LC-MS/MS Analysis of Chlortetracycline (CTC) in Environmental Samples

Jonathan Bailey

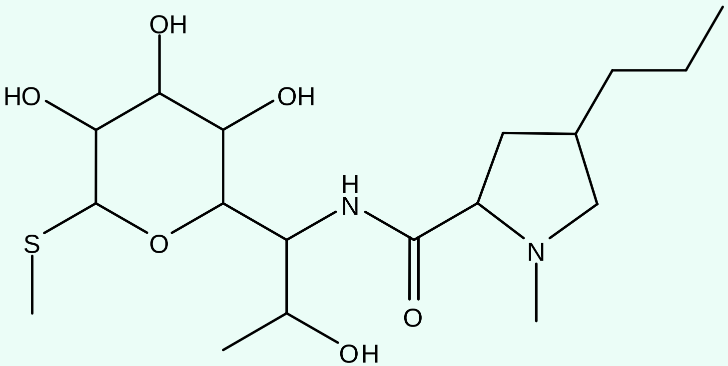
Kerry M. Peru, Allan J. Cessna and John V. Headley

National Water Research Institute (NWRI)

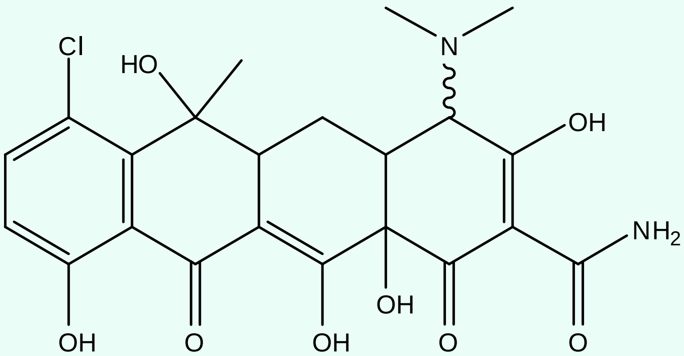
Saskatoon, SK, Canada

Multi-residue LC-MS/MS Antibiotic Method

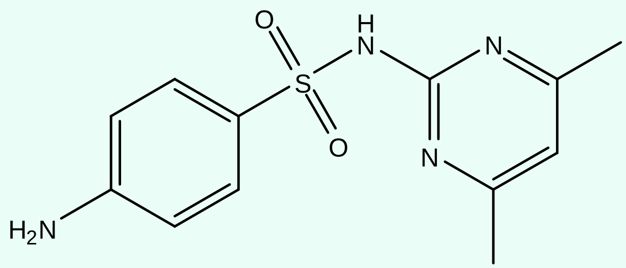
Lincomycin



Chlortetracycline



Sulfamethazine



LC Separation

Liquid Chromatographic Separation

LC Instrument: Waters 2695 Alliance HPLC System

Column: Waters Xterra: C18: 2.1mm x 100mm

Particle size: 3.5u

Column temp: 30^{o}C

Mobile system: 10:90 (Acetonitrile / H2O)

Modifier: 0.1 % formic acid

Flow rate: 200uL/min

Separation mode: isocratic

Injection volume: 20uL

MS/MS Analysis

MS/MS Parameters

- Instrument: Micromass Quattro Ultima
- Source temp: 90°C
- Electrospray Interface: (+) ion mode
- Cone Voltage: 42 V
- Capillary voltage setting: 3.76 kV
- Hexapole 1: 7.1 V
- Hexapole 2: 0 V
- Aperture Voltage: 0 V
- Desolvation temp: 220°C

