

# Synthetic and Analytical Challenges of Retigabine and N-Acetyl Retigabine



**Cerilliant**<sup>®</sup>  
Analytical Reference Standards

a **SIGMA-ALDRICH**<sup>®</sup> company

Heather Lima, Uma Sreenivasan, Isil Dilek, Josh  
Cooper, Brian Dockery and Genevieve Rodgers



# Background – Retigabine

- Prescribed as an anticonvulsant marketed under the trade names Potiga<sup>®</sup> and Trobalt<sup>®</sup>
- 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

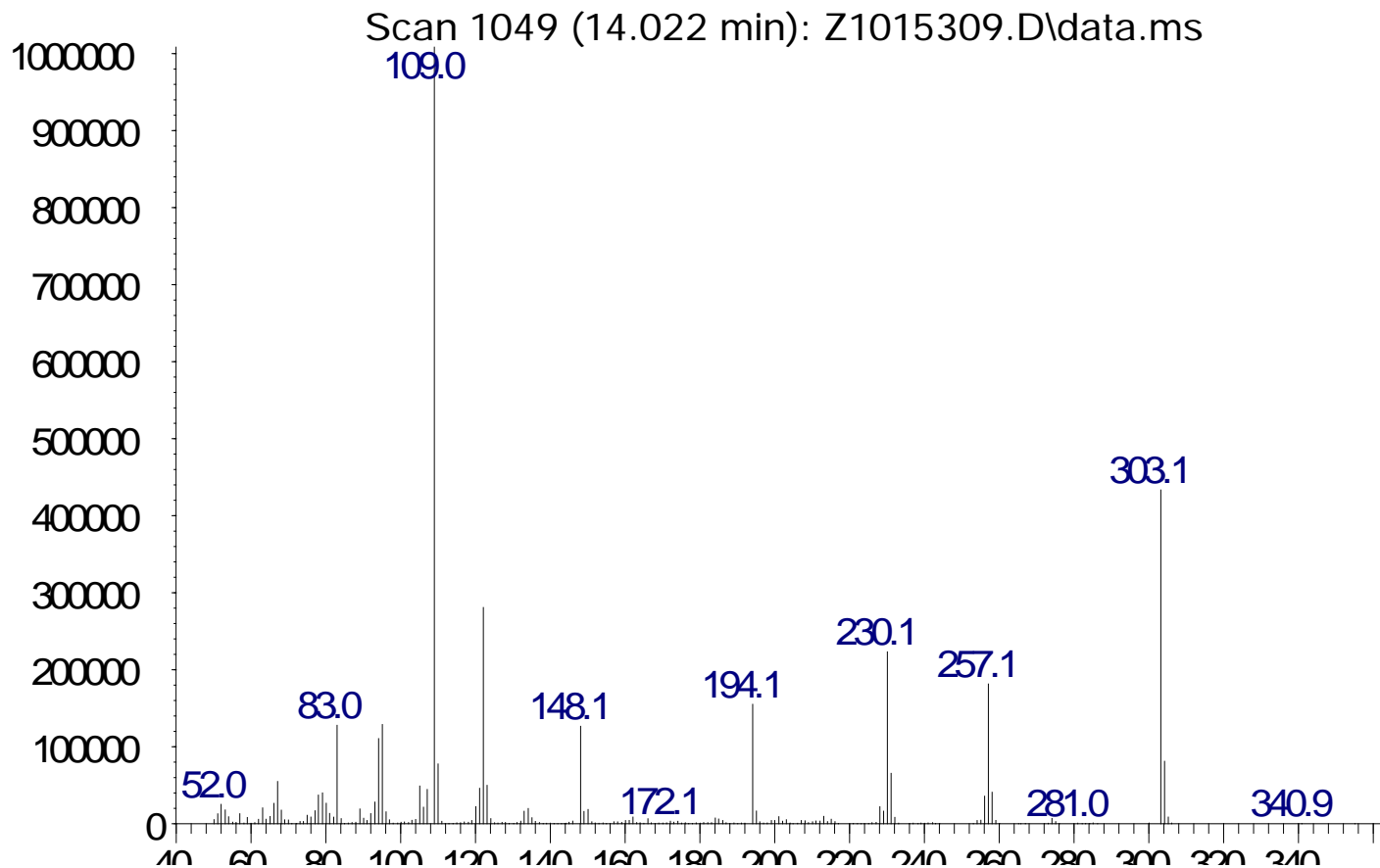
# Production of Retigabine CRM

- Retigabine was isolated from Potiga<sup>®</sup> 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

