Synthetic and Analytical Challenges of Retigabine and N-Acetyl Retigabine

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Analytical Reference Standards
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Background – Retigabine

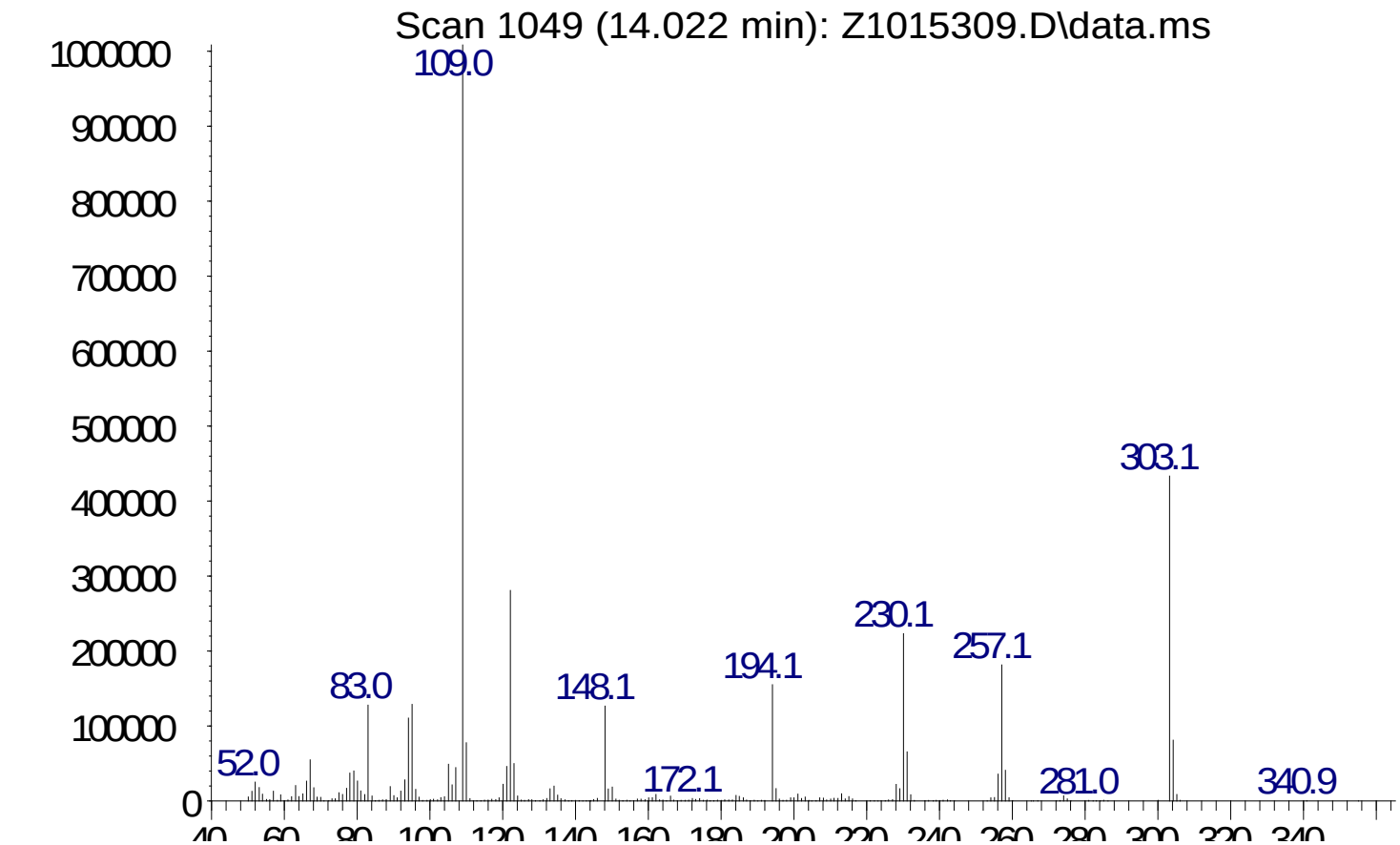
- Prescribed as an anticonvulsant marketed under the trade names Potiga© and Trobalt©
- Monitored in clinical and forensic applications due to side-effects associated with treatment and its DEA status (Schedule V)
- Certified Reference Materials (CRMs) for use in production of calibrators & controls were needed including: retigabine, an internal standard (IS) and the major metabolite *N*-acetyl retigabine
- A summary of the synthetic design, process, purification and analysis are described including the challenges encountered

Production of Retigabine CRM

- Retigabine was isolated from Potiga© tablets
- Purification provided material at acceptable purity for CRM production
  - Lesson learned: material sensitive to oxygen, light, acid & heat
- Analysis of the parent provides insights into the synthetic design for the IS
  - What is the optimum location for labeling based on the mass spec data?

