C, source and quadropole temperatures 280 °C and 180 °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 °C. Chromatographic separation as above (1). Split MS : IR = 1: 9.

MSD source EI = 70 eV. GC-MS transfer line T= 235 °C, source and quadropole temperatures 280 °C and 180 °C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300) amu.

IR (condensed (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

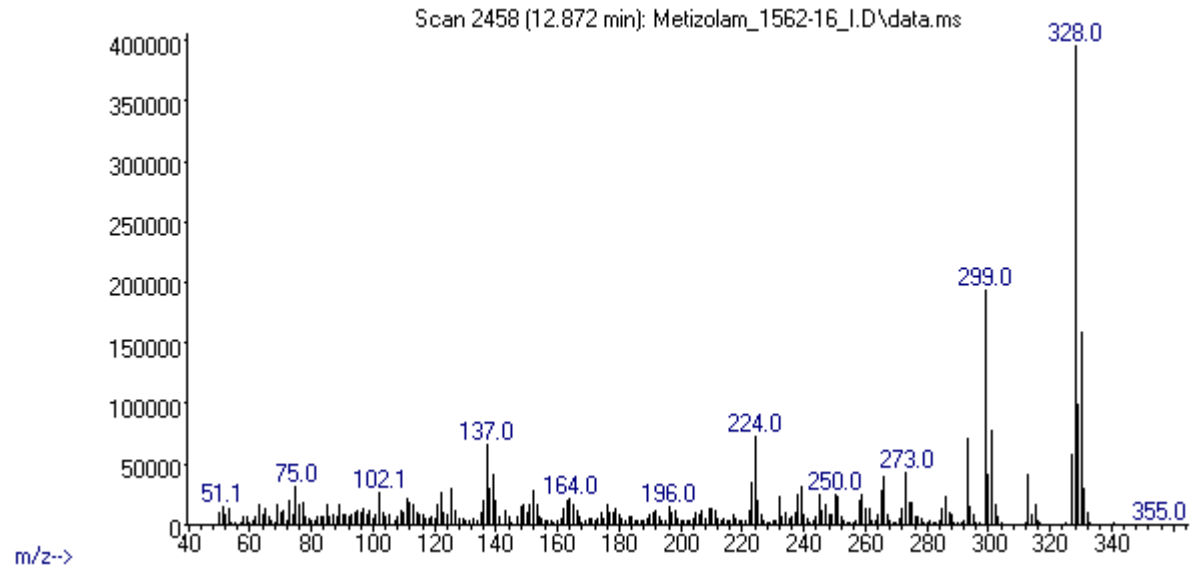
Solubility in	result/remark
CH ₂ Cl ₂	partially
MeOH	partially
H ₂ O	partially

Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 12,87 BP(1): 328; BP(2): 299,BP(3) :330,
HPLC-TOF	+	Exact mass (theoretical): 328,0549; measured value Δppm:0,28; formula:C16H13ClN4S
FTIR-ATR	-	direct measurement (sample as received)
FTIR (condensed phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	-	
validation		confirmed by reference material (KEM-ID:1576-16);
other		