Abundance



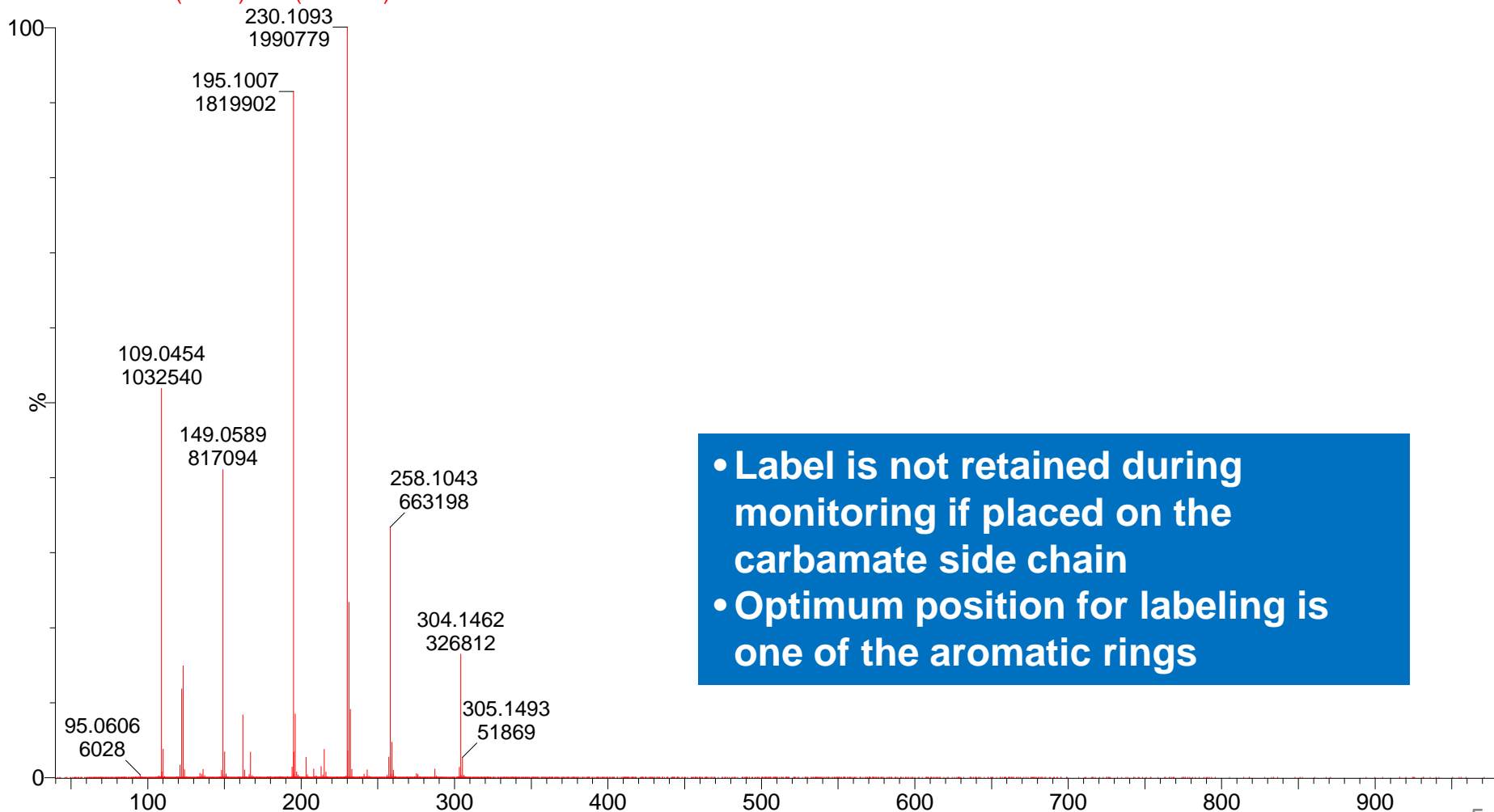
# Determining optimum location for label

## QTOF MS/MS analysis of Retigabine

RMR-017\_RMR-017-11C

Retigabine Ezogabine

W10221202 533 (3.164) Cm (532:535)



- 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<sub>4</sub> CRM

Native Distribution (%)		Isotopic Distribution (%)	
		Uncorrected values	Corrected for native distribution
		D <sub>0</sub>	0.01
		D <sub>1</sub>	0.28
M-2	2.25	D <sub>2</sub>	6.59
M-1	4.16	D <sub>3</sub>	9.36
M+1	93.60	D <sub>4</sub>	82.80
		D <sub>5</sub>	0.80
		D <sub>6</sub>	0.15
		D <sub>0</sub> /D <sub>4</sub>	0.013%

*(Isotopic distribution values are adjusted for the natural abundance of isotopes e.g. <sup>13</sup>C, <sup>15</sup>N...)*

