

Synthetic and Analytical Challenges of Retigabine and N-Acetyl Retigabine



Cerilliant[®]
Analytical Reference Standards

a **SIGMA-ALDRICH**[®] company

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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

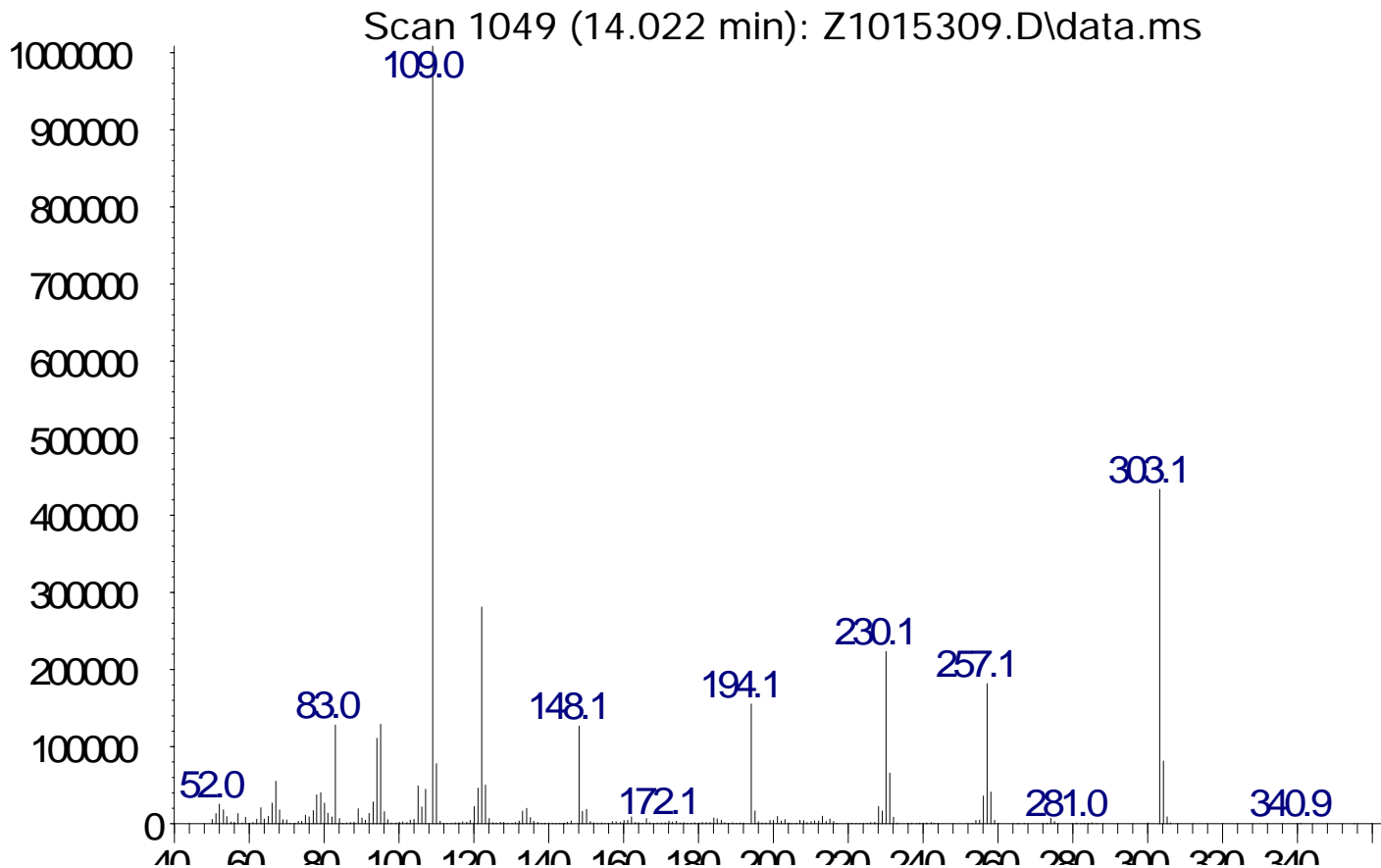
