NACIONALNI FORENZIČNI LABORATORIJ
NATIONAL FORENSIC LABORATORY

Vodovodna 95
1000 Ljubljana
SLOVENIJA

T: +386 (0)1 428 44 93
E: nfl@policija.si
www.policija.si

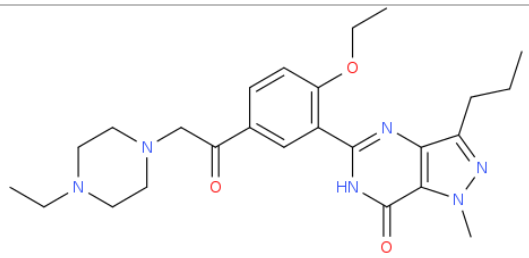
ANALYTICAL REPORT[1]

acetildenafil (C25H34N6O3)

5-{2-ethoxy-5-[2-(4-ethylpiperazin-1-yl)acetyl]phenyl}-1-methyl-3-propyl-1H,6H,7H-pyrazolo[4,3-d]pyrimidin-7-one

Remark – other NPS detected: none

Sample ID:	1542-16
Sample description:	powder - brown-yellowish
Sample type:	test purchase /RESPONSE -purchasing
Date of sample receipt (M/D/Y):	3/9/2016
Date of entry (M/D/Y) into NFL database:	3/17/2016
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	5-{2-ethoxy-5-[2-(4-ethylpiperazin-1-yl)acetyl]phenyl}-1-methyl-3-propyl-1H,6H,7H-pyrazolo[4,3-d]pyrimidin-7-one
Other names	HONGDENAFIL;
Formula (per base form)	C25H34N6O3
M_w (g/mol)	466,59
Salt form/anions detected	base
StdInChIKey	RRBRQNALHKQCAI-UHFFFAOYSA-N
Compound Class	Piperazine derivates
Other NPS detected	none
Add.info (purity..)	pure by GC-MS not pure, TOF: 10% C27H36N6O5 Mw=524,3

[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 (RT=9.53 min). Injection volume 1 ml and split mode (1:50) . Injector temperature: 280 ^{0}C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickens 0.25 mm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 ^{0}C for 1 min, followed by heating up to 293 ^{0}C at a rate of 18 ^{0}C/min, hold for 6.1 min, than 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) 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 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