# Synthesis of N-Acetyl Retigabine CRM

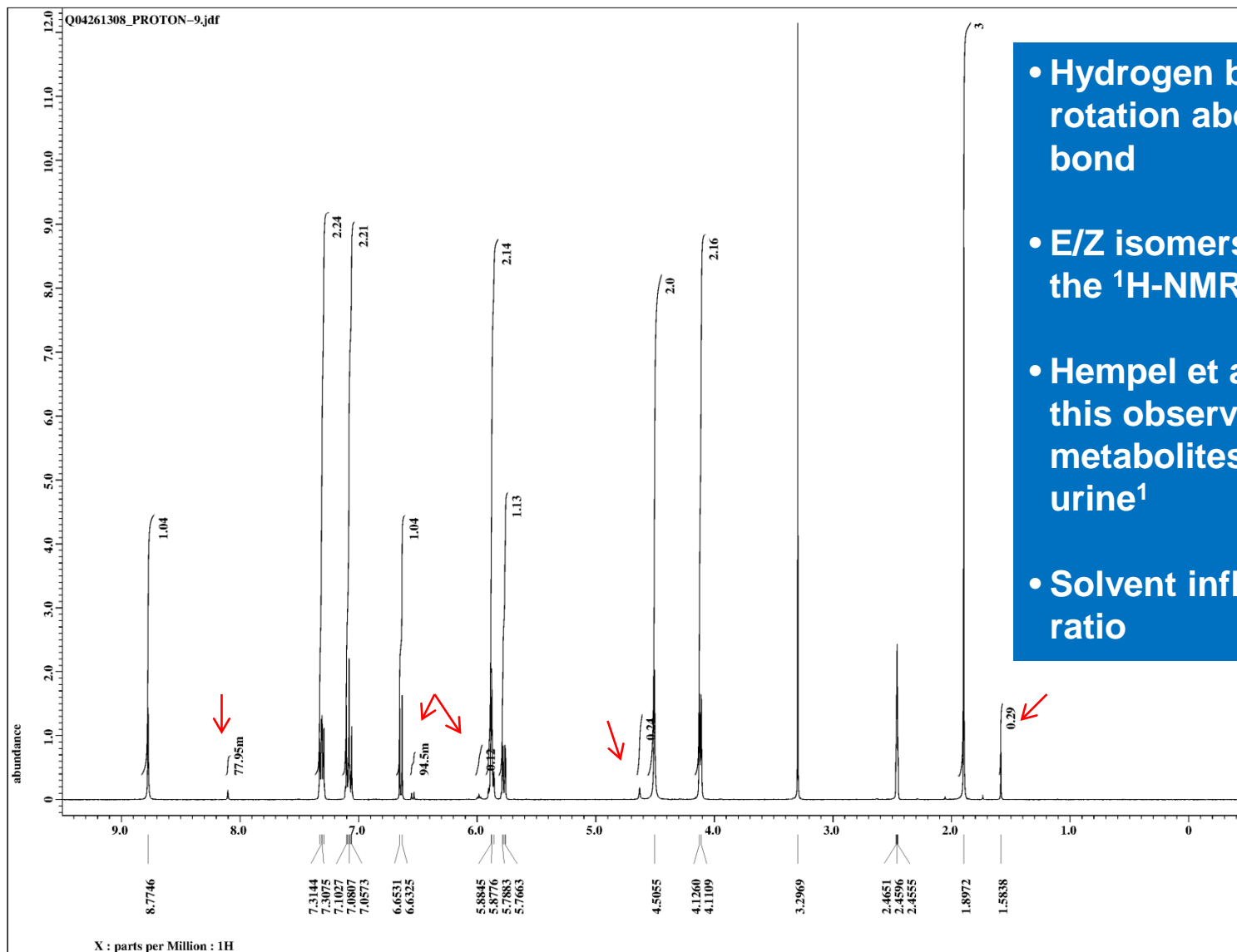
- Little to no literature information available
- Synthesizing the correct regio-isomer was difficult – synthetic design & conditions as well as control-point analysis critical