Determining optimum location for label

GC/MS Analysis of Retigabine



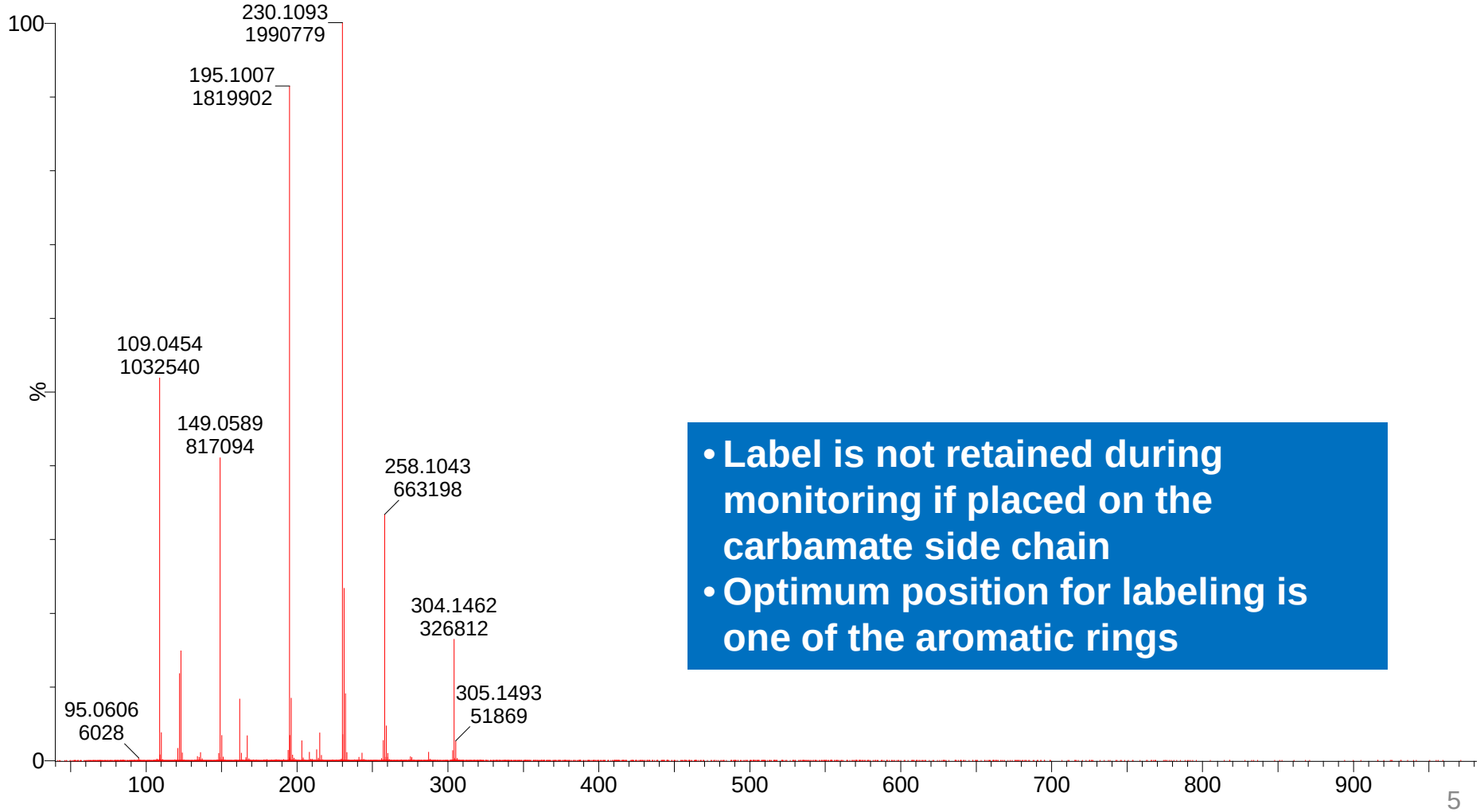
Determining optimum location for label

QTOF MS/MS analysis of Retigabine

RMR-017_RMR-017-11C

W10221202 533 (3.164) Cm (532:535)

Retigabine Ezogabine



- **Label is not retained during monitoring if placed on the carbamate side chain**
- **Optimum position for labeling is one of the aromatic rings**

Synthesis of Retigabine-D_4 CRM

Native Distribution (%)		Isotopic Distribution (%)		
			Uncorrected values	Corrected for native distribution
		D_0	0.01	0.01
		D_1	0.28	0.30
M-2	2.25	D_2	6.59	4.64
M-1	4.16	D_3	9.36	5.56
M+1	93.60	D_4	82.80	88.47
		D_5	0.80	0.86
		D_6	0.15	0.16
		D_0/D_4		0.013%

(Isotopic distribution values are adjusted for the natural abundance of isotopes e.g.^{13}C, ^{15}N…)

