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

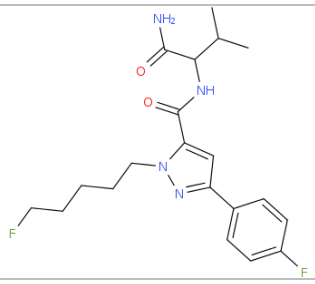
# ANALYTICAL REPORT

## 5F-3,5-AB-PFUPPYCA (C20H26F2N4O2)

**2-{[1-(5-fluoropentyl)-3-(4-fluorophenyl)-1H-pyrazol-5-yl]formamido}-3-methylbutanamide**

Remark – other NPS detected: none

| | |
|---|---|
| Sample ID: | 1668-16 |
| Sample description: | powder |
| Sample type: | test purchase /RESPONSE -purchasing (sample was purchased as AZ-037) |
| Date of sample receipt (M/D/Y): | 9/7/2016 |
| Date of entry (M/D/Y) into NFL database: | 4/10/2017 |
| Report[1] 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 | 2-{[1-(5-fluoropentyl)-3-(4-fluorophenyl)-1H-pyrazol-5-yl]formamido}-3-methylbutanamide |
| Other names | N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide; 5F-3,5-AB-FUPPYCA; 5-fluoro AB-FUPPYCA; AZ-037, AZ-037-isomer |
| Formula (per base form) | C20H26F2N4O2 |
| $M_w$ (g/mol) | 392,45 |
| Salt form/anions detected | base<br>(remark: traces of $Cl^-$ ions were detected by IC. However, concentration is below the stoichiometric amount required for HCl form of the compound. ) |
| StdInChIKey (per base form) | JPKXVUNTSWGYKJ-UHFFFAOYSA-N |
| Other NPS detected | none |
| Additional info (purity..) | impurities - organic by GCMS, HPLC-TOF and NMR |

[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) |
|---|---|
| 08/06/2017 | Explanations added (blue text). |
| | |
| | |
| | |
| | |

## **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 7.1 min, then heating at 50 $^0$C/min up to 325 $^0$C and finally 6.1 min isothermal (may be extended on as needed basis). 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 | partially |
| $H_2O$ | low (bad) |

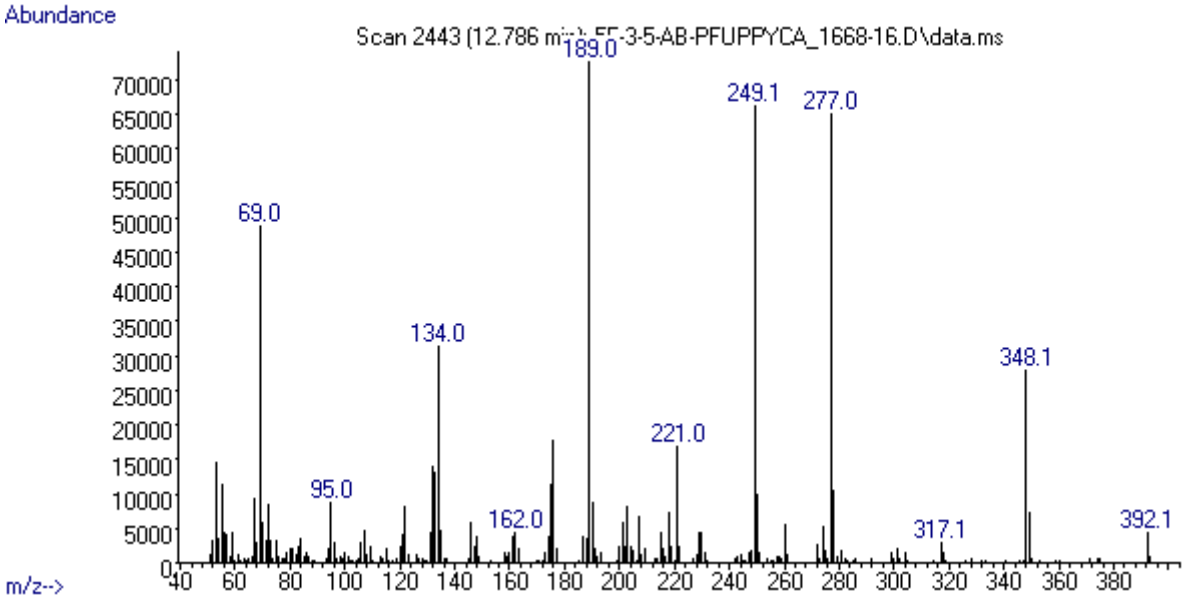
| Analytical technique: | applied | remarks |
|---|---|---|
| GC-MS (EI ionization) | + | NFL GC-RT (min): 12,79<br>BP(1): 189; BP(2): 249,BP(3) :277, |
| HPLC-TOF | + | Exact mass (theoretical): 329,2024;<br>measured value Δppm:0,24;<br>formula:C20H26F2N4O2 |
| FTIR-ATR | + | direct measurement (sample as received) and measurement of dried solid sample after the extraction from alkaline solution into butyl acetate and drying at $70^0$C until constant weight. |
| FTIR (condensed phase) always as base form | + | The spectrum of test purchased sample complies by reference materials purchased from Cayman and Chiron. |
| IC (anions) | + | |
| NMR (in FKKT) | + | NMR did not reveal a clear positions of ring substituent (5F-5,3-AB-PFUPPYCA or 5F-3,5-AB-PFUPPYCA); see the attached report and validation section below. |

The identity of compound was confirmed by REFERENCE materials.

