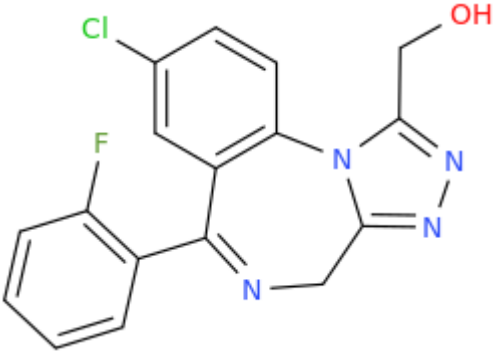


## ANALYTICAL REPORT

alpha-hydroxy Flualprazolam (C<sub>17</sub>H<sub>12</sub>ClFN<sub>4</sub>O)**[12-chloro-9-(2-fluorophenyl)-2,4,5,8-tetraazatricyclo[8.4.0.02,6]tetradeca-1(14),3,5,8,10,12-hexaen-3-yl]methanol**

Remark – other active cpd. detected

Sample ID:	2220-20
Sample description:	powder - white
Sample type:	RM-reference material
Comments:	CAY Lot#0581550-14, for GC-MS compound was derivatized by MSTFA: GC-RT and MS spectrum refer to TMS derivative
Date of entry (DD/MM/YYYY):	06/07/2021

Substance identified-structure <sup>1</sup> (base form)	
Systematic name:	[12-chloro-9-(2-fluorophenyl)-2,4,5,8-tetraazatricyclo[8.4.0.02,6]tetradeca-1(14),3,5,8,10,12-hexaen-3-yl]methanol
Other names:	8-chloro-6-(2-fluorophenyl)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine-1-methanol; $\alpha$ -hydroxy Flualprazolam
Formula (per base form)	C <sub>17</sub> H <sub>12</sub> ClFN <sub>4</sub> O
M <sub>w</sub> (g/mol)	342,76
Salt form:	base
StdInChIKey (per base form)	GOSZRMNDJKRVJJ-UHFFFAOYSA-N
Other active cpd. detected	
Add.info (purity..)	≥98%

<sup>1</sup> Created by OPSIN free tool: <http://opsin.ch.cam.ac.uk/> DOI: 10.1021/ci100384d

## Report updates

date	comments (explanation)

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## Supporting information

Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 13,14 BP(1): 399; BP(2): 401,BP(3) :414,
FTIR-ATR	+	direct measurement
GC-IR (condensed phase)	+	always as base form
HPLC-TOF	+	exact mass theoretical: 342,0684 / measured $\Delta$ ppm: -1,37

**1. GC-MS** (Agilent): GC-method is RT locked to tetracosane (9.258 min). Injection volume 1  $\mu$ l and split mode (1:50). Injector temperature: 280  $^{\circ}$ C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickness 0.25  $\mu$ m. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170  $^{\circ}$ C for 1 min, followed by heating up to 190  $^{\circ}$ C at rate 8  $^{\circ}$ C/min, then heating up to 293  $^{\circ}$ C at a rate of 18  $^{\circ}$ C/min, hold for 7.1 min, then heating at 50  $^{\circ}$ C/min up to 325  $^{\circ}$ C and finally 6.1 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235 $^{\circ}$ C, source and quadropole temperatures 280 $^{\circ}$ C and 180 $^{\circ}$ C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300 until 6 min) amu.

**2. FTIR-ATR** (Perkin Elmer): scan range 4000-400  $\text{cm}^{-1}$ ; resolution 4 $\text{cm}^{-1}$

**3. GC- (MS)-IR** condensed phase (GC-MS (Agilent) & IR (Spectra analyses-Danny)

GC-method: Injection volume 1  $\mu$ l and split mode (1:5). Injector temperature 280  $^{\circ}$ C. Chromatographic separation as above **(1)**. Split MS: IR = 1 : 9.

MSD source EI = 70 eV. GC-MS transfer line T= 235 $^{\circ}$ C, source and quadropole temperatures 280 $^{\circ}$ C and 180 $^{\circ}$ 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  $\text{cm}^{-1}$ .