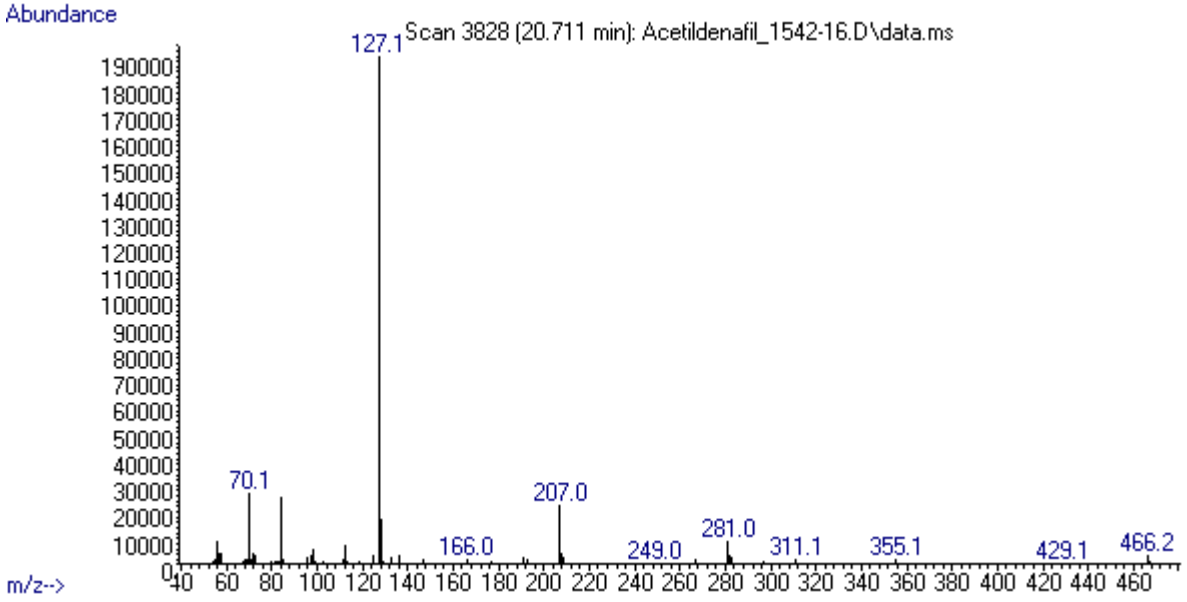
Supporting information

Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	partially

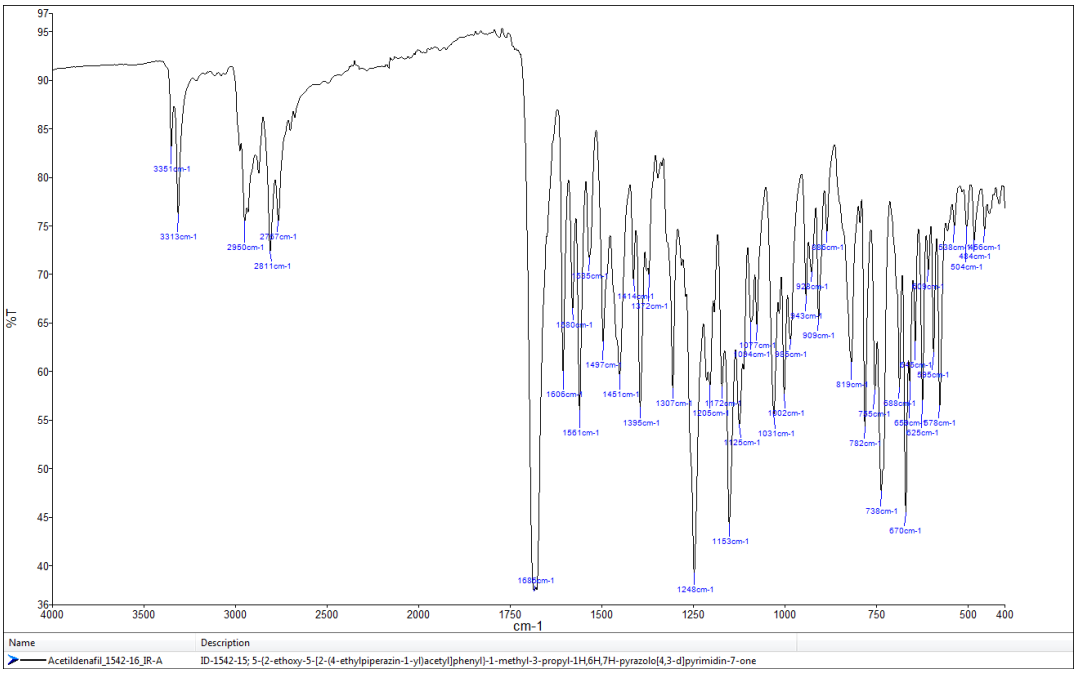
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 20,72 BP(1): 127; BP(2): 70,BP(3) :84,
HPLC-TOF	+	Exact mass (theoretical): 466,2629; measured value Δppm:-0,02; formula:C25H34N6O3
FTIR-ATR	+	direct measurement (sample as received)
FTIR (condensed phase) always as base form	+	
IC (anions)	-	
NMR (in FKKT)	-	
validation		
other		Confirmed by Cayman and SWGDRUG MS libraries.

ANALYTICAL RESULTS

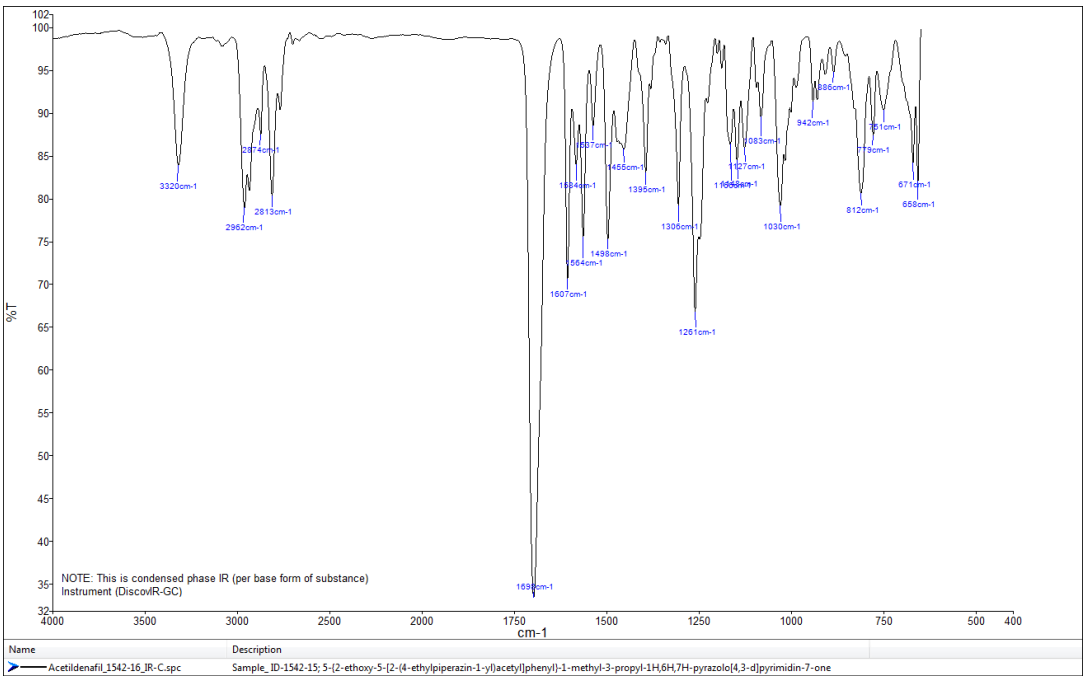
MS (EI)