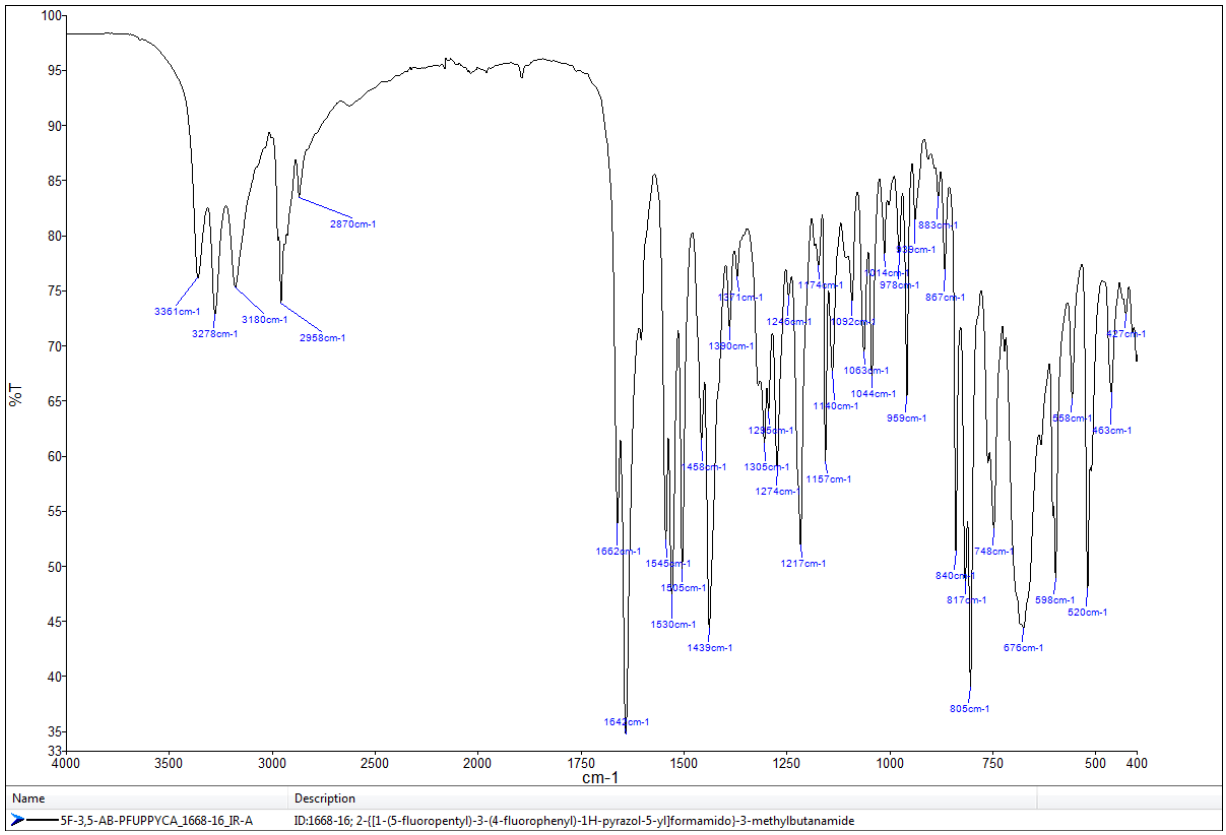
<table>
<tr>
<td>validation</td>
<td></td>
<td>
Comparison of the results by reference materials obtained from:
<ul>
<li>Cayman (https://www.caymanchem.com/product/17181 . Two batches were ordered and analyzed (batch # 0472185-14 (NFL:</li>
<li>Chem-ID 1800-17) and batch #0494763 (NFL Chem-ID 1800-17B).</li>
</ul>
Chiron http://shop.chiron.no/main.aspx?page=article&artno=C11355.20-10MG&gid=&gidlevel=&pid= ) (NFL Chem-ID: (1786-17)<br>
GC-MS: test sample was spiked by three reference materials at very low concentrations. Single peak was observed. MS spectra of test and spiked samples was in agreement by spectra scanned on reference materials.<br>
1. FTIR-ATR: direct measurement (the correlations between test sample and references were below the acceptance limits. Additionally, also the FTIR spectra from different vendors were not in good agreement. Furthermore FTIR spectra from two batches of Cayman`s were not in agreement as well, but both were in agreement with data reported in the certificate of particular batch.<br><br>
Table1: FTIR correlation (cosine function correlation coefficients) – test sample (direct measurement – sample as received) vs reference material
<table>
<tr><td>CHEM-ID</td><td>1786-16 (Chiron)</td><td>1800-17 (Cayman – batch # 0472185-14)</td><td>1800-17 B (Cayman – batch # #0494763)</td></tr>
<tr><td>1668-16 (test sample)</td><td>0,83</td><td>0,67</td><td>0,82</td></tr>
</table>
2. FTIR –ATR: dried test sample extracted from butyl acetate<br>
Table 2: FTIR correlation (cosine function correlation coefficients) – test sample vs reference materials . FTIR-ATR is sensitive to crystalinic forms and polimorfic modifications, therefore we implemented GC-FTIR condensed phase anaysis which is free of those effects in the next step.
<table>
<tr><td>CHEM-ID</td><td>1786-16 (Chiron)</td><td>1800-17 (Cayman – batch # 0472185-14)</td><td>1800-17 B (Cayman – batch # #0494763)</td></tr>
<tr><td>1668-16 (test sample)</td><td>0,90</td><td>0,63</td><td>0,99</td></tr>
</table>
3. FTIR (condensed phase): all spectra (test sample and reference materials listed above were in very good agreement (correlation coefficients >0.98). This fact combined with other analitical findings confirmed the identity of the tested compound as 5F-3,5-AB-PFUPPYCA.
</td>
</tr>
<tr>
<td>other</td>
<td></td>
<td>Declared purities of reference materials were > 98%.</td>
</tr>
</table>