# Regio-isomer/rearrangement complications

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

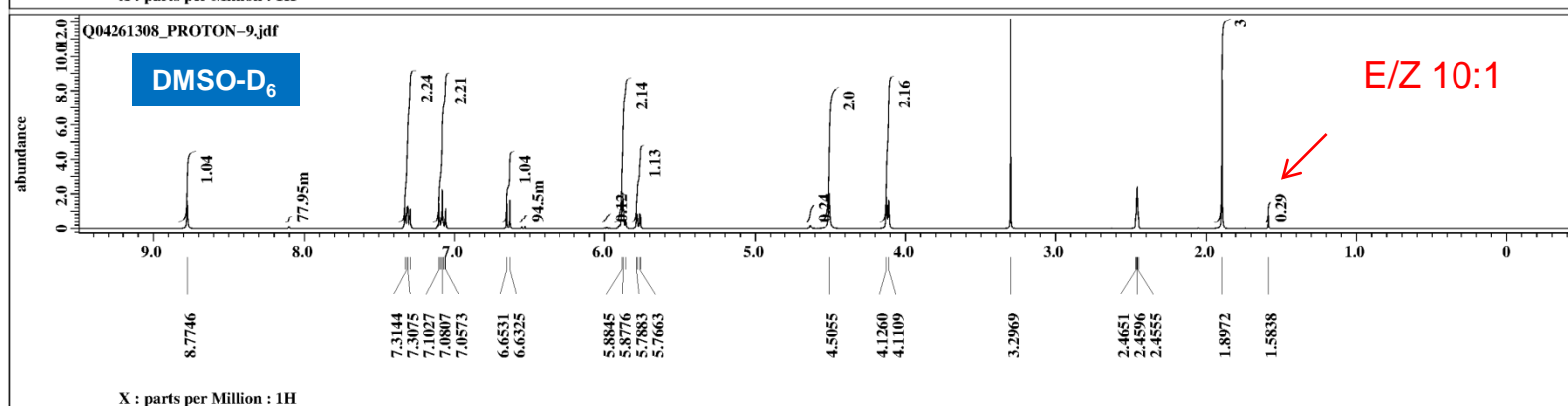
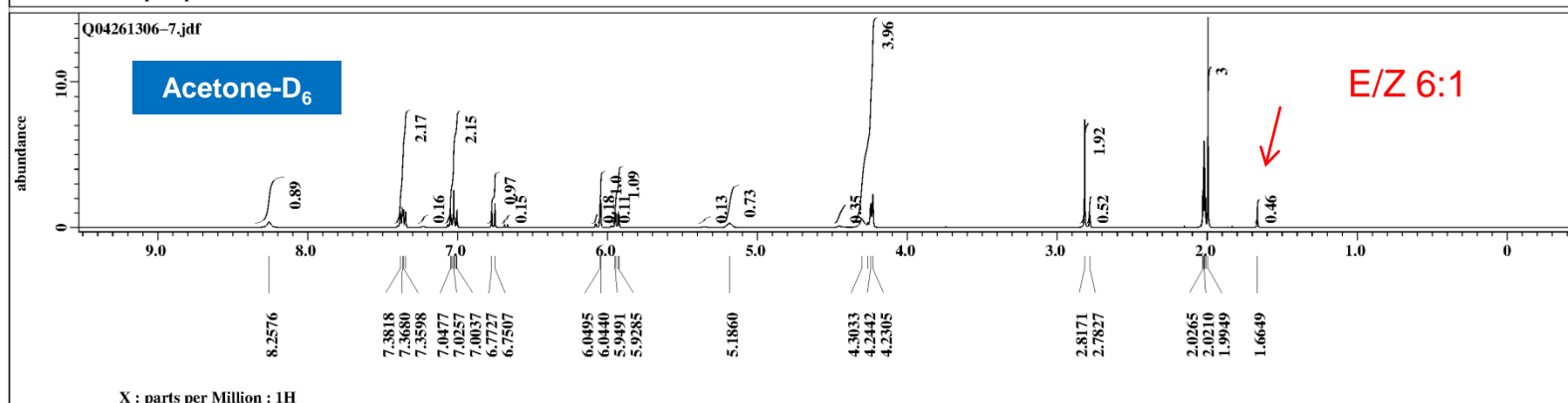
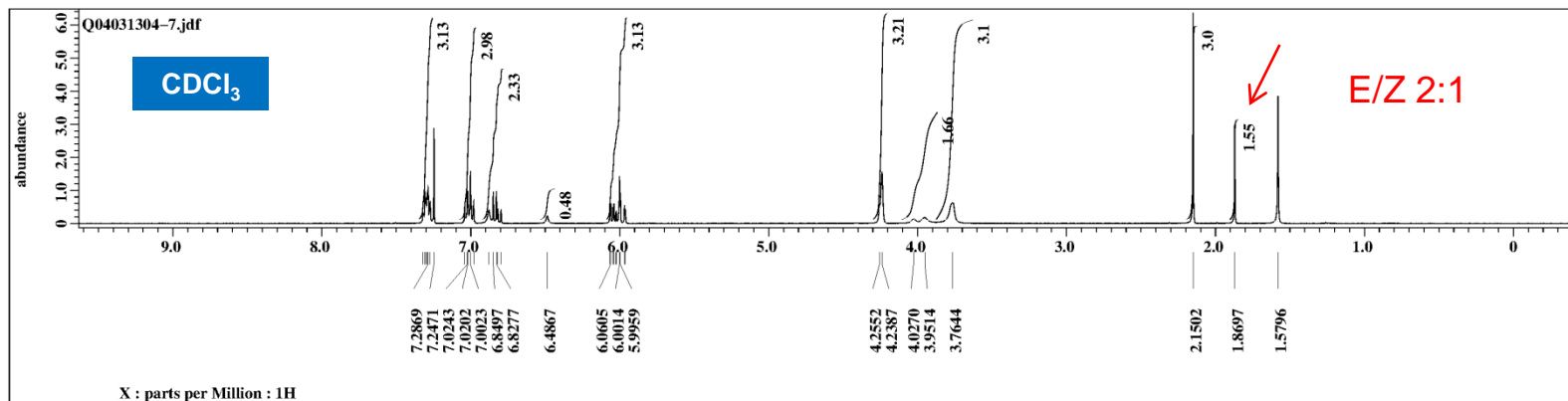
**Possible intramolecular rearrangement:**