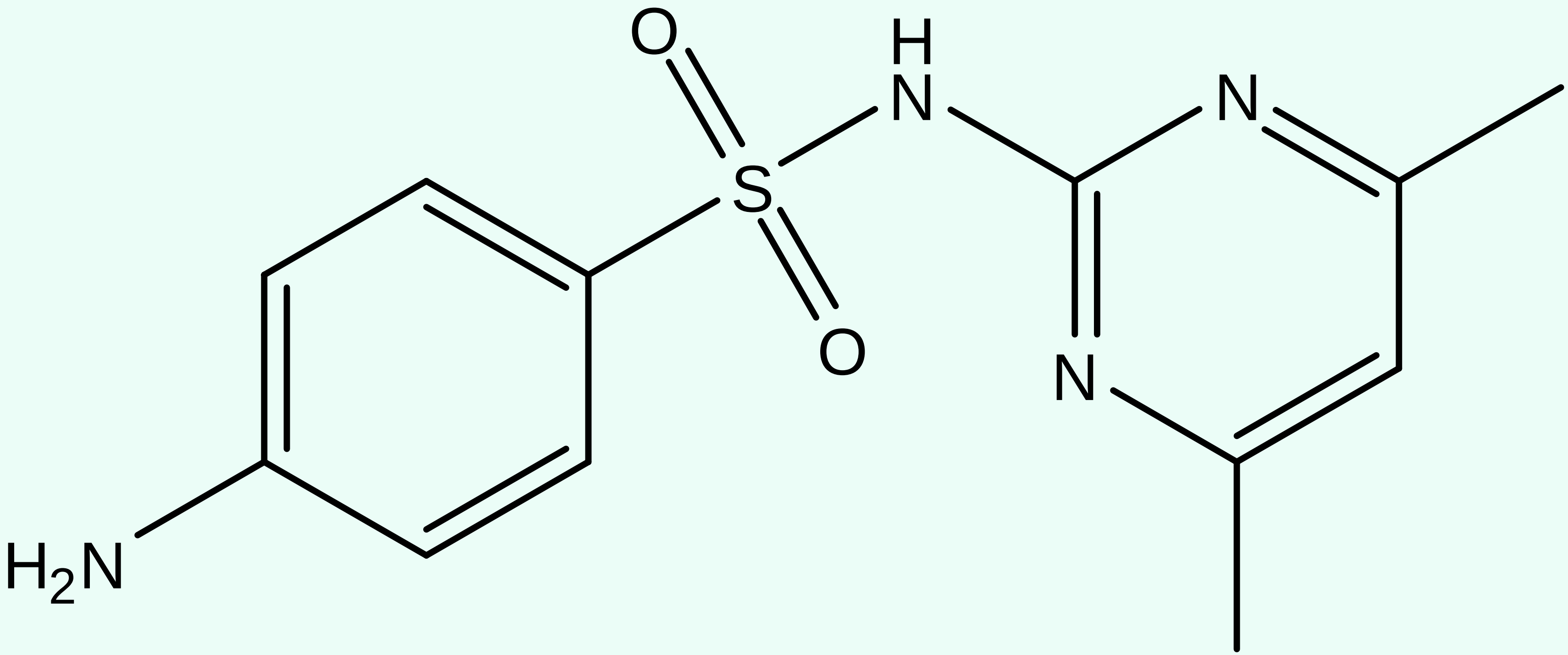
MS/MS Parameters Cont.

- Desolvation gas – N_2 @ 488 L h^{-1}
- Cone gas – N_2 @ 154 L h^{-1}
- Quadrupole 1: unit mass resolution
- Quadrupole 3: ~2.0 m/z resolution
- Photomultiplier voltage - 650 V
- Collision gas: Argon
- Collision gas pressure: 3.53 x 10^{-4} torr.
- Collision energy: 30 eV

