

# Test report

Number

Colorless liquid

Object of analysis: **Colorless liquid**

Customer: Samples: 1

Batch: Arrived on: 2020/09/21

Subject: Identification, other ingredients, density measurement, quantification

Method(s): NMR (nuclear magnetic resonance spectroscopy)  
qNMR (quantitative NMR)

Instrumentation: Bruker Avance III 700 MHz NMR spectrometer  
Mettler Toledo ME204 analytical balance  
Hamilton 1 mL gastight syringe, model 1001 TLL

Sample / Analysis	Method	Status	Result	Value
<b><u>Colorless liquid:</u></b>				
Identification	NMR	N	Etizolam [40054-69-1]	Identified
Other ingredients	NMR	N	Ethanol [64-17-5] Water [7732-18-5]	Identified
Density	Mass divided by volume	N	$\rho_{oil} = \frac{0.6815 \text{ g}}{0.84 \text{ mL}} = 0.81 \frac{\text{g}}{\text{mL}}$	$0.81 \frac{\text{g}}{\text{mL}}$
Quantification	qNMR	N	<b>Component</b> Etizolam Ethanol Water	<b>Mass%</b> 0.6% 90.7% 7.0% <b>mg/mL</b> 5.0 mg/mL 736 mg/mL 57 mg/mL

Method status: G = GMP A = accredited V = generally validated P = validated on product N = not validated  
E = external lab

Remarks: This liquid was etizolam solution in ethanol (5.0 mg/mL).

The uncertainty of volume measurement using Hamilton 1 mL syringe was  $\pm 0.01$  mL.

Signatures:

Date:

2020/09/22

## Quantitative analysis of the colorless liquid in a glass vial

0.1665 g of the colorless liquid and 0.0540 g of 1,4-dimethoxybenzene (99+% purity) were directly weighed into an NMR tube, and 0.6 mL of acetone-d<sub>6</sub> was added. Shaked at room temperature, giving a homogeneous solution.

For the <sup>1</sup>H qNMR experiment, 128 scans were acquired, recycling delay was set to 30 seconds, 90° pulse was used.

Compound [CAS number]	Molar mass	Weight taken	Chemical shift of chosen <sup>1</sup> H NMR signal	Integral of that <sup>1</sup> H NMR signal	Number of H's under that signal	Content in the oil (mass%)	Sum (mass%)	Content in the oil (mg/mL)
Ethanol [64-17-5]	46.07 g/mol	0.1665 g	1.15 ppm	2538.1817	3	90.7%	98.3%	735.7 mg/mL
Etizolam [40054-69-1]	342.85 g/mol		2.67 ppm	2.2970	3	0.6%		5.0 mg/mL
Water [7732-18-5]	18.02 g/mol		3.68 ppm	333.6279	2	7.0%		56.7 mg/mL
1,4-Dimethoxybenzene [150-78-7]	138.17 g/mol	0.0540 g	6.84 ppm	400.0001	4	99.0%		

\* This liquid was etizolam solution in ethanol (5.0 mg/mL).