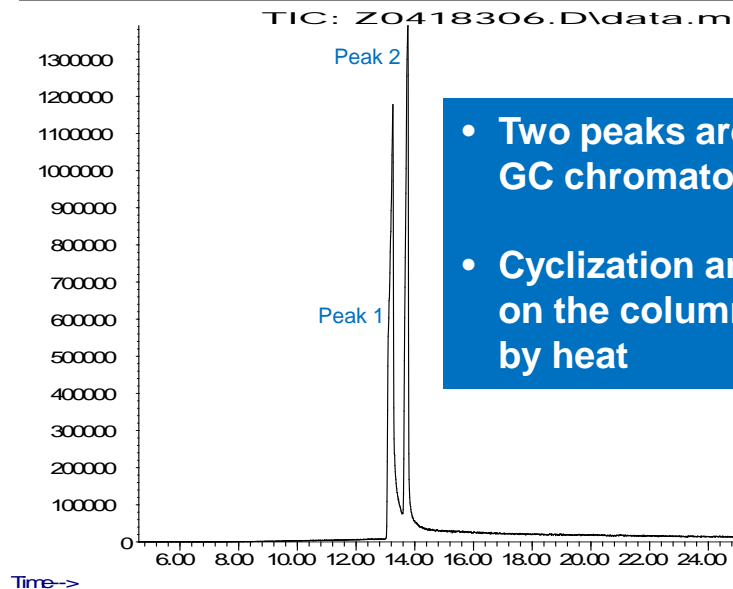
- Hydrogen bonding hinders rotation about the amide bond
- E/Z isomers are visible in the  $^1\text{H}$ -NMR
- Hempel et al. described this observation in metabolites isolated from urine<sup>1</sup>
- Solvent influences the E/Z ratio