Production of Retigabine CRM

- 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

Abundance



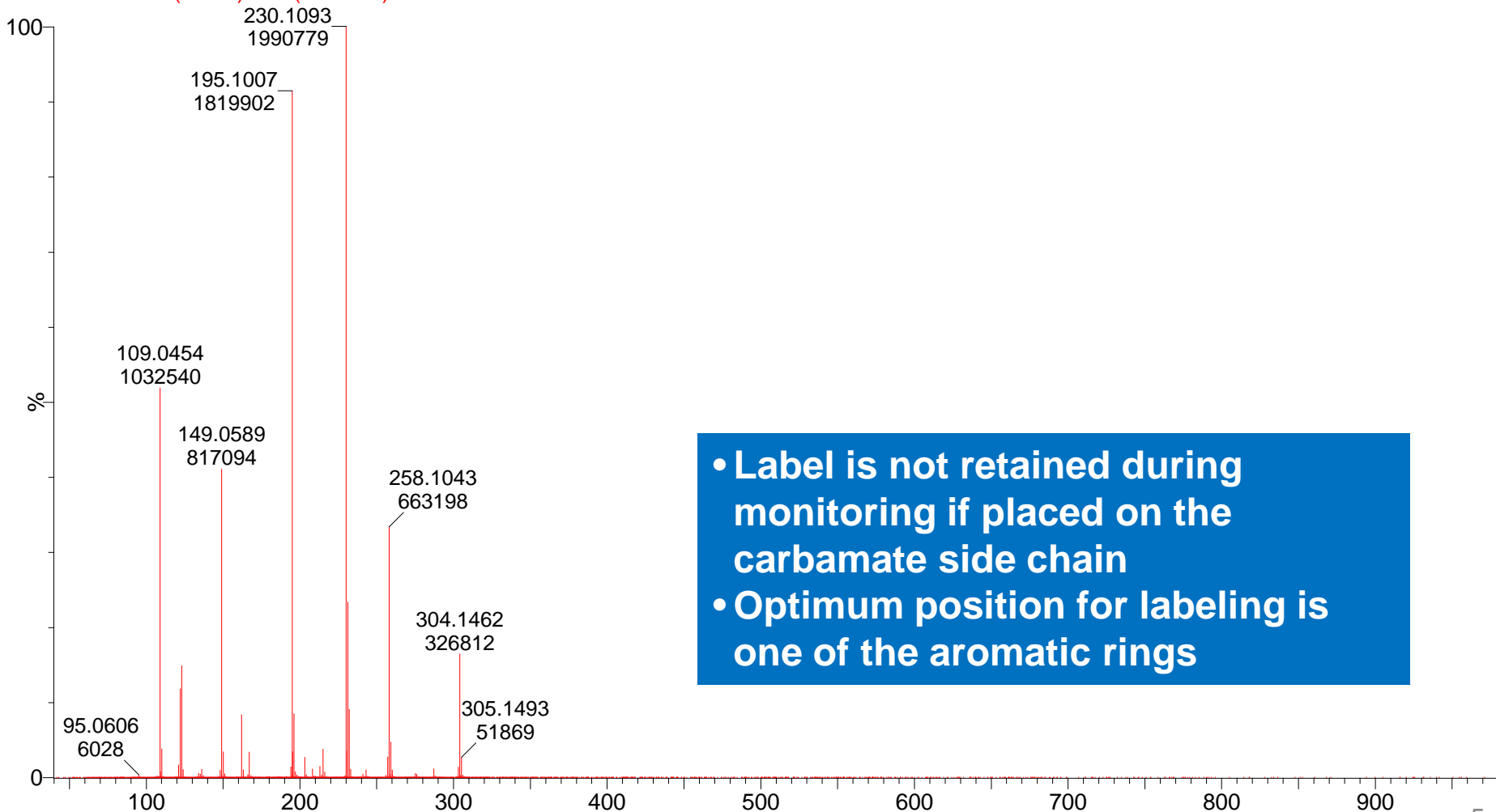
Determining optimum location for label

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-D₄ 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. ¹³C, ¹⁵N...)