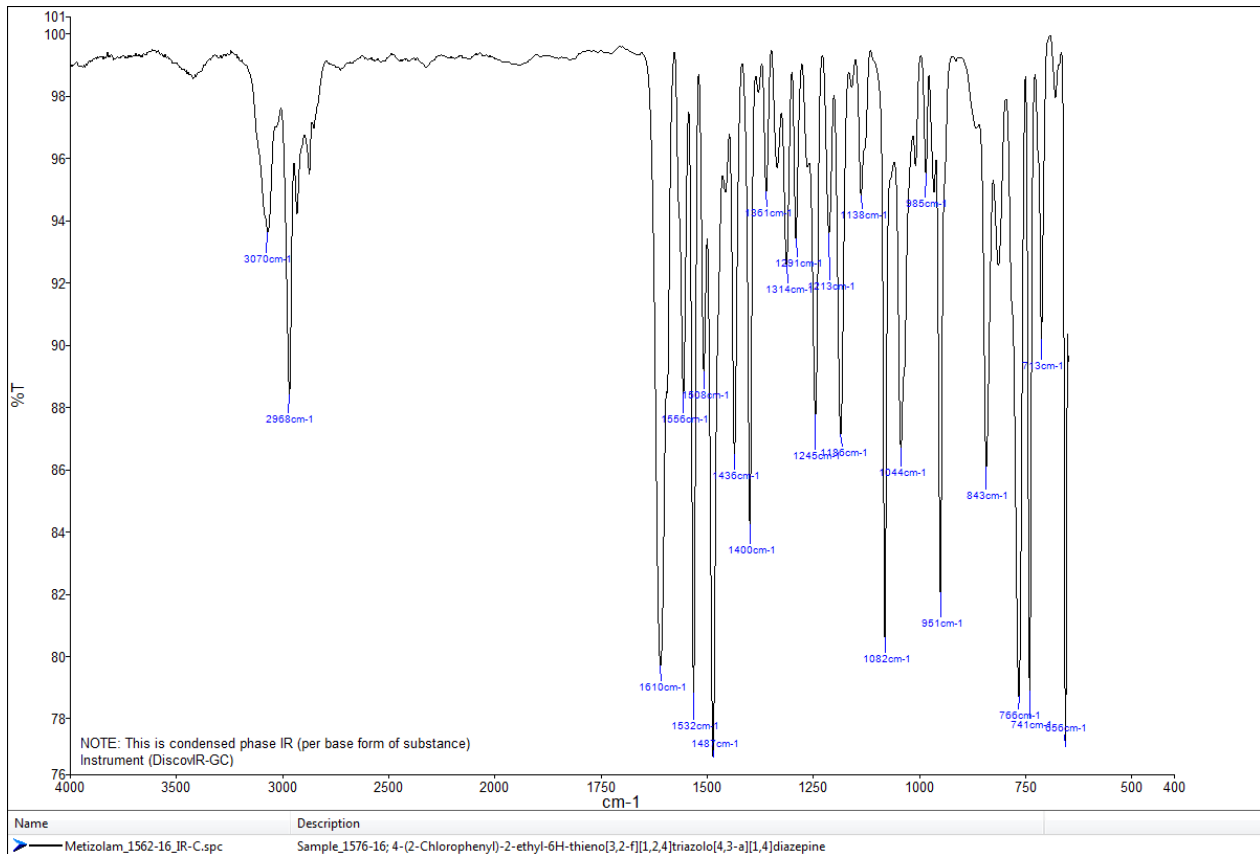
ANALYTICAL RESULTS

MS (EI)

Abundance



IR (condensed phase – after chromatographic separation)



TOF REPORT

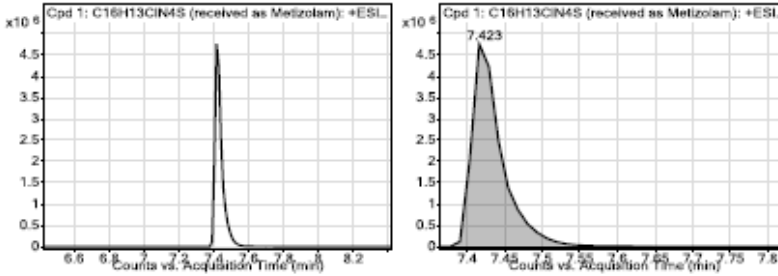
Data File	Metizolam_1562-16_TOF.d	Sample Name	ID_1562-16
Sample Type	Sample	Position	P1-F6
Instrument Name	62308 TOF LC-MS	User Name	TG
Acq Method	general-1512015-XDB-C18-ESI-poz-pod.m	Acquired Time	4/15/2016 11:37:11 AM
IRM Calibration Status	Success	DA Method	Drugs_NFL.m
Comment	extract in MeOH		

Compound Table

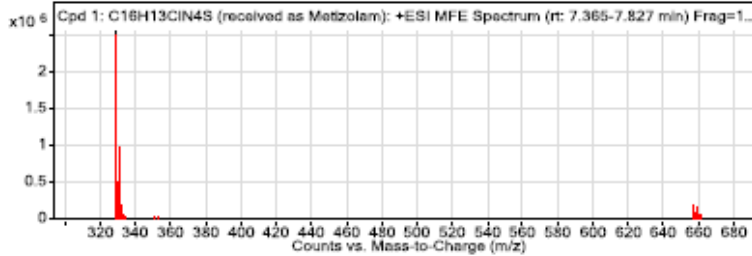
Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 1: C16H13ClN4S (received as Metizolam)	C16H13ClN4S (received as Metizolam)	C16 H13 Cl N4 S	7.423	328.0548

Name	Obs. m/z	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
C16H13ClN4S (received as Metizolam)	329.0619	7.423	328.0548	7.42	C16 H13 Cl N4 S	328.0549	0.28

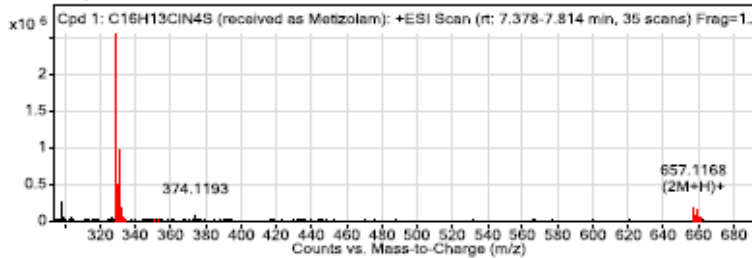
Compound Chromatograms



MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Charge	Abund	Formula	Ion/Isotope
329.0619	1	2553923	C16 H13 Cl N4 S	(M+H)+
330.0653	1	473826.86	C16 H13 Cl N4 S	(M+H)+
331.0599	1	952852.76	C16 H13 Cl N4 S	(M+H)+
332.0619	1	167525.14	C16 H13 Cl N4 S	(M+H)+
333.0577	1	44734.6	C16 H13 Cl N4 S	(M+H)+
657.1172	1	189164.39	C16 H13 Cl N4 S	(2M+H)+
658.1197	1	69381.87	C16 H13 Cl N4 S	(2M+H)+
659.1149	1	149660.16	C16 H13 Cl N4 S	(2M+H)+
660.1165	1	49685.86	C16 H13 Cl N4 S	(2M+H)+
661.1124	1	36284.43	C16 H13 Cl N4 S	(2M+H)+

--- End Of Report ---

Peak Integration Report

Sample Name:	Metizolam_1562-16_IC	Inj. Vol.:	25,00
Injection Type:	Unknown	Dilution Factor:	1,0000
Program:	ANIONI	Operator:	kemija
Inj. Date / Time:	18-apr-2016 / 16:39	Run Time:	42,00

No.	Time min	Peak Name	Peak Type	Area $\mu\text{S} \cdot \text{min}$	Height μS	Amount mg/L
1,00	8,34	Chloride	BMB	16,56	76,30	n.a.
TOTAL:				16,56	76,30	0,00