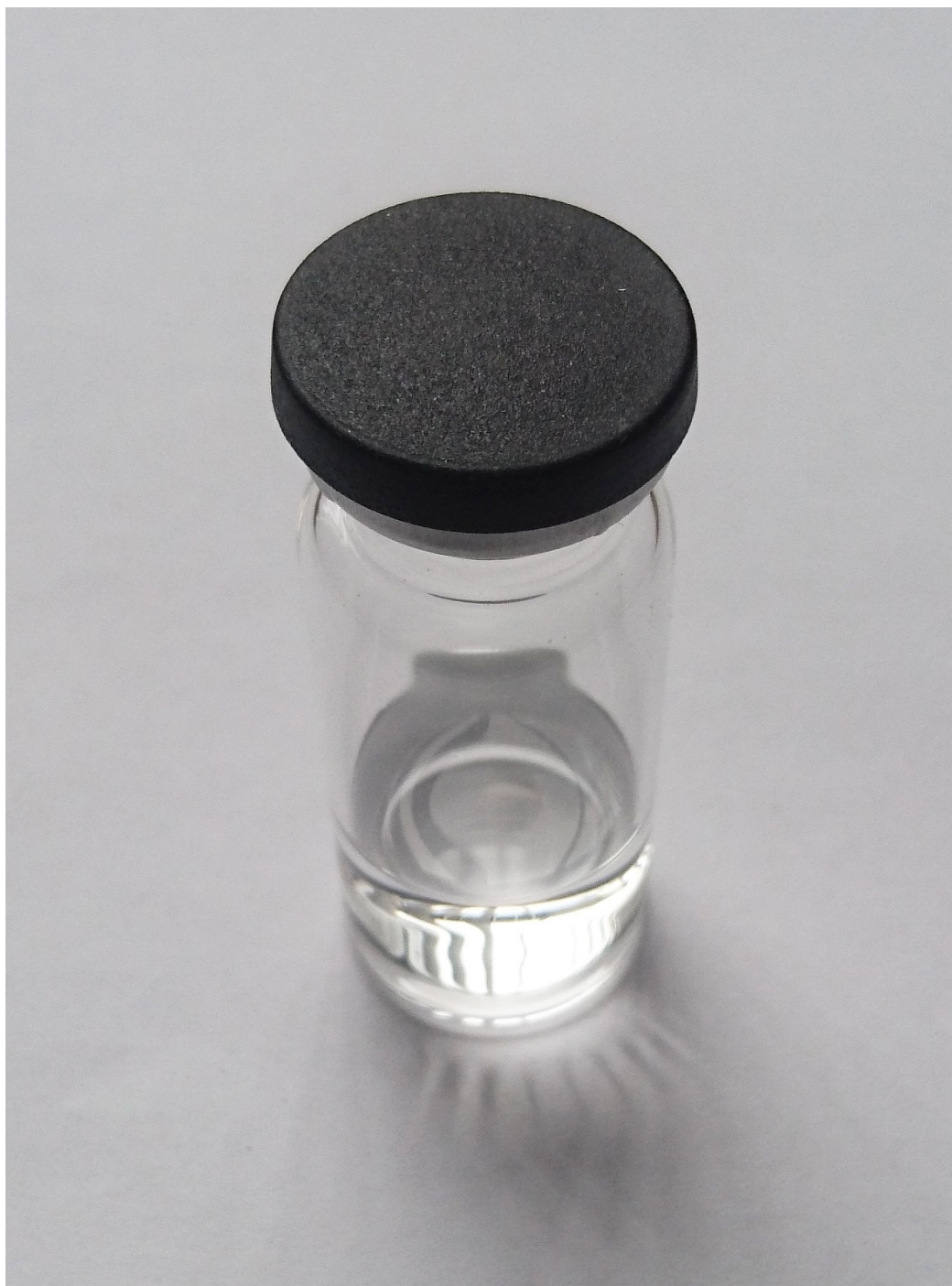
### Reference for the calculation method:

Rundlöf, T.; Mathiasson, M.; Bekiroglu, S.; Hakkarainen, B.; Bowden, T.; Arvidsson, T. Survey and qualification of internal standards for quantification by <sup>1</sup>H NMR spectroscopy, *J. Pharm. Biomed. Anal.* 2010, 52, 5, 645-651. <https://doi.org/10.1016/j.jpba.2010.02.007>