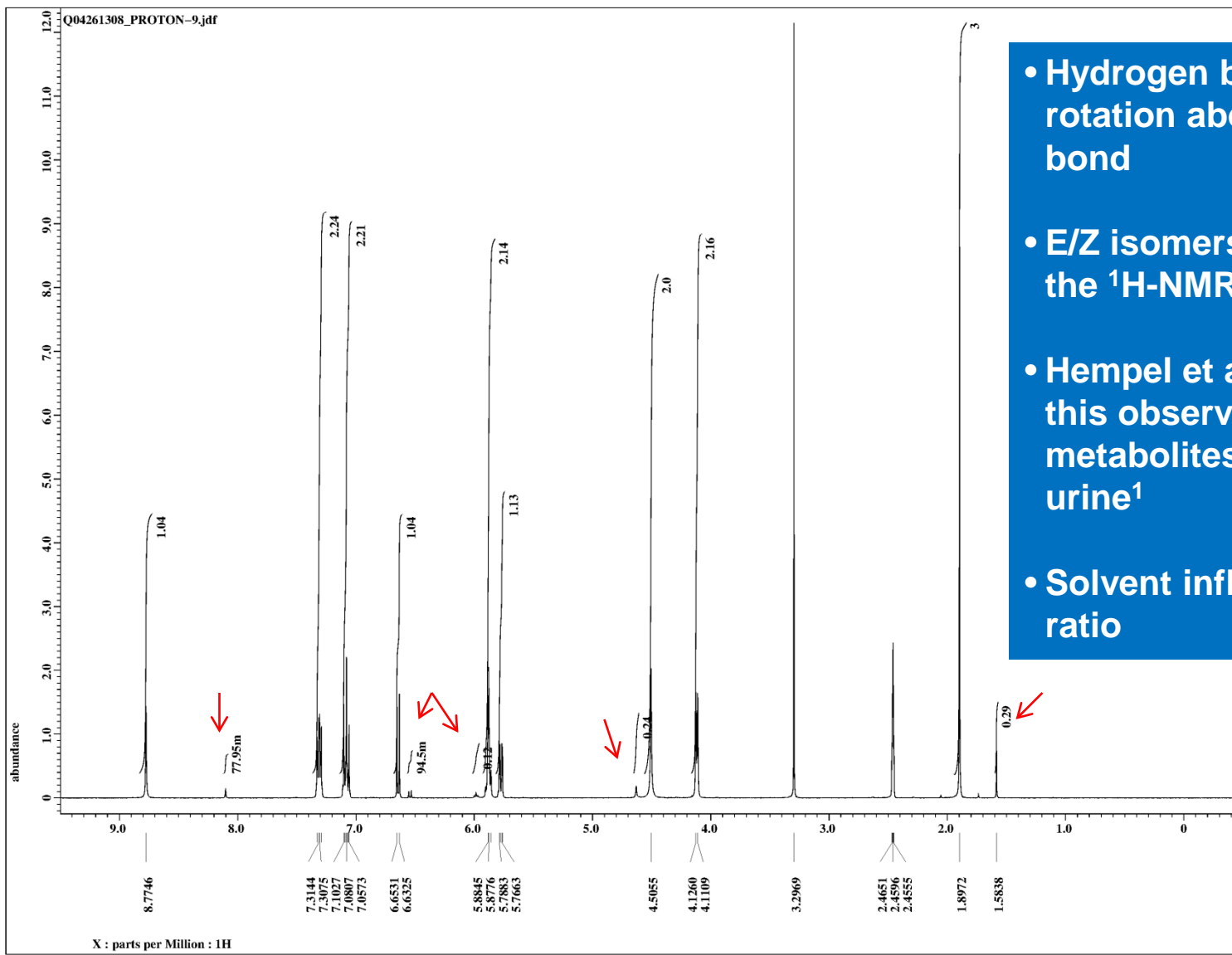
Synthesis of N-Acetyl Retigabine CRM

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

Regio-isomer/rearrangement complications

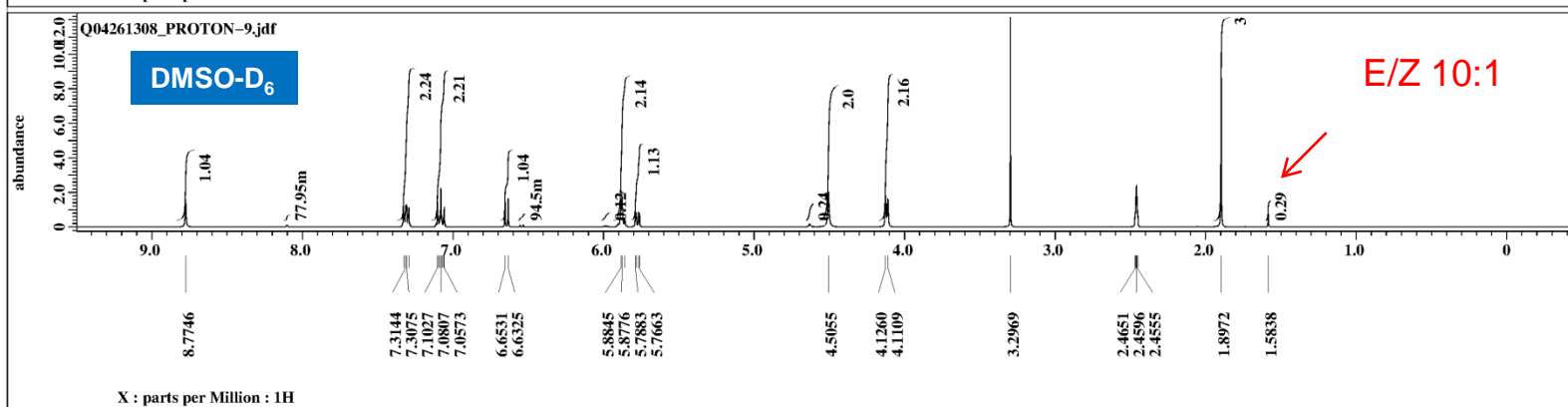