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

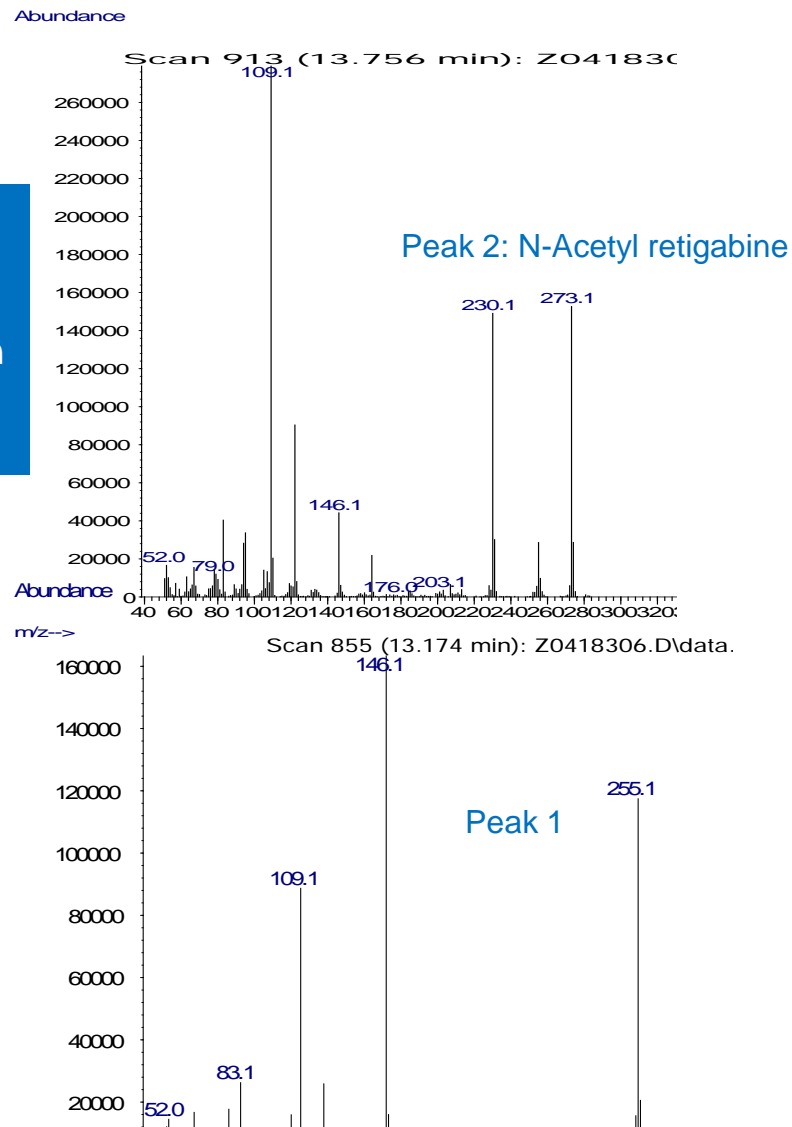


# Analytical challenges

## GC/MS data for N-Acetyl Retigabine

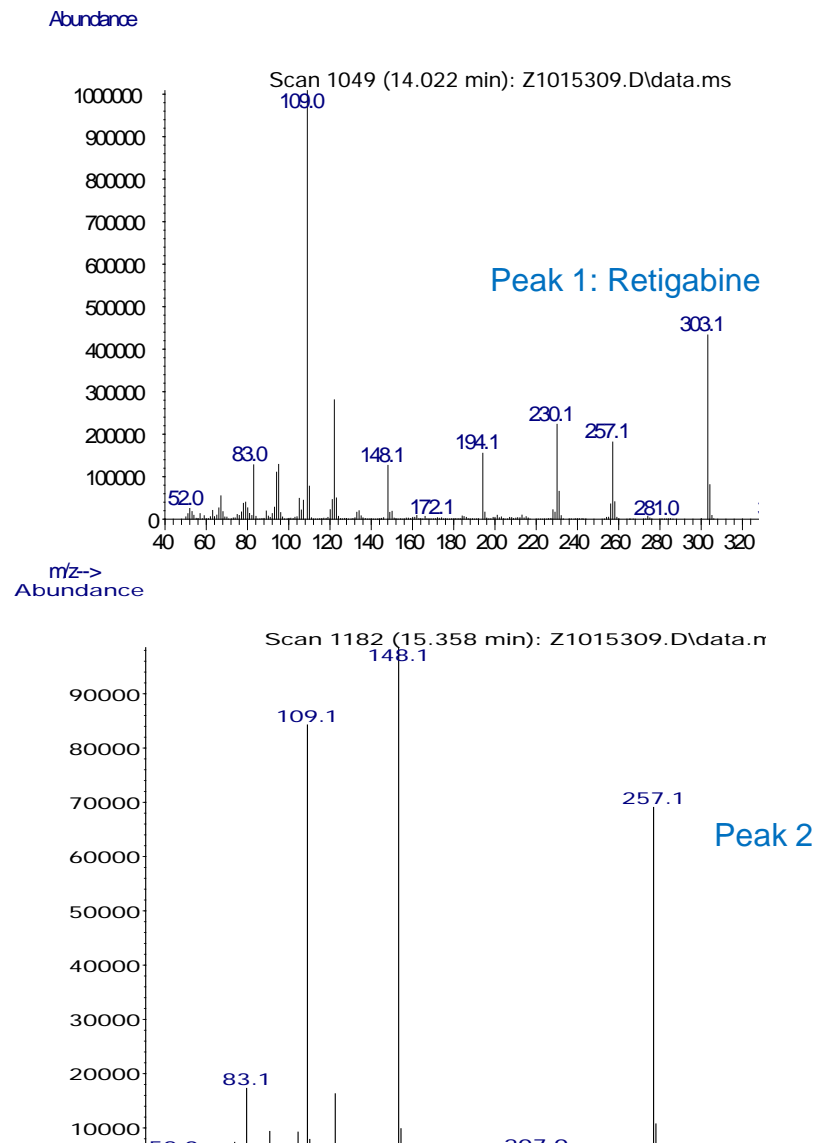
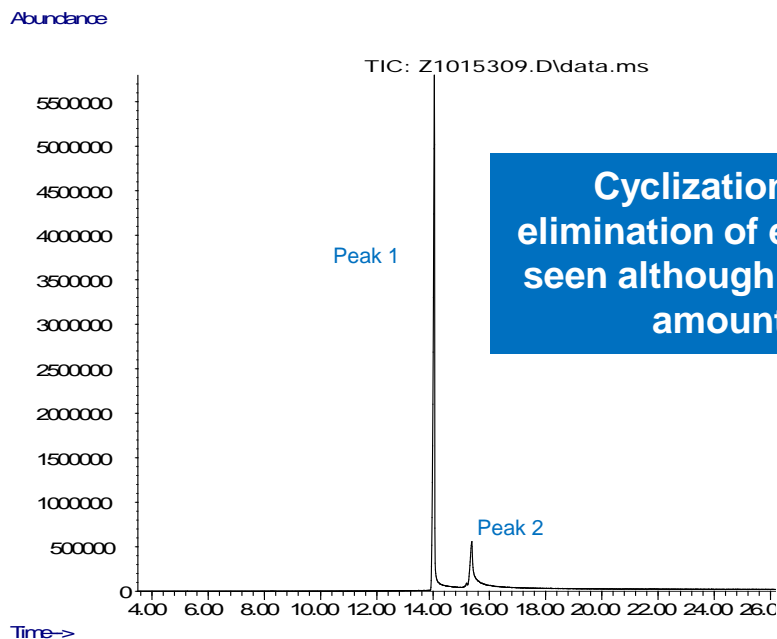


- Two peaks are seen in the GC chromatogram
- Cyclization and dehydration on the column accelerated by heat