## ANALYTICAL RESULTS

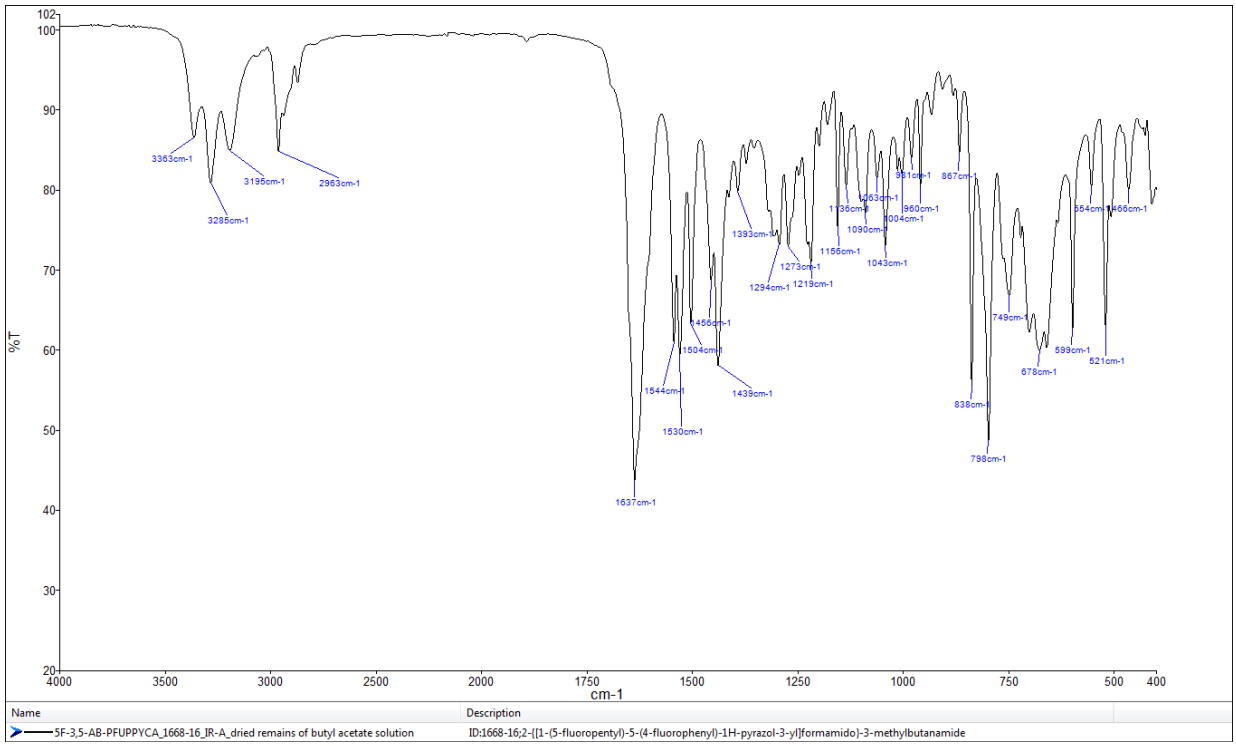
MS spectrum



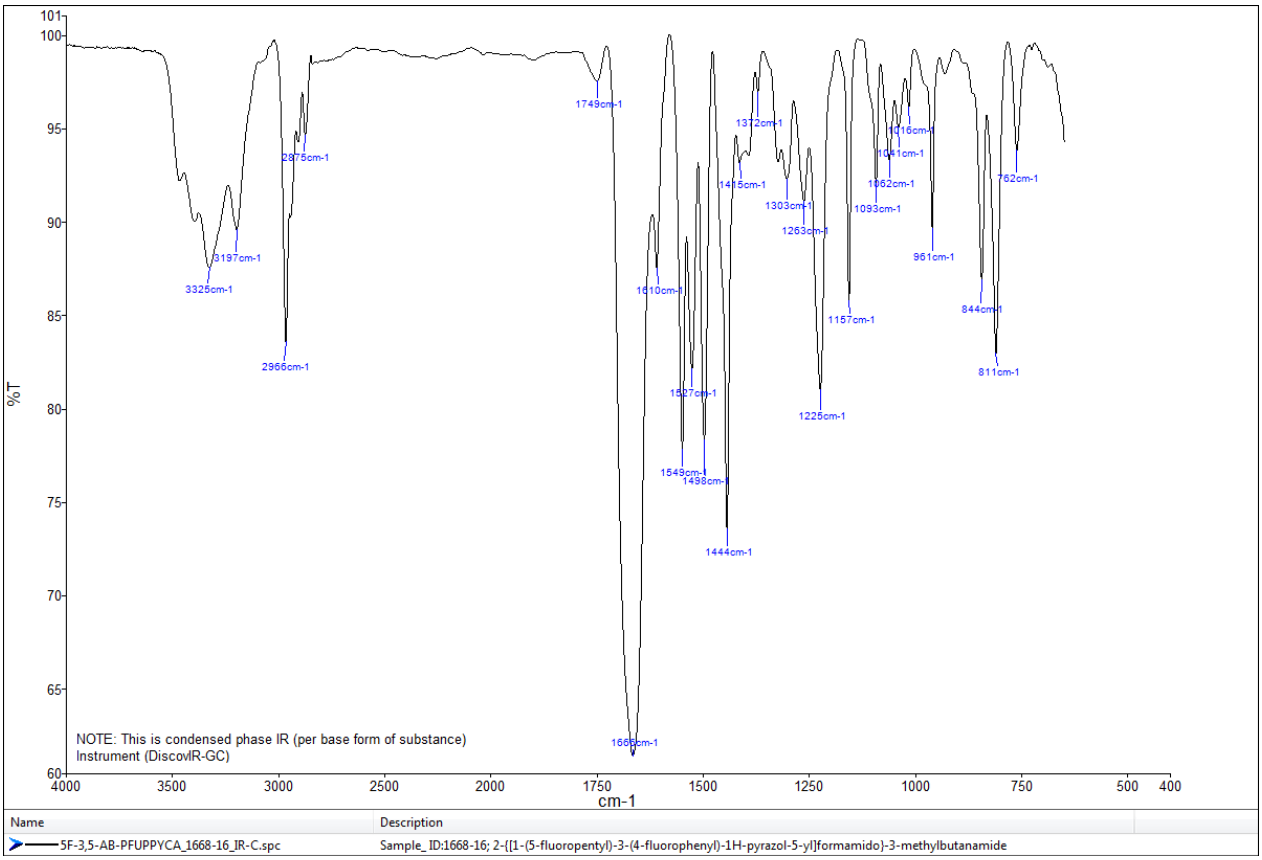
FTIR-ATR - direct measurement (sample as received)




FTIR-ATR - dried powder of test sample after the extraction from alkaline solution into butyl acetate



IR (condensed phase – after chromatographic separation)



# TOF REPORT

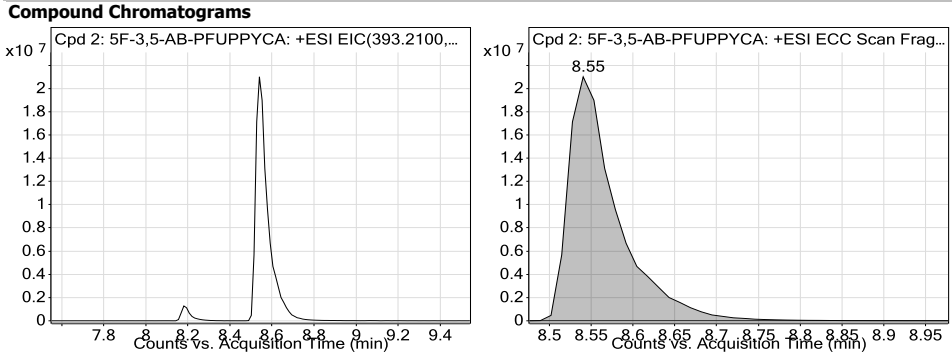
| | | | |
|---|---|---|---|
| **Data File** | 5F-3_5-AB-PFUPPYCA_1668-16_TOF.d | **Sample Name** | ID_1668-16 |
| **Sample Type** | Sample | **Position** | P1-D5 |
| **Instrument Name** | 6230B TOF LC-MS | **User Name** | TG |
| **Acq Method** | general-24_08_2016-XDB-C18-ESI-poz-soft.m | **Acquired Time** | 9/26/2016 12:29:01 PM |
| **IRM Calibration Status** | Success | **DA Method** | Drugs_NFL.m |
| **Comment** | extract in MeOH | | |