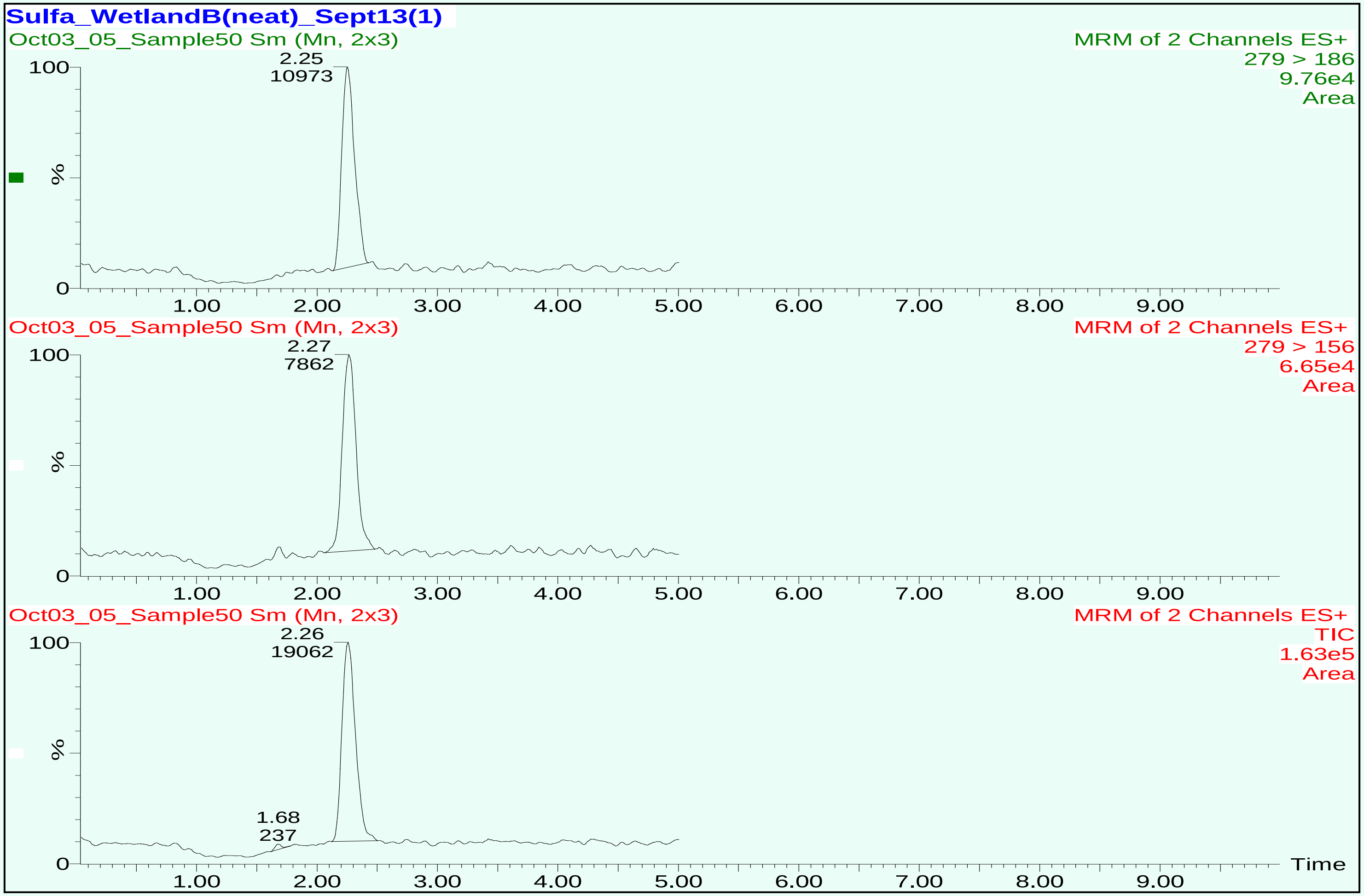
Sulfamethazine MS/MS

- Precursor Ion [M+H] (m/z 279)
- Product Ion 1: (m/z 186)
- Product Ion 2: (m/z 156)
- TIC of the two product ion channels was used for quantitation