# Concerns with cyclization of Retigabine

## GC/MS data for Retigabine

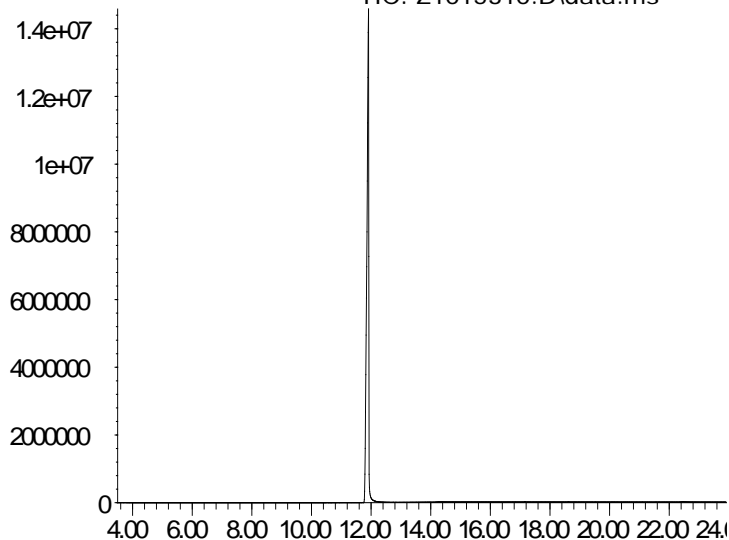


# Identifying the Retigabine-D<sub>4</sub> regio-isomer

## GC/MS Data

Abundance

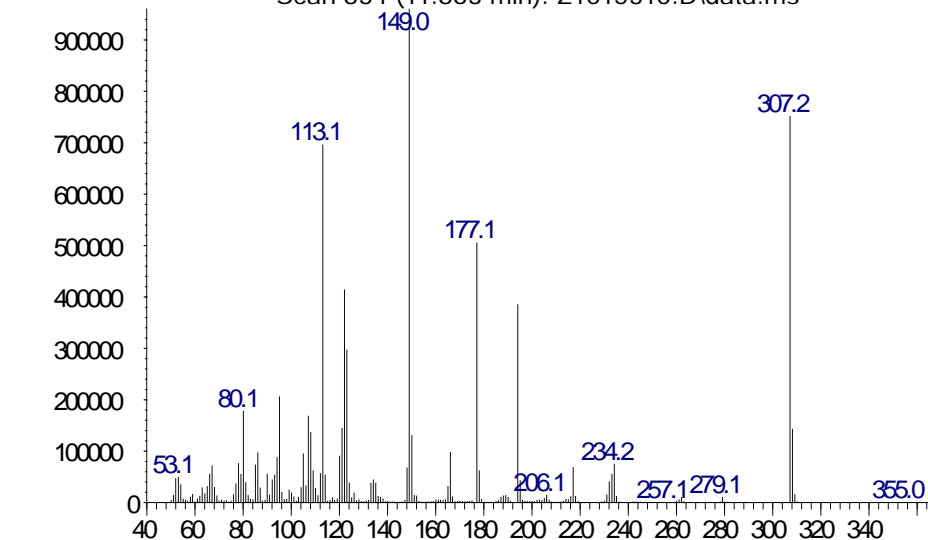
TIC: Z1015310.D\data.ms



Time-->

Abundance

Scan 834 (11.863 min): Z1015310.D\data.ms



m/z-->

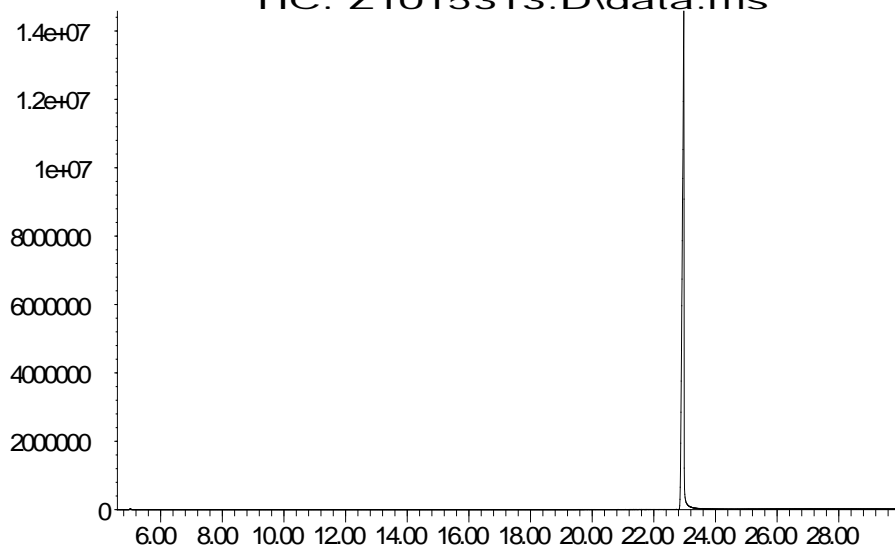
**Regio-isomer exhibits different fragmentation patterns and is chromatographically different from retigabine**