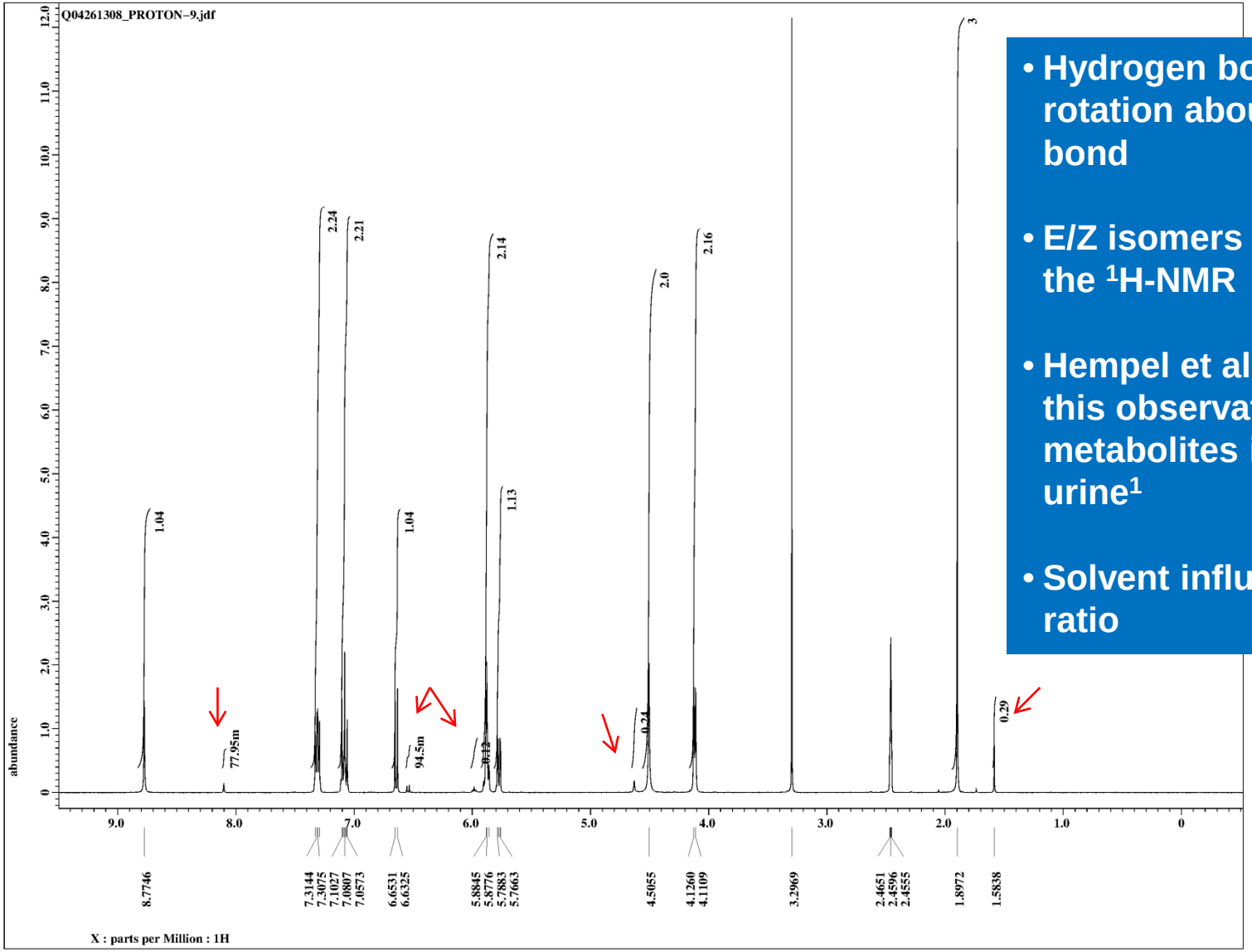
Synthesis of N-Acetyl Retigabine CRM

- Little to no literature information available
- Synthesizing the correct regio-isomer was difficult – synthetic design & conditions as well as control-point analysis critical