**Compound Table**

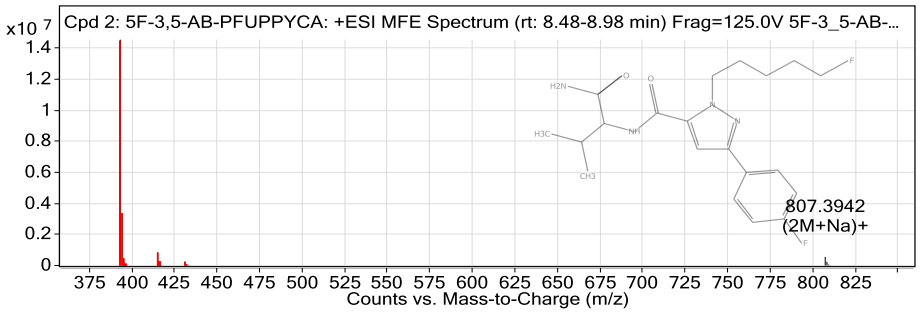
| Label | Compound Name | MFG Formula | Obs. RT | Obs. Mass |
|---|---|---|---|---|
| Cpd 2: 5F-3,5-AB-PFUPPYCA | 5F-3,5-AB-PFUPPYCA | C20 H26 F2 N4 O2 | 8.55 | 392.2023 |

| Name | *Obs. m/z* | Obs. RT | Obs. Mass | DB RT | DB Formula | DB Mass | DB Mass Error (ppm) |
|---|---|---|---|---|---|---|---|
| **5F-3,5-AB-PFUPPYCA** | 393.2095 | 8.55 | 392.2023 | 8.55 | C20 H26 F2 N4 O2 | 392.2024 | 0.24 |