# Identifying the N-Acetyl retigabine regio-isomer

## GC/MS Data

Abundance

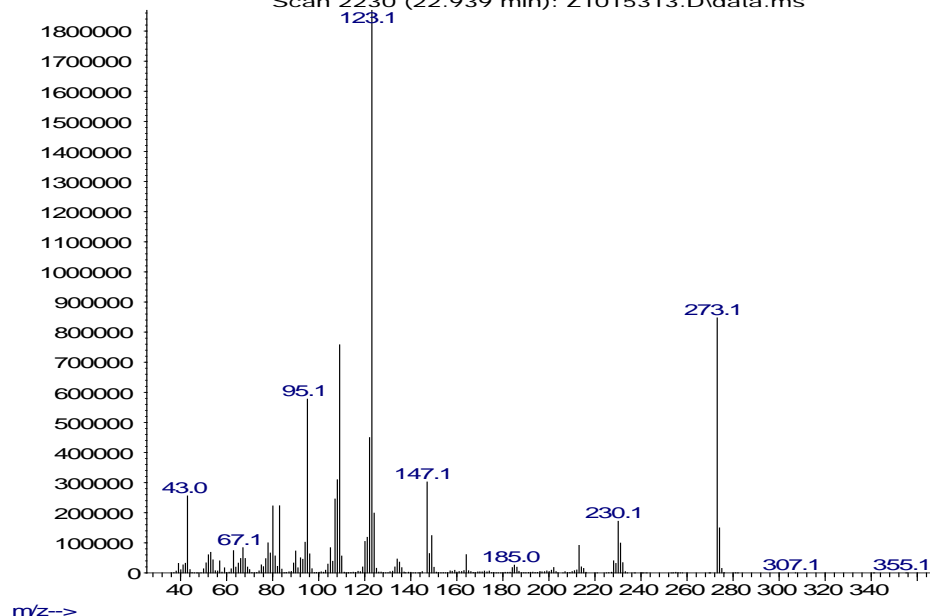
TIC: Z1015313.D\data.ms



Time-->

Abundance

Scan 2230 (22.939 min): Z1015313.D\data.ms



- 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 <sub>0</sub> = >99.9%			
	t <sub>1</sub> = 1 day	t <sub>2</sub> = 4 days	t <sub>3</sub> = 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 <sub>0</sub> = >99.9 %				
	t <sub>1</sub> = 1 day	t <sub>2</sub> = 4 days	t <sub>3</sub> = 7 days	t <sub>4</sub> = 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 <sub>0</sub> = >99.9 %			
	t <sub>1</sub> = 3 days	t <sub>2</sub> = 5 days	t <sub>3</sub> = 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 <sub>0</sub> = >99.9 %				
	t <sub>1</sub> = 3 days	t <sub>2</sub> = 5 days	t <sub>3</sub> = 7 days	t <sub>4</sub> = 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<sub>4</sub> and *N*-acetyl retigabine were successfully developed**

# Thank You!

Contact info:

[heather\\_lima@cerilliant.com](mailto:heather_lima@cerilliant.com)

## Cerilliant

800.848.7837 | 512.238.9974

[www.cerilliant.com](http://www.cerilliant.com)

[techserv@cerilliant.com](mailto:techserv@cerilliant.com)

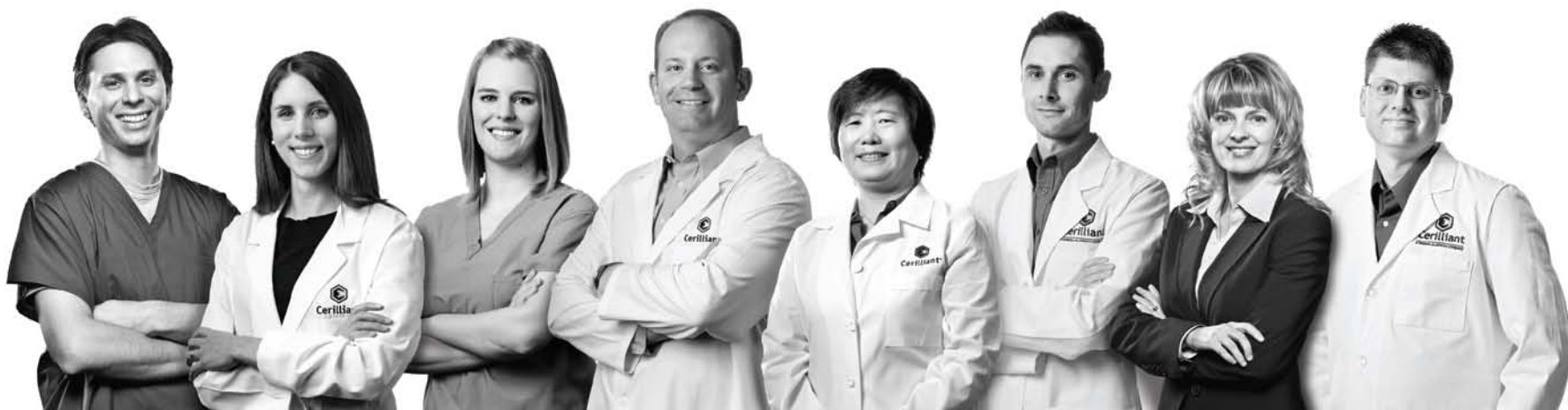
[custserv@cerilliant.com](mailto:custserv@cerilliant.com)



## Cerilliant®

Analytical Reference Standards

a **SIGMA-ALDRICH®** company



**Cerilliant®**

**SIGMA-ALDRICH®**