Regio-isomer/rearrangement complications

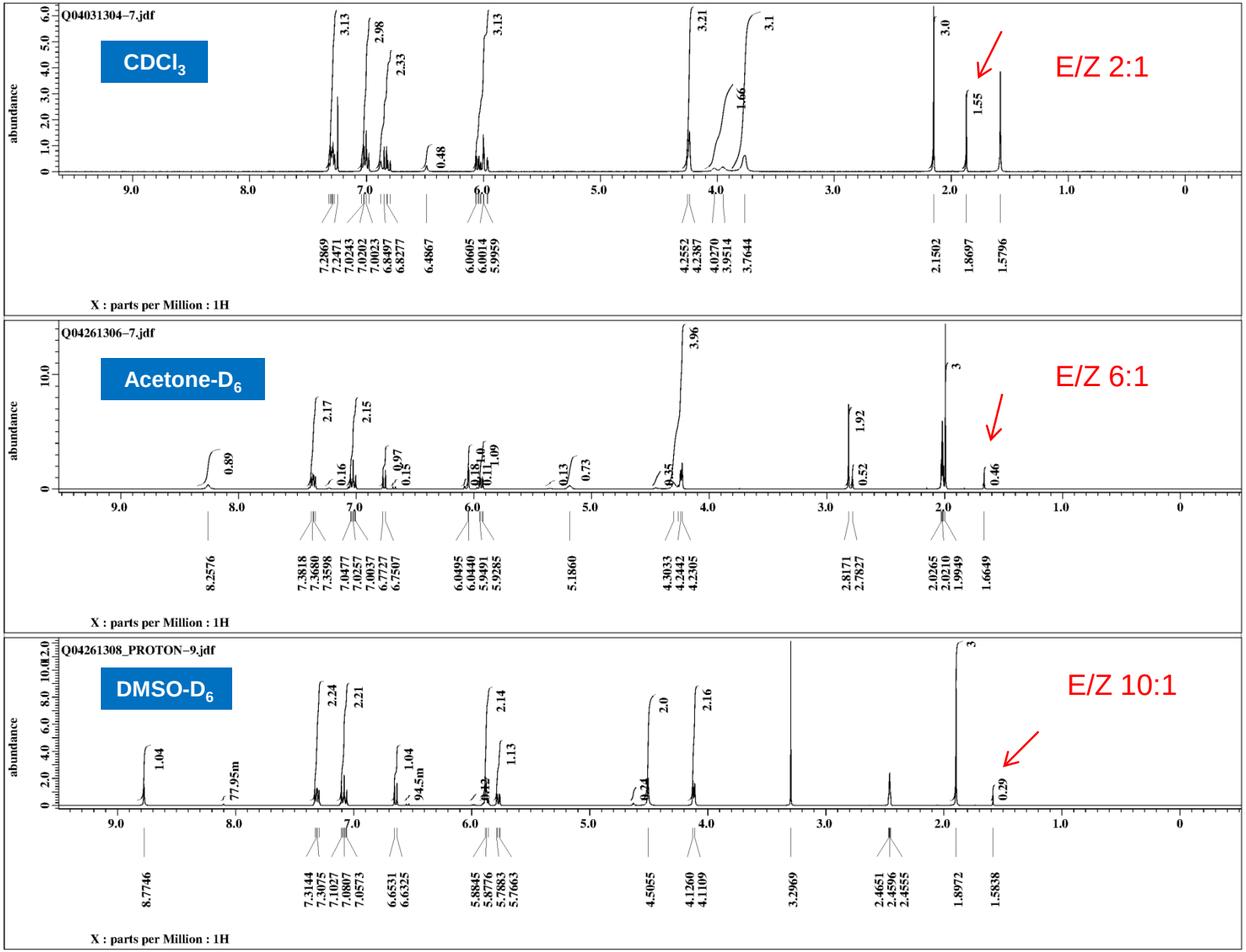
- Regio-isomers formed during the synthesis – identifiable by ^{1}H-NMR
- Possible intramolecular rearrangement; how do we detect and control it?

Possible intramolecular rearrangement:



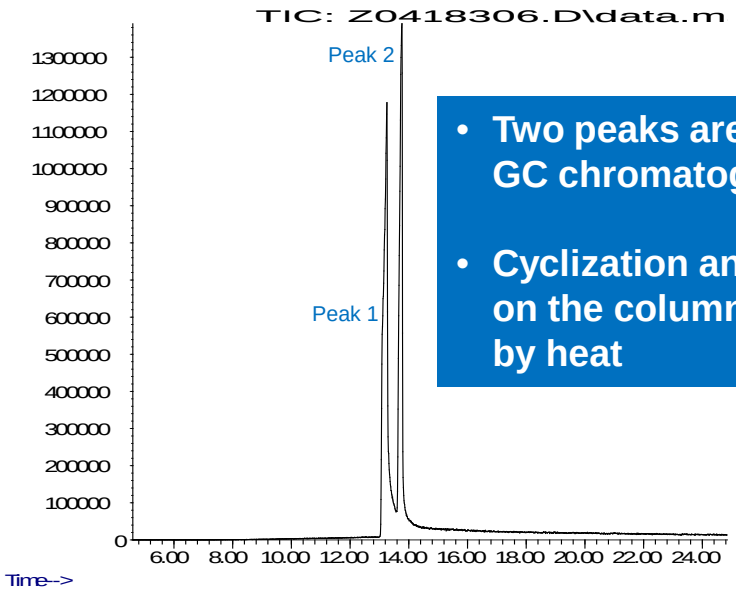
- **Hydrogen bonding hinders rotation about the amide bond**
- **E/Z isomers are visible in the ^{1}H-NMR**
- **Hempel et al. described this observation in metabolites isolated from urine[1]**
- **Solvent influences the E/Z ratio**

1) Hempel, R.; et al. Drug Metabolism and Disposition, 1999, 27, 613-622.