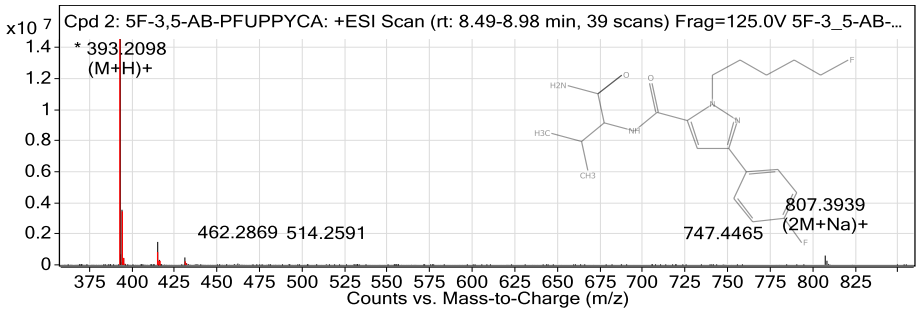
**Compound Chromatograms**




MFE MS Zoomed Spectrum




MS Zoomed Spectrum




**MS Spectrum Peak List**

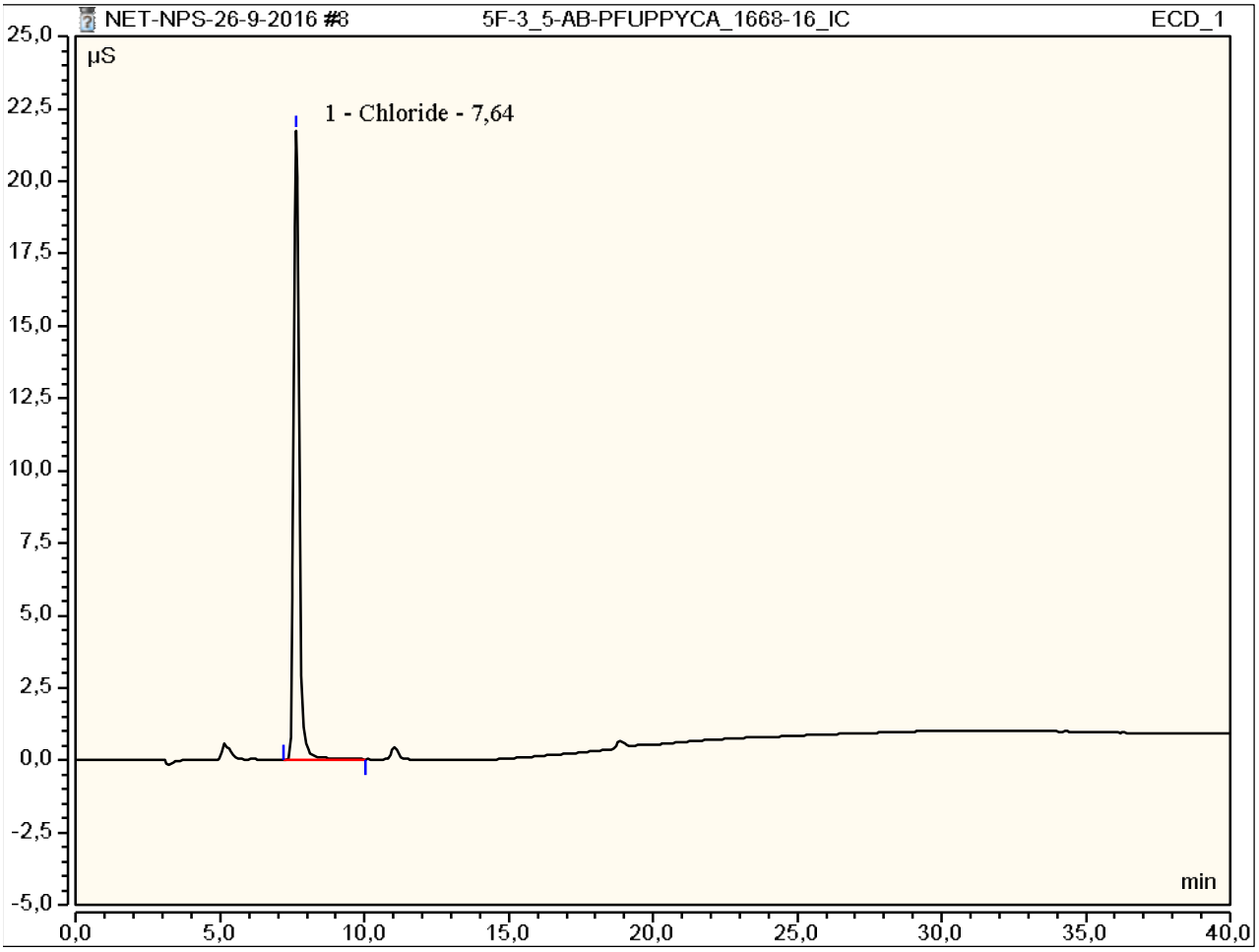
| *Obs. m/z* | Charge | Abund | Formula | Ion/Isotope |
|---|---|---|---|---|
| 393.2095 | 1 | 14548641 | C20 H26 F2 N4 O2 | (M+H)+ |
| 394.2129 | 1 | 3372866.95 | C20 H26 F2 N4 O2 | (M+H)+ |
| 395.216 | 1 | 382932.04 | C20 H26 F2 N4 O2 | (M+H)+ |
| 415.192 | 1 | 837285.06 | C20 H26 F2 N4 O2 | (M+Na)+ |
| 416.1947 | 1 | 181416.02 | C20 H26 F2 N4 O2 | (M+Na)+ |
| 431.1656 | 1 | 260034.2 | C20 H26 F2 N4 O2 | (M+K)+ |
| 432.1683 | 1 | 55826.2 | C20 H26 F2 N4 O2 | (M+K)+ |
| 807.3942 | 1 | 550406.31 | | (2M+Na)+ |
| 808.397 | 1 | 244032.14 | | (2M+Na)+ |
| 809.3994 | 1 | 55794.99 | | (2M+Na)+ |

--- End Of Report ---

# Peak Integration Report

| | | | |
|---|---|---|---|
| *Sample Name:* | **5F-3_5-AB-PFUPPYCA_1668-16_IC** | *Inj. Vol.:* | **25,00** |
| *Injection Type:* | **Unknown** | *Dilution Factor:* | **1,0000** |
| *Program:* | **ANIONI** | *Operator:* | **kemija** |
| *Inj. Date / Time:* | **27-sep-2016 / 09:29** | *Run Time:* | **42,00** |

| No. | Time min | Peak Name | Peak Type | Area µS*min | Height µS | Amount mg/L |
|---|---|---|---|---|---|---|
| 1,00 | 7,64 | **Chloride** | BMB | 4,62 | 21,73 | n.a. |
| | | **TOTAL:** | | 4,62 | 21,73 | 0,00 |



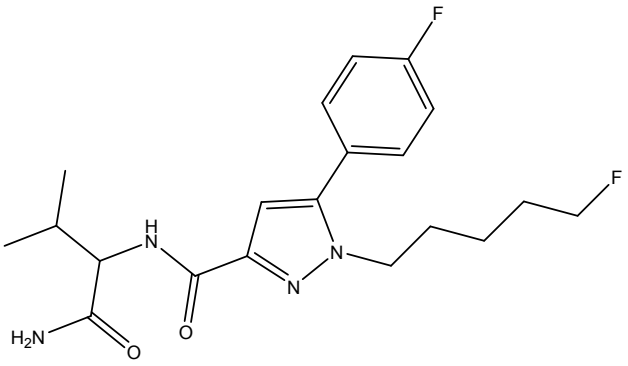
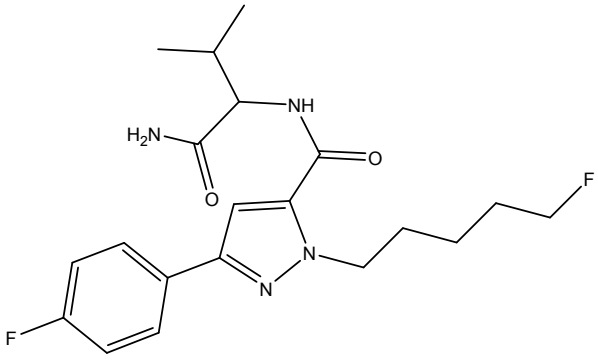
University
*of Ljubljana*