FTIR-ATR - direct measurement (sample as received)



IR (condensed phase – after chromatographic separation)



TOF REPORT

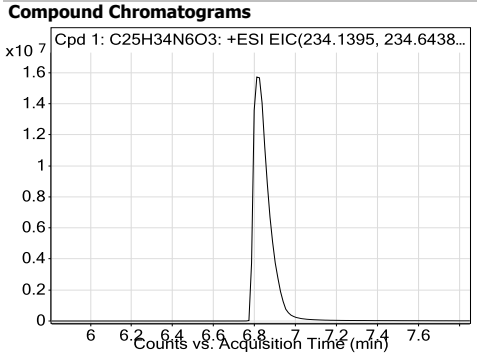
Data File	Acetildenafil-1542-16_TOF.d	**Sample Name**	ID_1542-16
Sample Type	Sample	**Position**	P1-E5
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-1512015-XDB-C18-ESI-poz-pod.m	**Acquired Time**	3/13/2016 11:08:59 AM
IRM Calibration Status	Success	**DA Method**	Drugs_NFL.m
Comment	extract in MeOH		

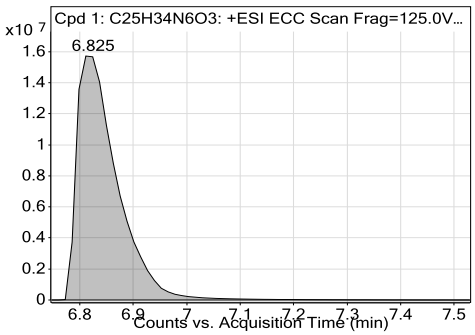
Compound Table

Label	Compound Name	Obs. RT	Obs. Mass
Cpd 1: C25H34N6O3	C25H34N6O3	6.825	466.2692
Cpd 2: C27H36N6O5	C27H36N6O5	6.965	524.2748

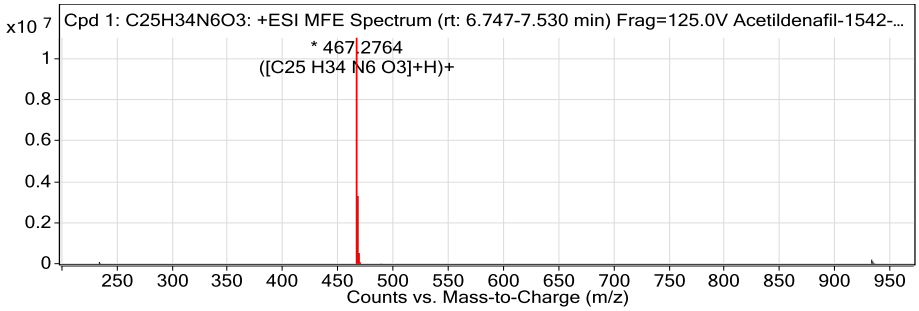
Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
C25H34N6O3	467.2764	6.825	466.2692	6.83	C25 H34 N6 O3	466.2692	-0.02