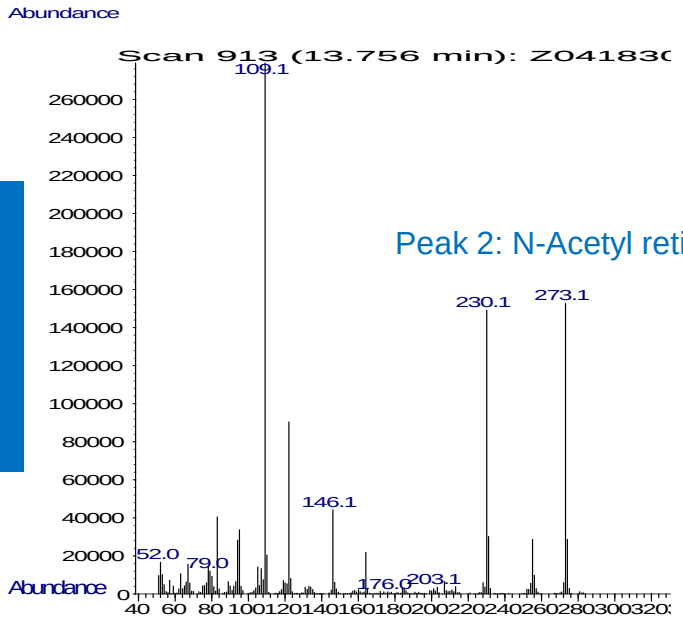

Q04031304–7.jdf
CDCl3
E/Z 2:1
abundance
X : parts per Million : 1H
Q04261306–7.jdf
Acetone-D6
E/Z 6:1
abundance
X : parts per Million : 1H
Q04261308_PROTON–9.jdf
DMSO-D6
E/Z 10:1
abundance
X : parts per Million : 1H

Analytical challenges

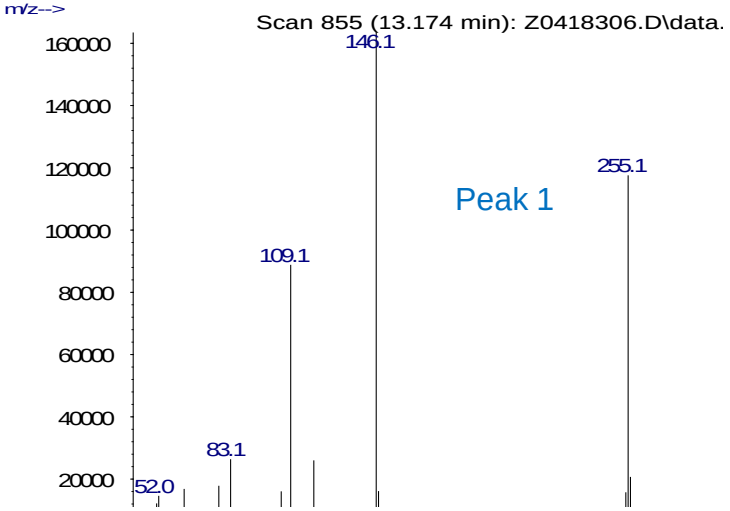
GC/MS data for N-Acetyl Retigabine



- Two peaks are seen in the GC chromatogram
- Cyclization and dehydration on the column accelerated by heat