Faculty *of Chemistry and Chemical Technology*

*Večna pot 113*
*P. O. Box 537*
*SI-1001 Ljubljana*
*Slovenia*
*Phone: +386 1 479 8558*
*janez.kosmrlj@fkkt.uni-lj.si*

Co-funded by the Prevention of and Fight against Crime Programme of the European Union

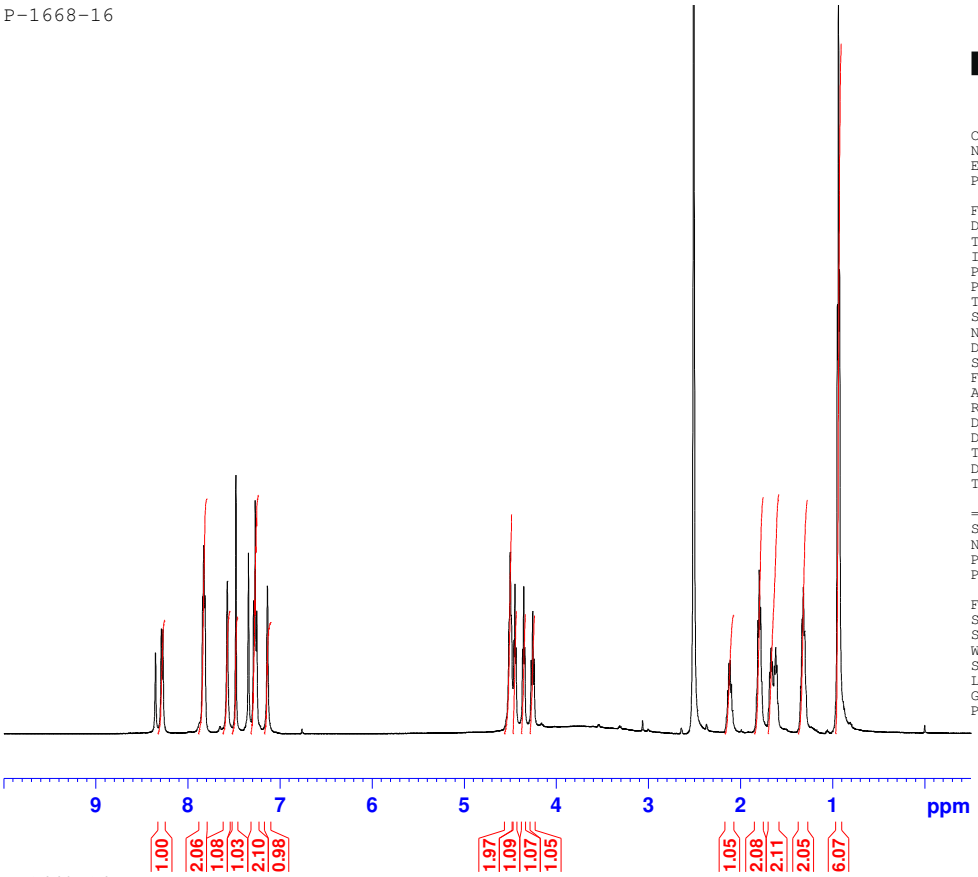
# REPORT

| | |
|---|---|
| Sample ID: | **1668-16** |
| Our notebook code: | P-1668-16 |
| NMR sample preparation: | 15 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, $^1$H–$^1$H *gs*-NOESY |
| Proposed structure: |  <br>*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1 *H*-pyrazole-3-carboxamide<br>  <br>*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide |

*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 publication are the sole responsibility of the Author and can in no way be taken to reflect the views of the European Commission.*