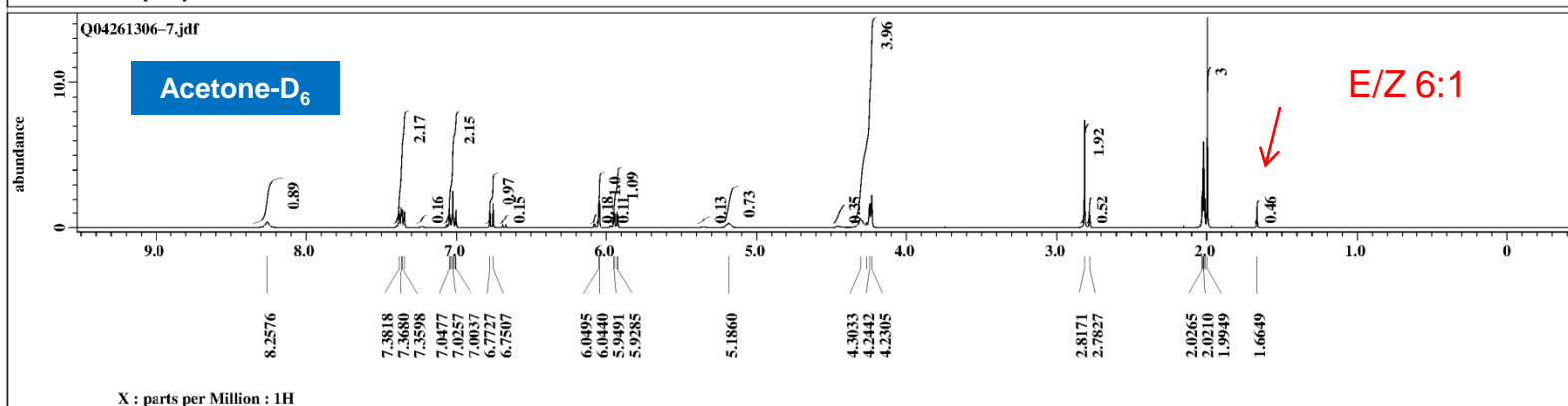
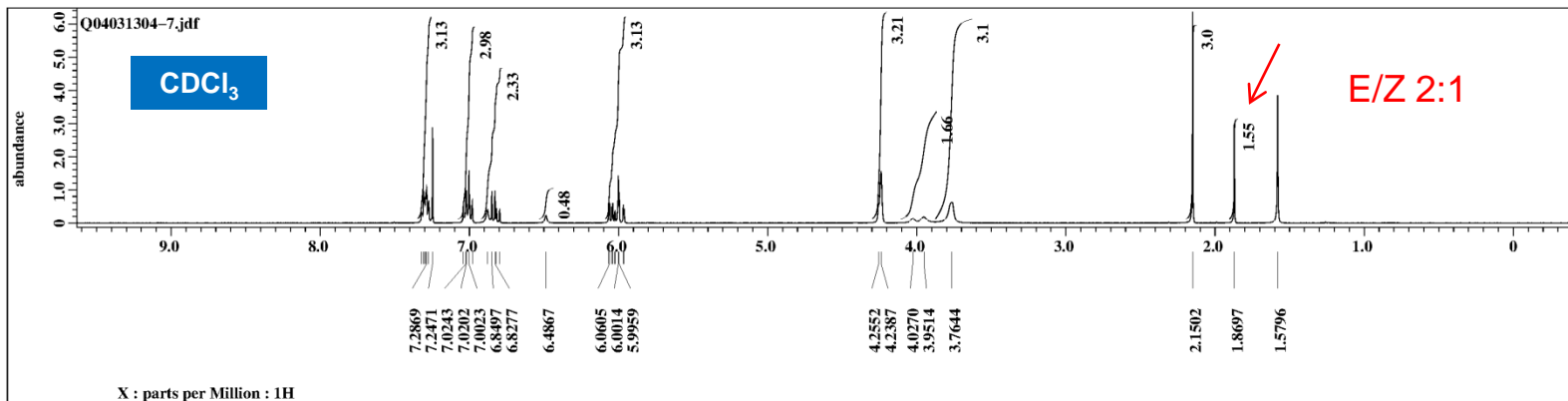
- Regio-isomers formed during the synthesis – identifiable by $^1\text{H-NMR}$
- Possible intramolecular rearrangement; how do we detect and control it?