## Photos of the sample

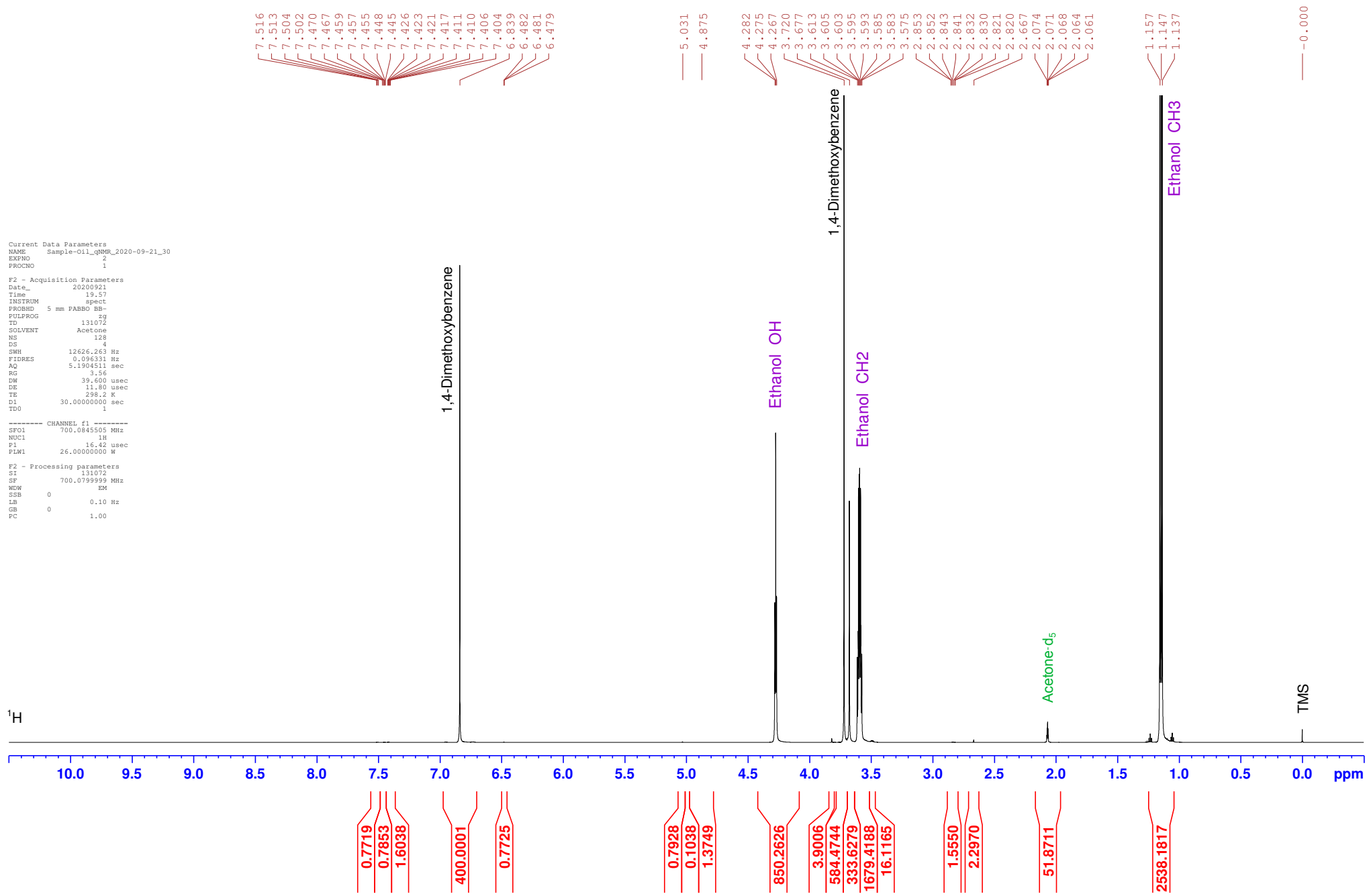
Colorless liquid

arrived 21/09/2020



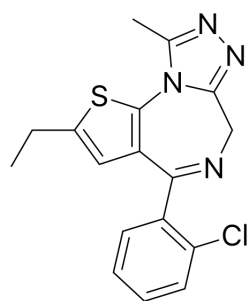
# qNMR

Sample: Colorless liquid (0.1665 g) + 1,4-dimethoxybenzene (0.0540 g, internal standard, 99+% purity) in acetone-d<sub>6</sub>