| | |
|---|---|
| Chemical name: | *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1*H*-pyrazole-3-carboxamide (structure A)<br><br>*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide (structure B) |
| Comments: | - Structure elucidation based on 1D and 2D NMR spectra<br><br>- The sample is not pure as evident by NMR; it contains a nitrogen-containing impurity in an appreciable amount ($^{1}$H NMR signals at 8.35 and 7.34 ppm and $^{13}$C NMR signals at 135.2 and 121.0 ppm).<br><br>- The sample contains just one of both possible isomers (either A or B). However, to distinguish between them on the basis of NMR spectra was not possible. Even the results of the NOESY spectrum are inconclusive as there are no cross-peaks for the correlations between the aromatic protons (of the 4-fluorophenyl group) and the $CH_2$ groups of the fluoropentyl group observed (if such cross-peaks would be observed, this would represent some support for the structure A). However, the absence of this signals is not a sufficient proof for the structure B.<br><br>- NMR prediction tools (ChemBioDraw Ultra) provide the following $^{13}$C NMR estimations:<br><br>structure A: for pyrazole carbon 3 (where amide is bound): 140.1 ppm and pyrazole carbon 5 (where fluorophenyl is bound): 143.1 ppm.<br><br>structure B: for pyrazole carbon 5 (where amide is bound): 132.0 ppm and pyrazole carbon 3 (where fluorophenyl is bound): 150.2 ppm.<br><br>Experimentally observed values are 136.99 and 147.90 ppm - somewhat nearer those estimated for the sturcture B. But such evidence is not sufficient. Additionally, other NMR prediction tools (for example NMR predictor on www.nmrdb.org) provide somewhat different estimates (142; 153 ppm for A and 137; 149 ppm for B), though still hinting to the structure B.<br><br>- To solve this conundrum it would be necessary to have either:<br><br>(a) authentic samples of both isomers and compare their NMR spectra with the one observed for this sample or<br><br>(b) obtain a single-crystal X-ray diffraction analysis. |
| Supporting information: | Copies of $^{1}$H and $^{13}$C NMR spectra |
| Author: | Prof. Dr. Janez Košmrlj, Doc. Dr. Krištof Kranjc |
| Date of report: | November 29, 2016 |

P-1668-16



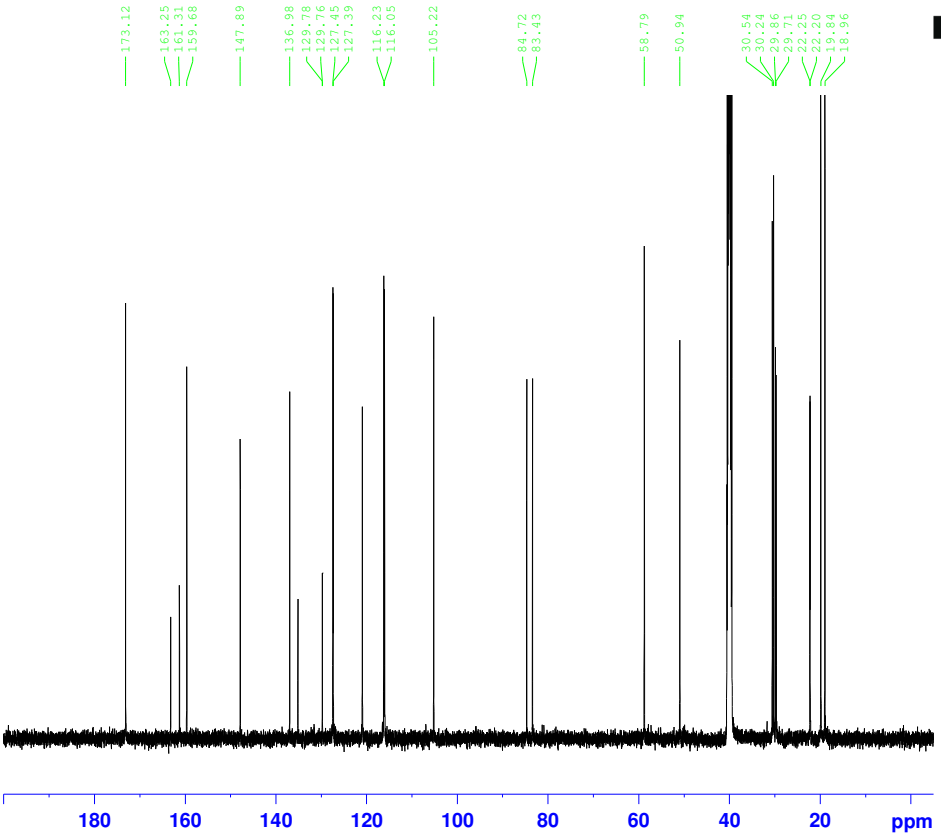

```
Current Data Parameters
NAME                  P-1668-16
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20161125
Time                      16.50
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                   zg30
TD                       65536
SOLVENT                   DMSO
NS                          16
DS                           2
SWH                  10000.000 Hz
FIDRES                0.152588 Hz
AQ                    3.2768500 sec
RG                         71.8
DW                       50.000 usec
DE                        6.50 usec
TE                       298.0 K
D1                    1.00000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                500.1330885 MHz
NUC1                        1H
P1                        8.60 usec
PLW1                26.00000000 W

F2 - Processing parameters
SI                       65536
SF                  500.1300008 MHz
WDW                         EM
SSB                  0
LB                        0.30 Hz
GB                   0
PC                        1.00
```

P-1668-16




```
Current Data Parameters
NAME                  P-1668-16
EXPNO                         3
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20161125
Time                      18.23
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                   DMSO
NS                         5120
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                       298.0 K
D1                    1.00000000 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                  125.7577885 MHz
WDW                         EM
SSB                  0
LB                        1.00 Hz
GB                   0
PC                        1.40
```