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 urine¹
- Solvent influences the E/Z ratio

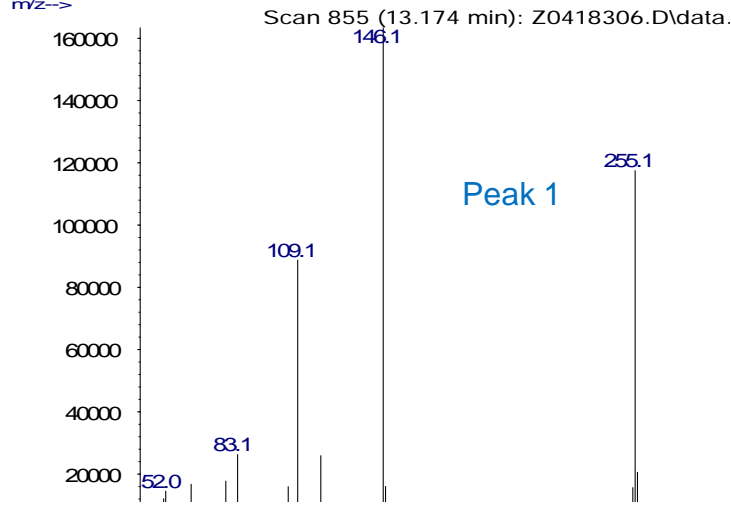
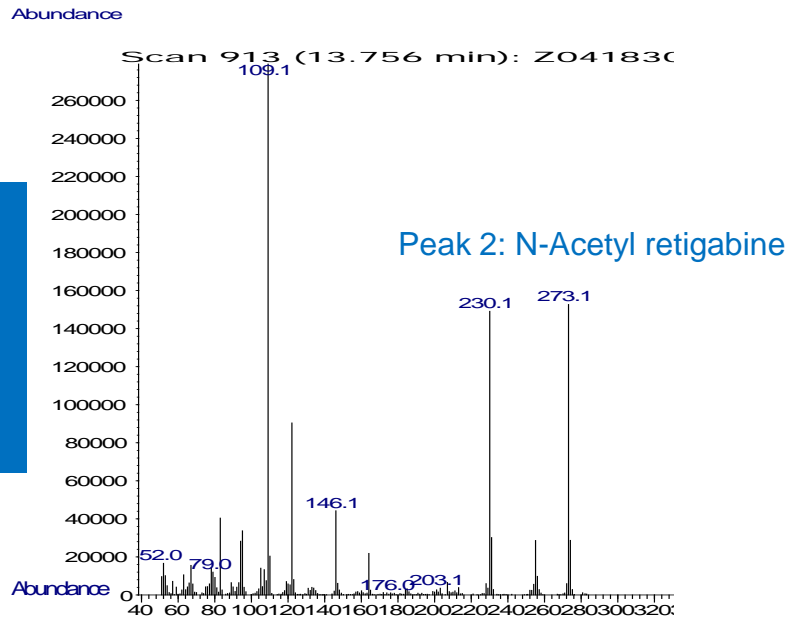
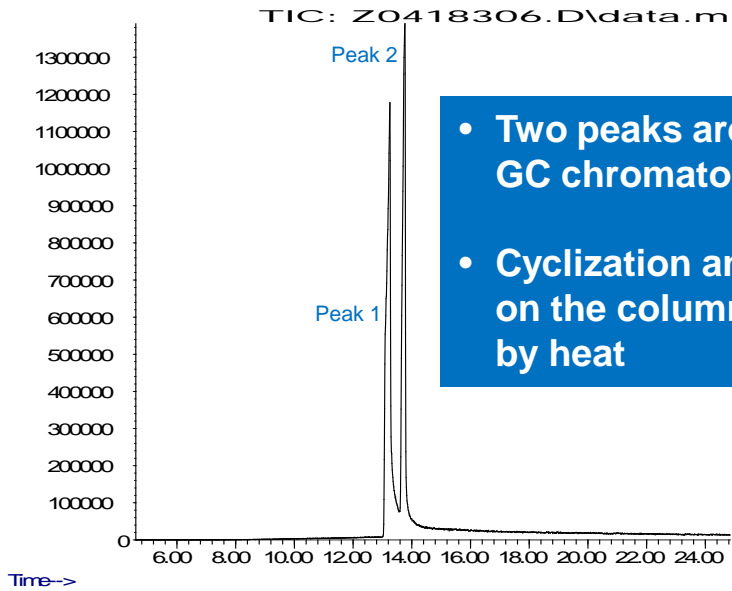
1) Hempel, R.; et al. Drug Metabolism and Disposition, 1999, 27, 613-622.