Sulfamethazine



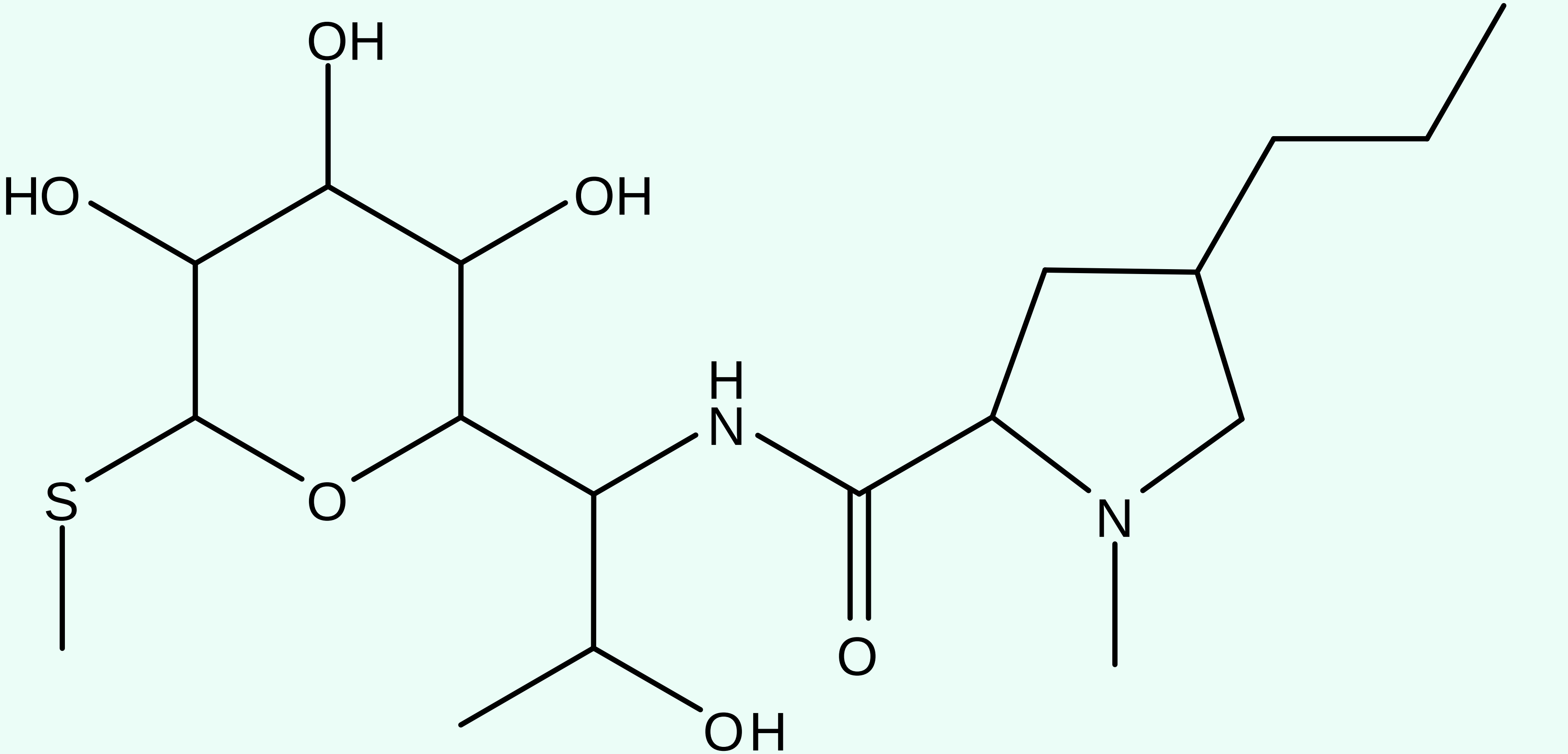
Chromatogram of Sulfamethazine



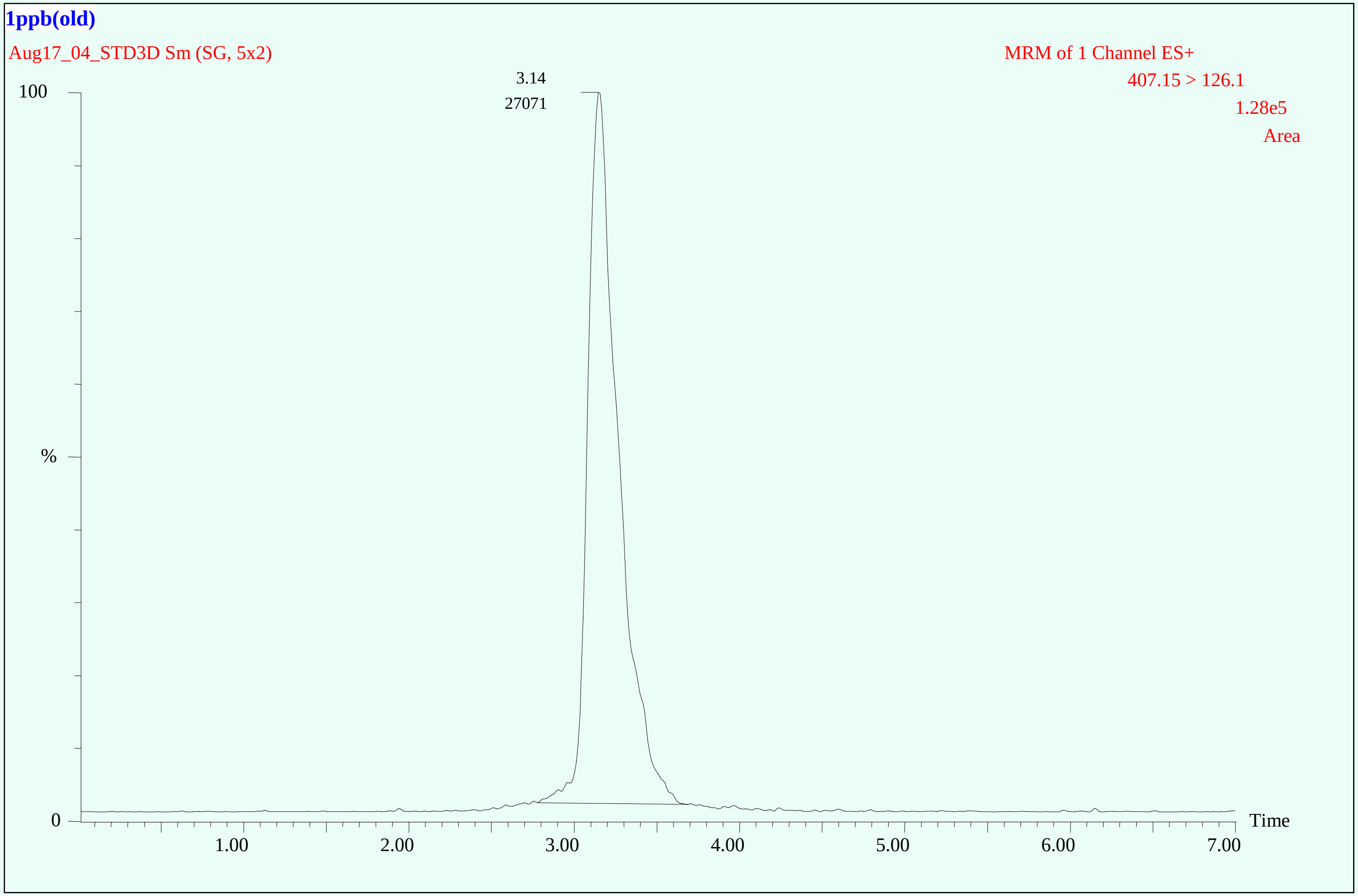
Lincomycin MS/MS

- Precursor Ion [M+H] (m/z 407.1)
- Product Ion 1: (m/z 126.1)
- Integration of Product Ion 1 was used for quantitation