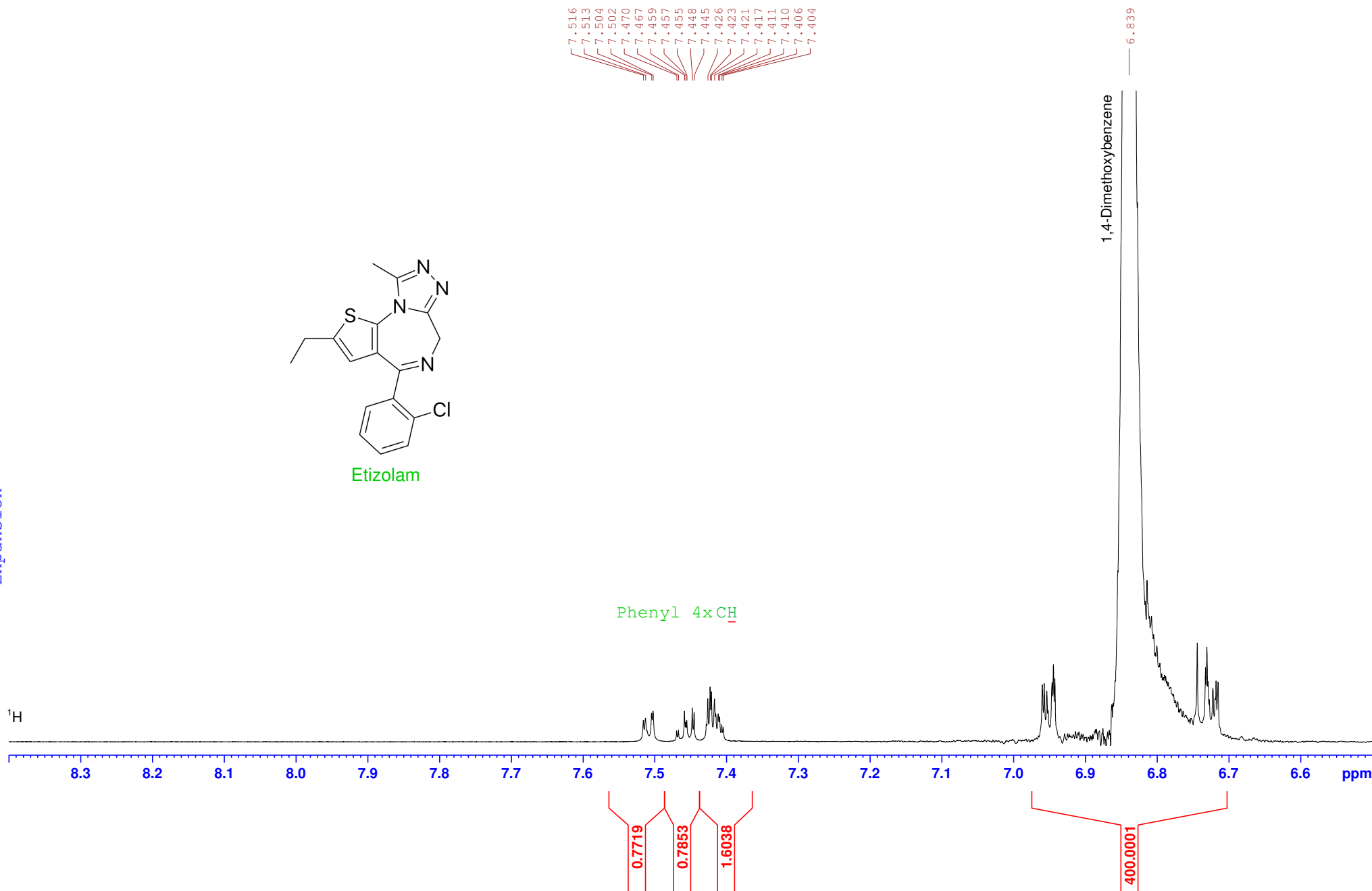
# qNMR

Sample: Colorless liquid (0.1665 g) + 1,4-dimethoxybenzene (0.0540 g, internal standard, 99+% purity) in acetone-d6