Analytical challenges

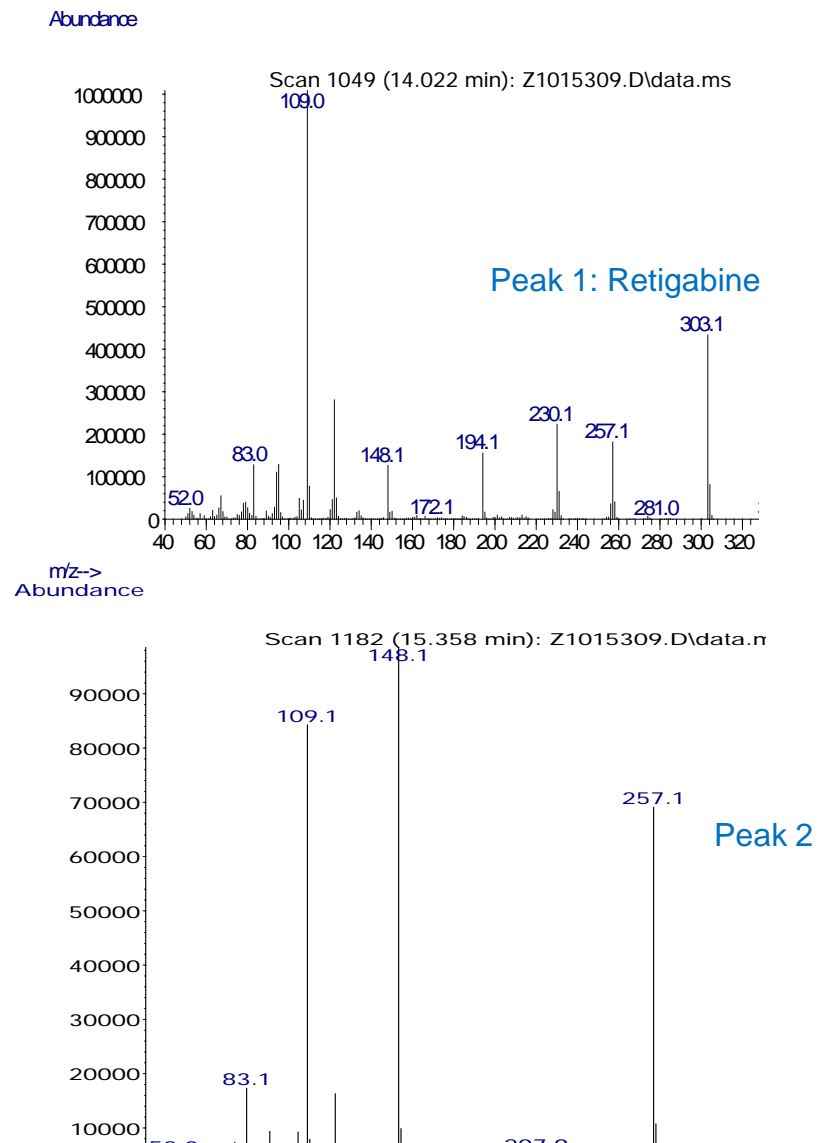
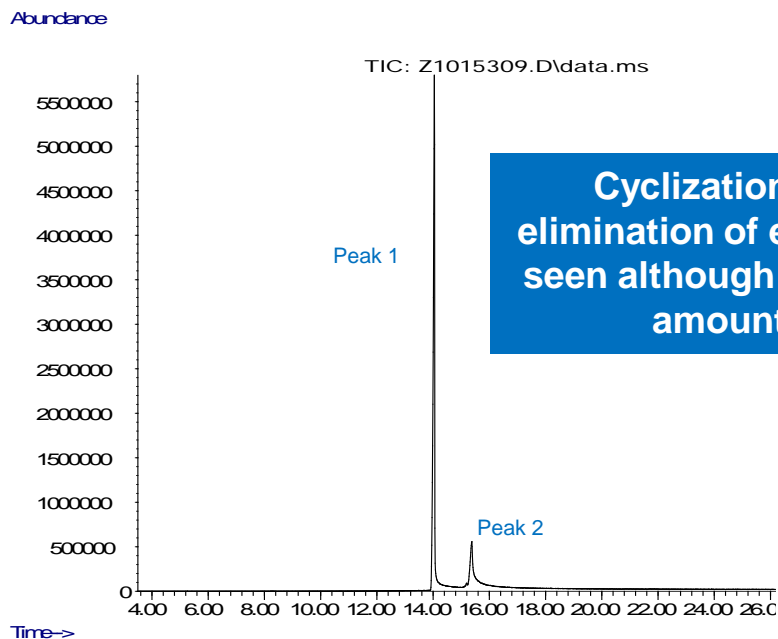
GC/MS data for N-Acetyl Retigabine