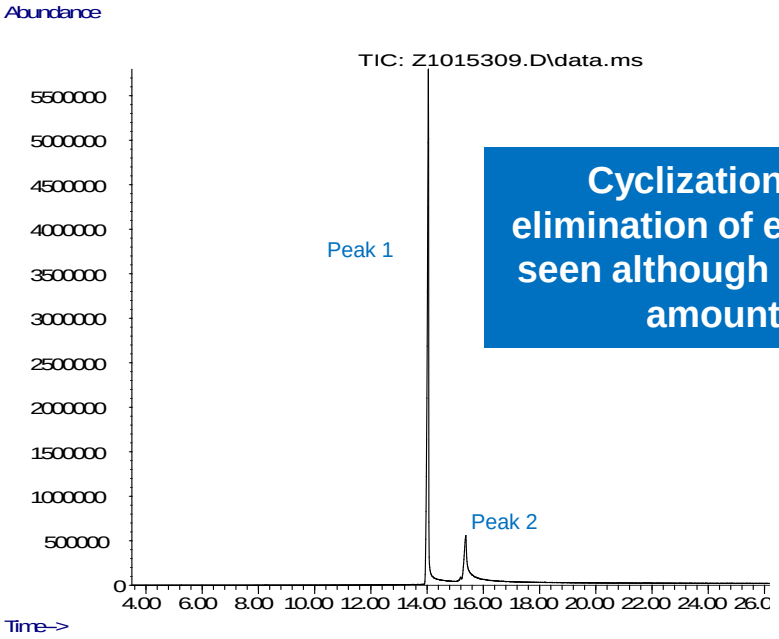
Peak 2: N-Acetyl retigabine



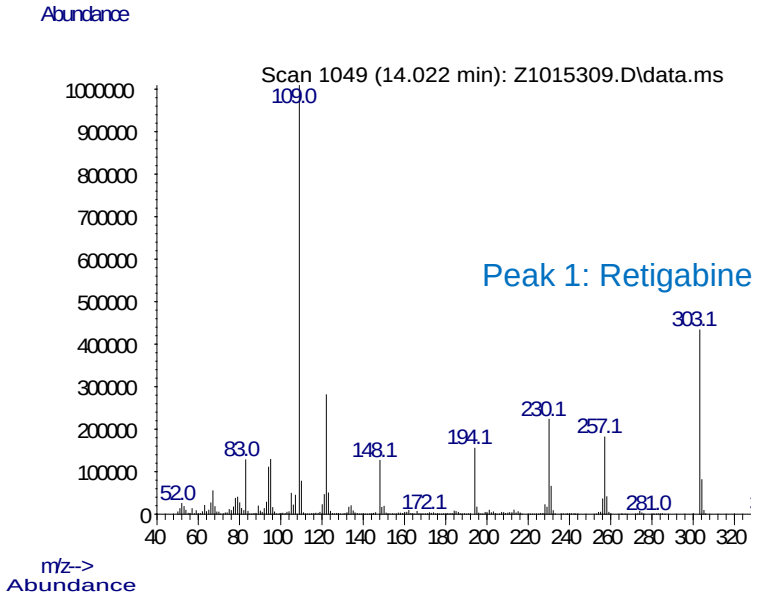
Peak 1

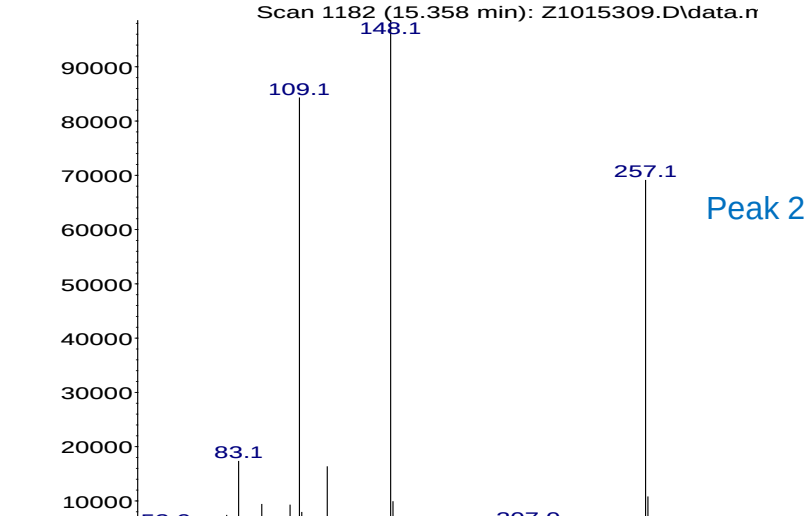
Concerns with cyclization of Retigabine

GC/MS data for Retigabine



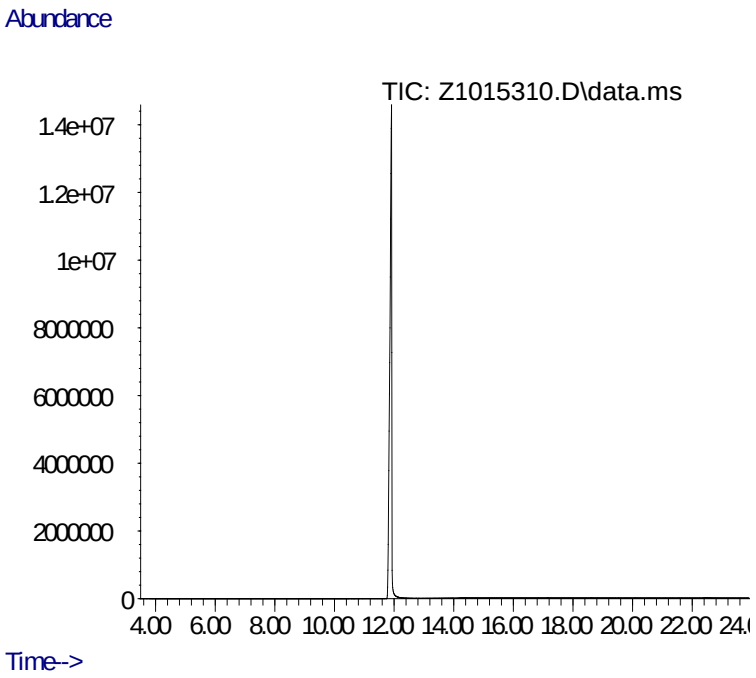
Cyclization and elimination of ethanol is seen although in lesser amounts