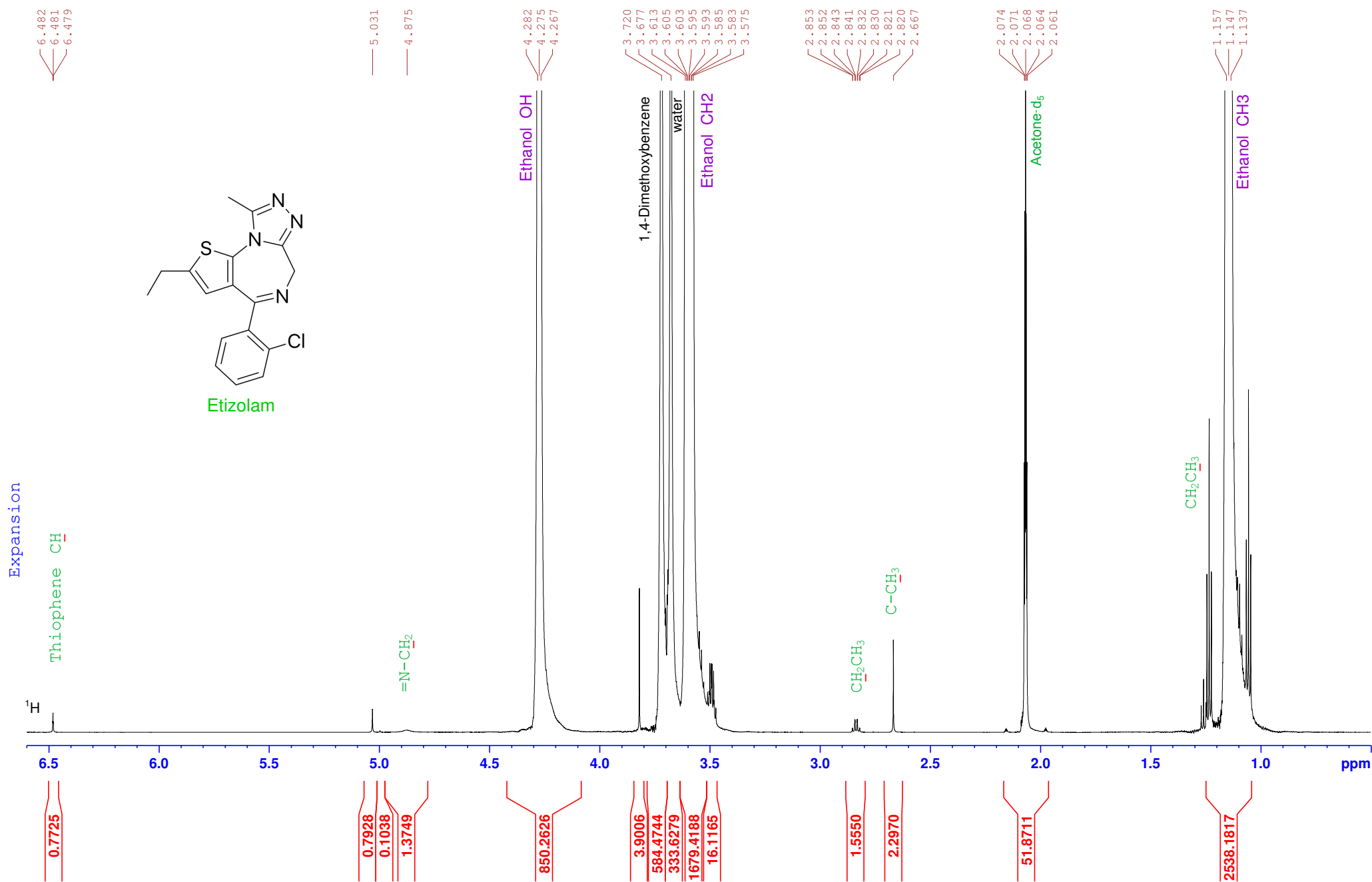
Etizolam

Expansion