**4. 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  $\mu$ l. 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 (N<sub>2</sub>) and sheath temperature 325  $^{\circ}$ 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.

## ANALYTICAL RESULTS

MS (EI)

Abundance

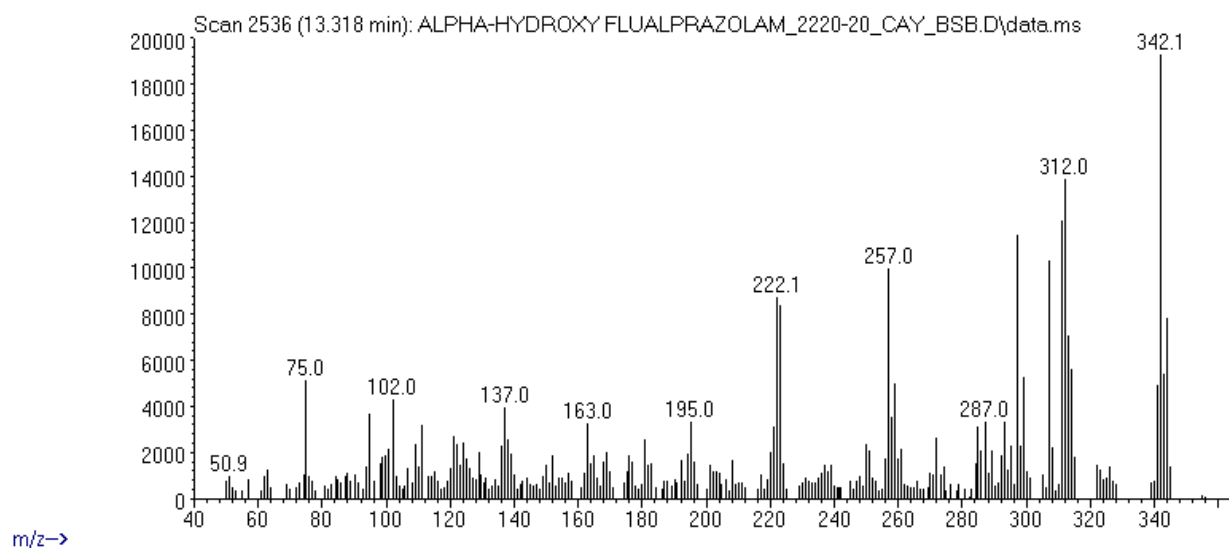