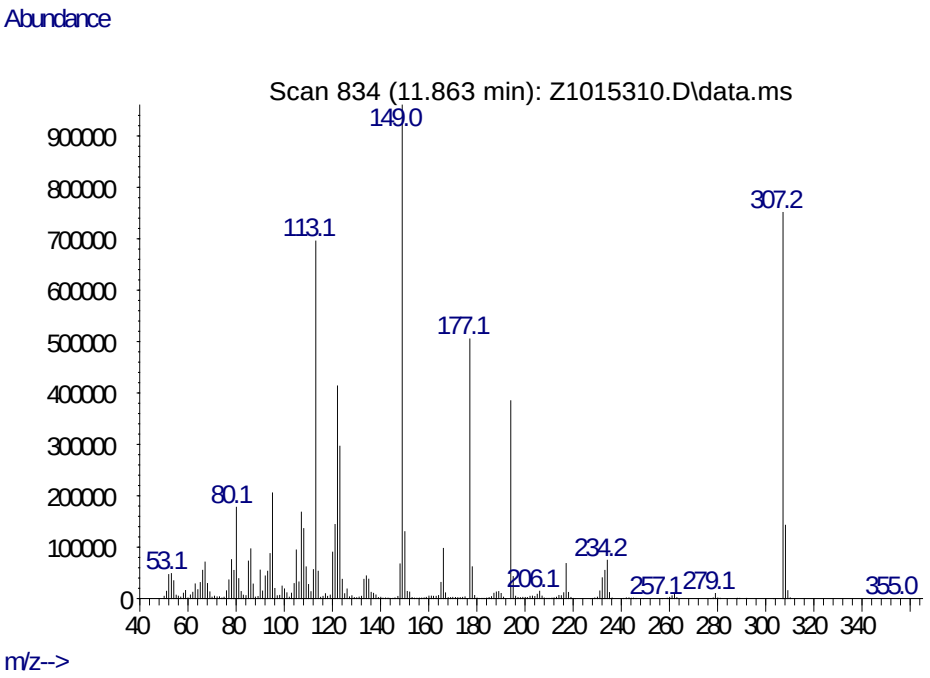




Identifying the Retigabine-D_4 regio-isomer

GC/MS Data

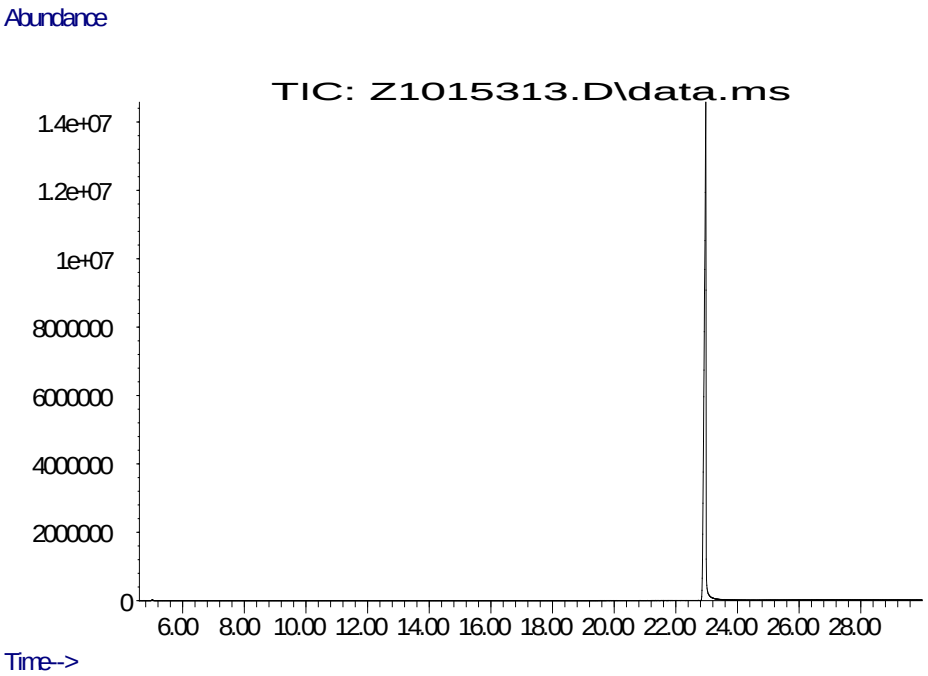


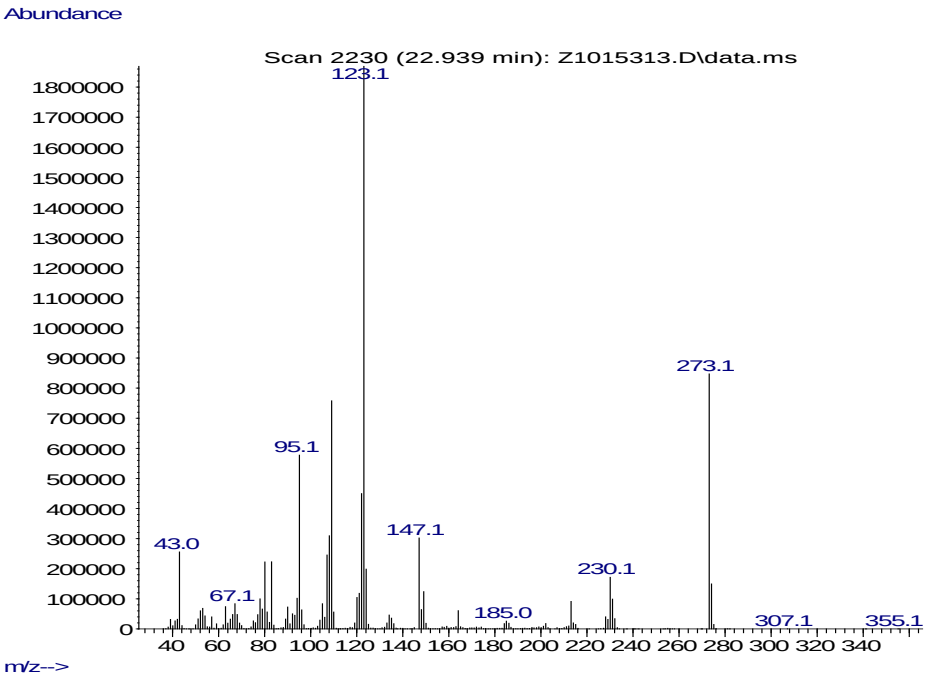


Regio-isomer exhibits different fragmentation patterns and is chromatographically different from retigabine

Identifying the N-Acetyl retigabine regio-isomer

GC/MS Data





- **Cyclization is not observed and fragmentation pattern is different**
- **The wrong regio-isomer is easily synthesized and could be encountered from commercial sources – impact on analysis must be considered**

Production of Certified Spiking Solutions® for use in manufacture of calibrators & controls

Stability concerns

Retigabine:

- Known to be oxygen and light sensitive, as well as acid and heat labile
- Steps were taken during synthesis and standard preparation to minimize exposure to air and light
- Choice of diluent was influenced by the observation that methanolic solutions turned pink – indication of degradation

N-Acetyl retigabine:

- Very little literature available – stability was unknown
- While material is similar to retigabine, the stability is slightly different
- Methanolic solutions turned pink upon sitting at room temperature in clear vials - solutions are light sensitive

Cerilliant Quality
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

Solution Stability

In acetonitrile – no degradation was observed after 1 month for either analytes

Retigabine

Solvent 1: Methanol 1 mg/mL			
t_0 = >99.9%			
	t_1 = 1 day	t_2 = 4 days	t_3 = 7 days
Freezer	>99.9 %	>99.9 %	>99.9 %
Refrigerator	>99.9 %	>99.9 %	>99.9 %
Room Temp	>99.9 %	>99.9 %	>99.9 %
40 °C	>99.9 %	>99.9 %	**>99.9 %**