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



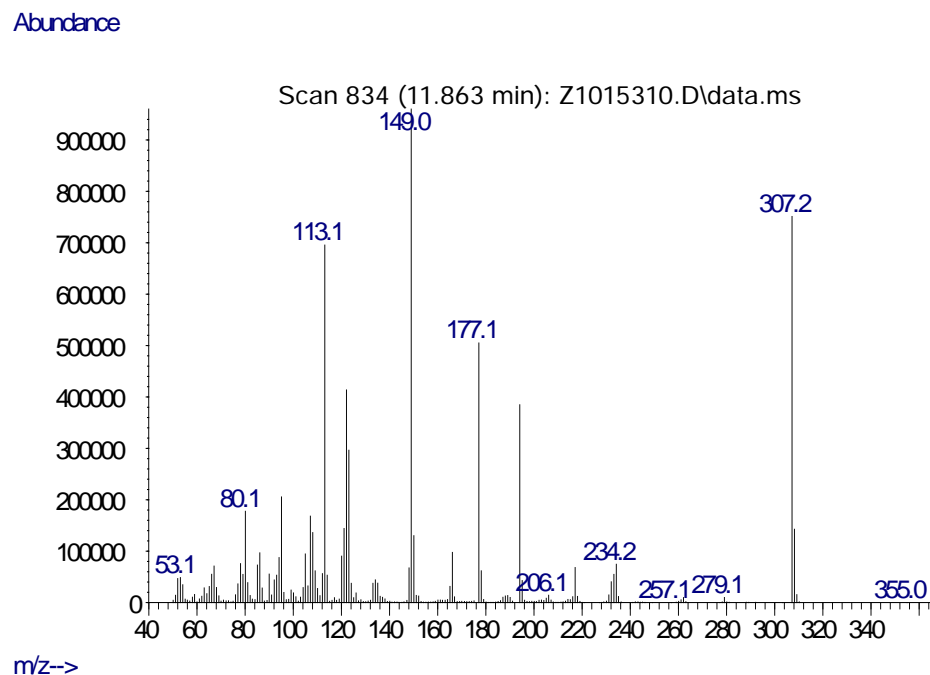
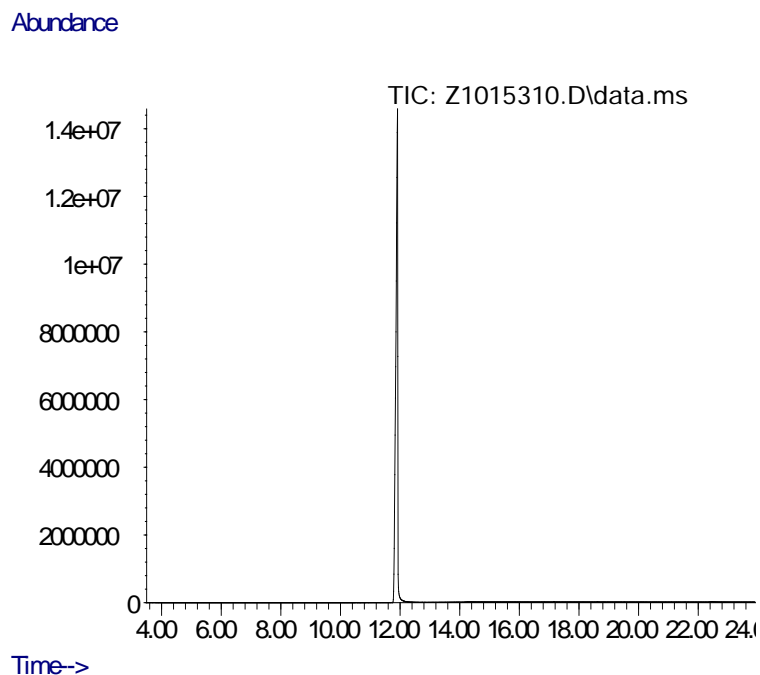
Concerns with cyclization of Retigabine