Fig 1: MS spectrum of non derivatized sample

Abundance

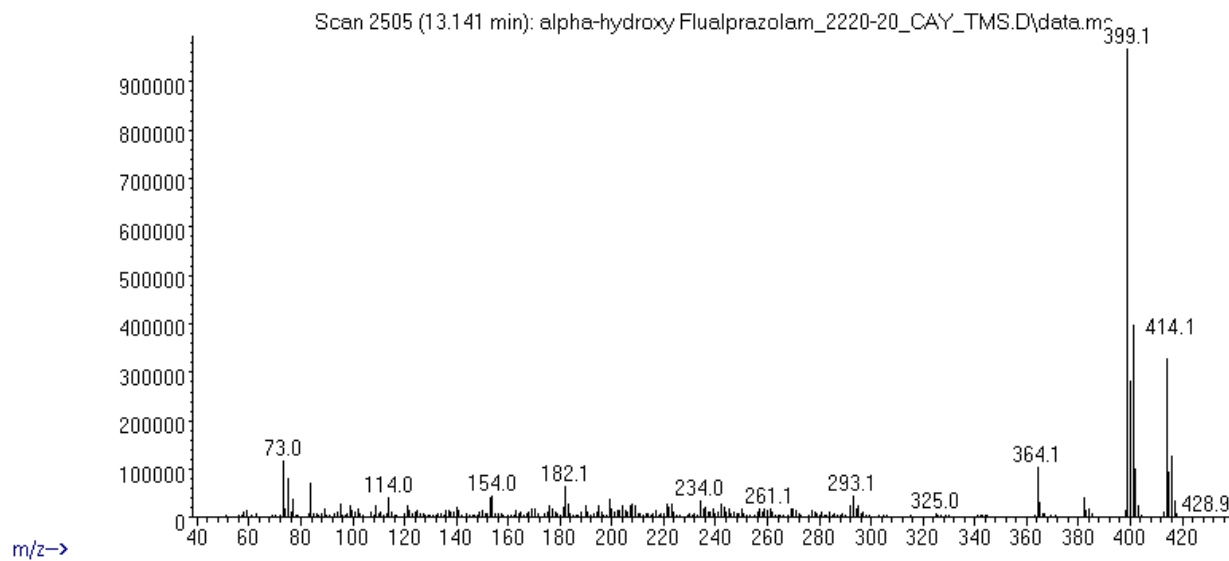
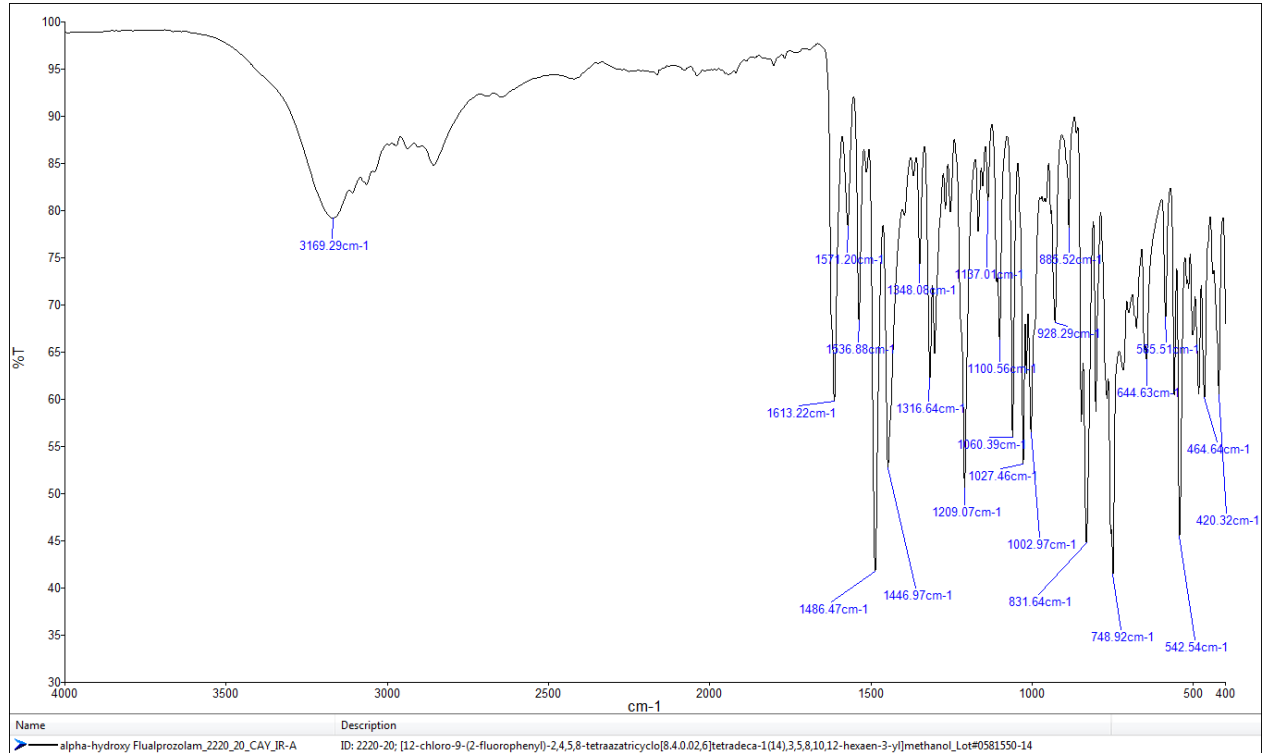


Fig. 2: MS spectrum of TMS derivative

FTIR-ATR (direct measurement – sample as received)



IR- (condensed (solid) phase – after chromatographic separation) - spectrum per base form of TMS derivative of compound