Compound Chromatograms

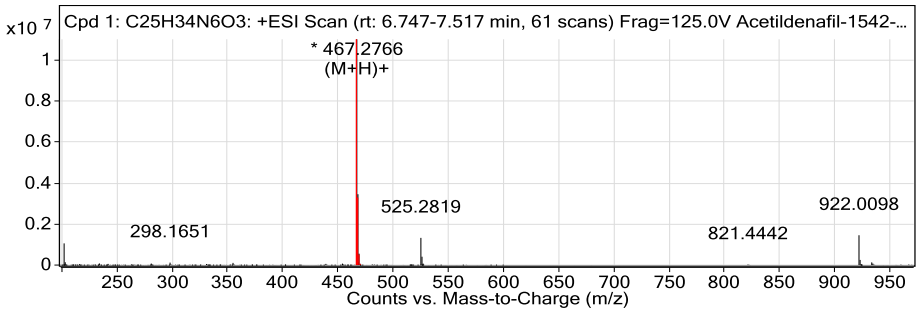




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



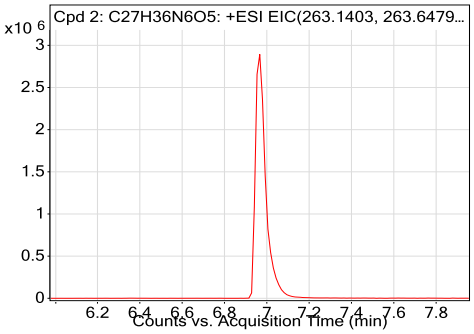
MS Spectrum Peak List

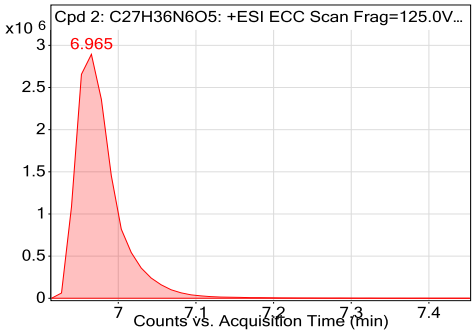
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
234.143	2	82664.06		(M+2H)+2
234.6442	2	27909.28		(M+2H)+2
467.2764	1	11007167	C25 H34 N6 O3	(M+H)+
468.2797	1	3297568.22	C25 H34 N6 O3	(M+H)+
469.2831	1	514451.74	C25 H34 N6 O3	(M+H)+
470.2849	1	57804.95	C25 H34 N6 O3	(M+H)+
489.2583	1	10039.62		(M+Na)+
933.546	1	200111.52		(2M+H)+
934.5487	1	110892.42		(2M+H)+
935.5506	1	31782.99		(2M+H)+

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
C27H36N6O5	525.2819	6.965	524.2748	6.97	C27 H36 N6 O5	524.2747	-0.22

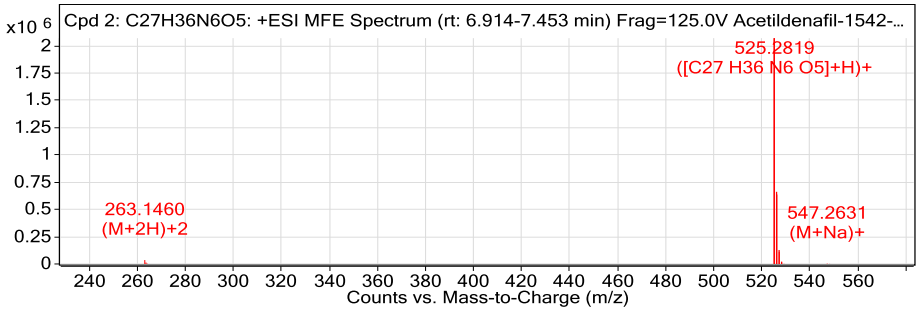
TOF REPORT

Compound Chromatograms

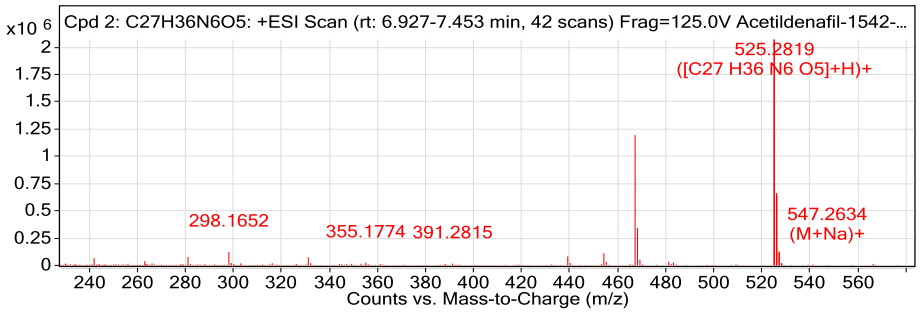




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Charge	Abund	Formula	Ion/Isotope
263.146	2	35610.11		(M+2H)+2
263.6468	2	13324.66		(M+2H)+2
264.1489	2	3130.62		(M+2H)+2
525.2819	1	2070495.5	C27 H36 N6 O5	(M+H)+
526.2858	1	637237.15	C27 H36 N6 O5	(M+H)+
527.2878	1	111810.69	C27 H36 N6 O5	(M+H)+
528.2894	1	14260.5	C27 H36 N6 O5	(M+H)+
529.2911	1	1450.93	C27 H36 N6 O5	(M+H)+
547.2631	1	3142.35		(M+Na)+
548.2681	1	1019.06		(M+Na)+

--- End Of Report ---