GC/MS data for Retigabine



Identifying the Retigabine-D₄ regio-isomer

GC/MS Data



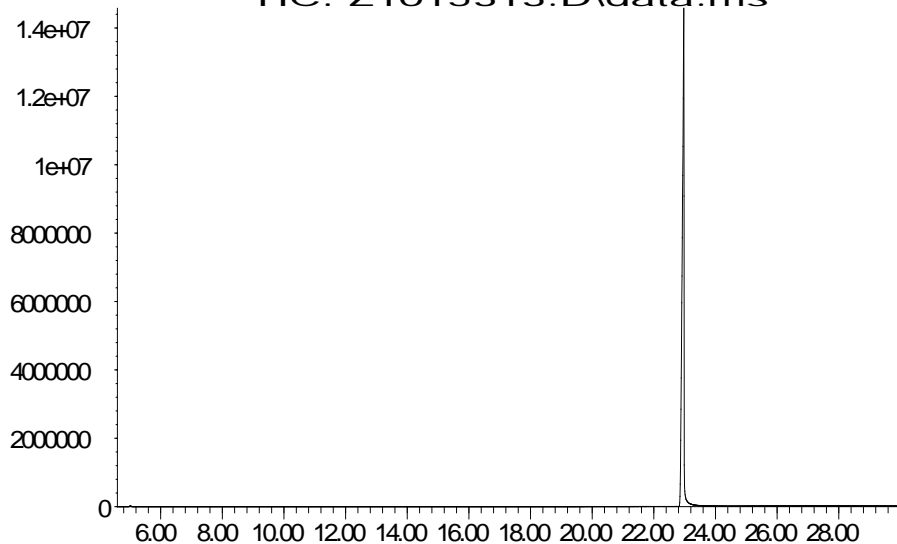
Regio-isomer exhibits different fragmentation patterns and is chromatographically different from retigabine

Identifying the N-Acetyl retigabine regio-isomer

GC/MS Data

Abundance

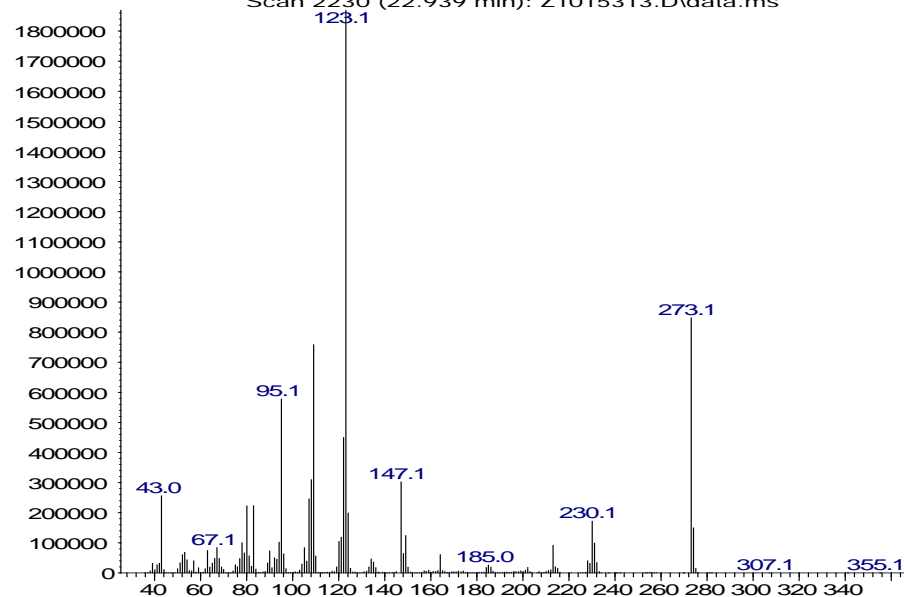
TIC: Z1015313.D\data.ms



Time-->

Abundance

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



m/z-->

- 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-D₄ and *N*-acetyl retigabine were successfully developed

Thank You!

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