Solvent 2: Acetonitrile 1 mg/mL				Accelerated Stability of standard
t_0 = >99.9 %				
	t_1 = 1 day	t_2 = 4 days	t_3 = 7 days	t_4 = 1 month
Freezer	>99.9 %	>99.9 %	>99.9 %	99.8%
Refrigerator	>99.9 %	>99.9 %	>99.9 %	99.9%
Room Temp	>99.9 %	>99.9 %	>99.9 %	99.9%
40 °C	>99.9 %	>99.9 %	>99.9 %	99.8%

N-Acetyl Retigabine

Solvent 1: Methanol 1 mg/mL			
t_0 = >99.9 %			
	t_1 = 3 days	t_2 = 5 days	t_3 = 7 days
Freezer	99.8%	99.1%	99.2%
Refrigerator	99.8%	99.8%	99.8%
Room Temp	99.8%	**99.8%**	**99.2%**
40 °C	99.8%	99.8%	99.8%

Solvent 2: Acetonitrile 1 mg/mL				Accelerated Stability of standard
t_0 = >99.9 %				
	t_1 = 3 days	t_2 = 5 days	t_3 = 7 days	t_4 = 1 month
Freezer	>99.9 %	99.6%	99.6%	99.6%
Refrigerator	>99.9 %	>99.9 %	>99.9 %	99.4%
Room Temp	>99.9 %	>99.9 %	>99.9 %	99.2%
40 °C	>99.9 %	>99.9 %	>99.9 %	98.9%

Indicates samples turned pink

Conclusions

- GC/MS and LC/MS/MS fragmentation patterns were critical in design of the internal standard – led to placing the label on the aromatic ring rather than the carbamate side-chain
- Careful analysis of spectrometric identification and analytical techniques was required to ensure the correct materials were prepared at the highest purity to provide structurally accurate CRMs
- A regio-isomer of *N*-acetyl retigabine is easily prepared and could impact analysis if incorrectly identified
- N-Acetyl retigabine prone to chemical rearrangement which could impact analytical results
- Material and solution stability influenced handling and solution standard design – materials are sensitive to light, air, acid and heat

Stable CRMs for retigabine, retigabine-D_4 and *N*-acetyl retigabine were successfully developed

Thank You!

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