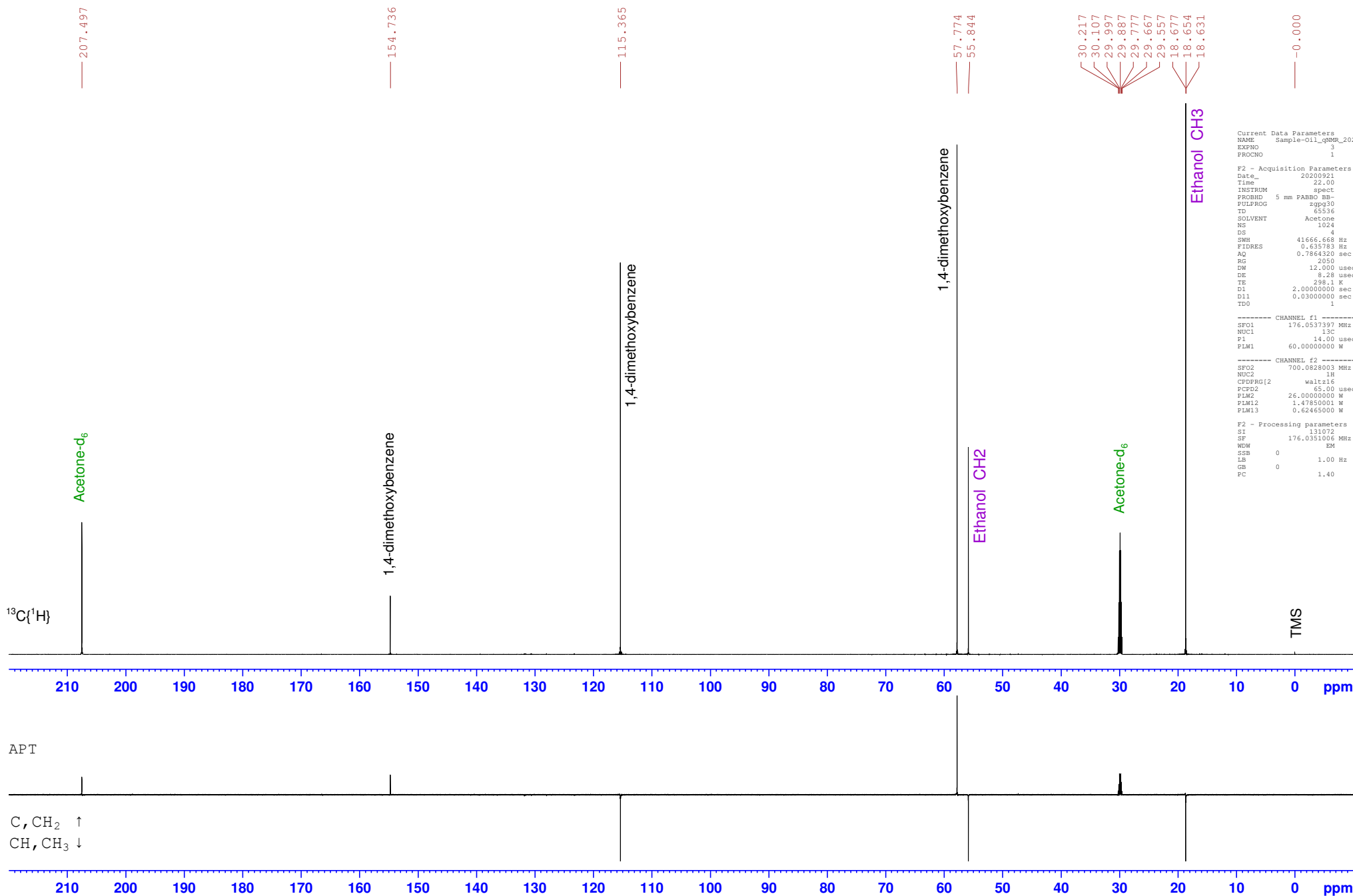
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