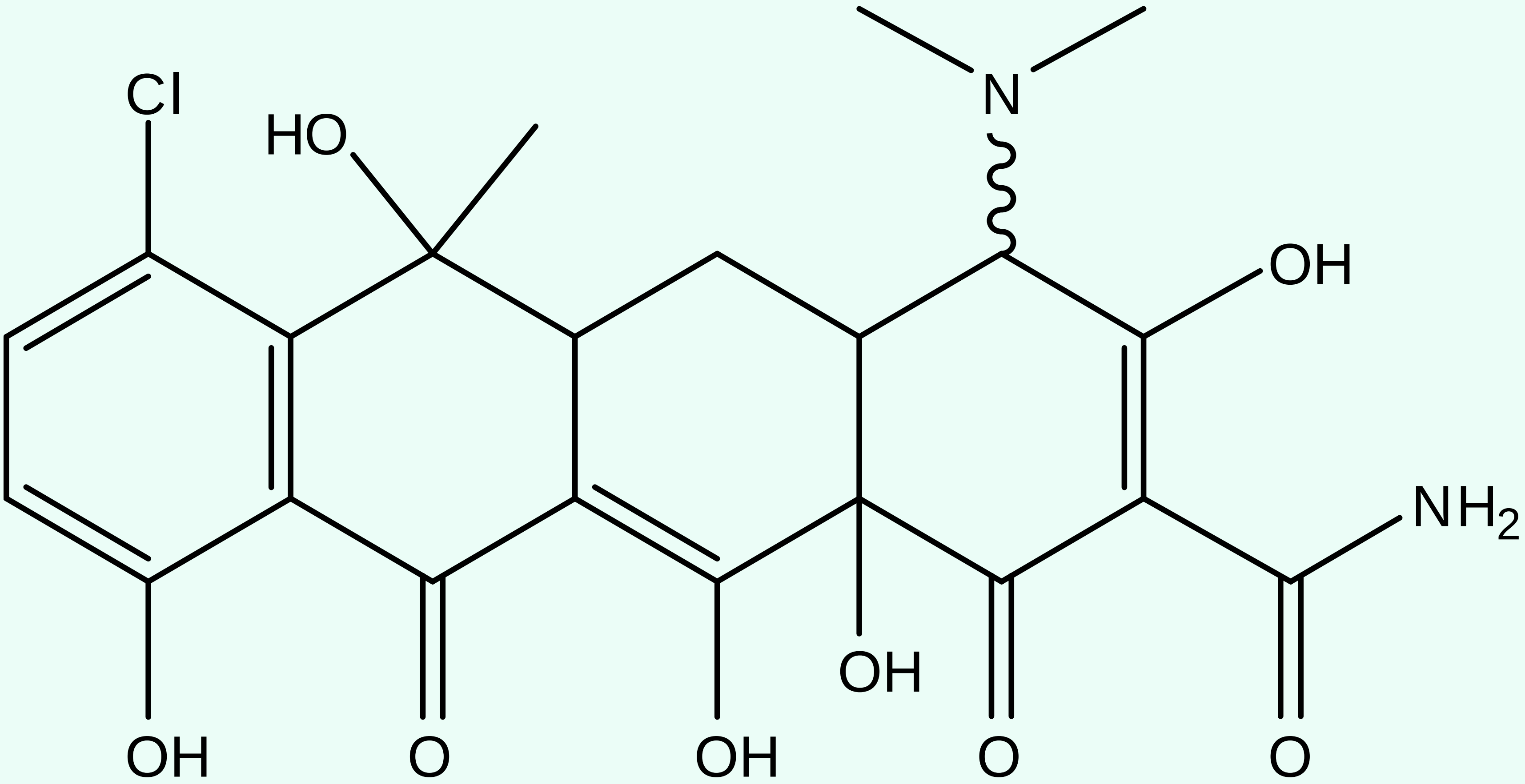
Lincomycin



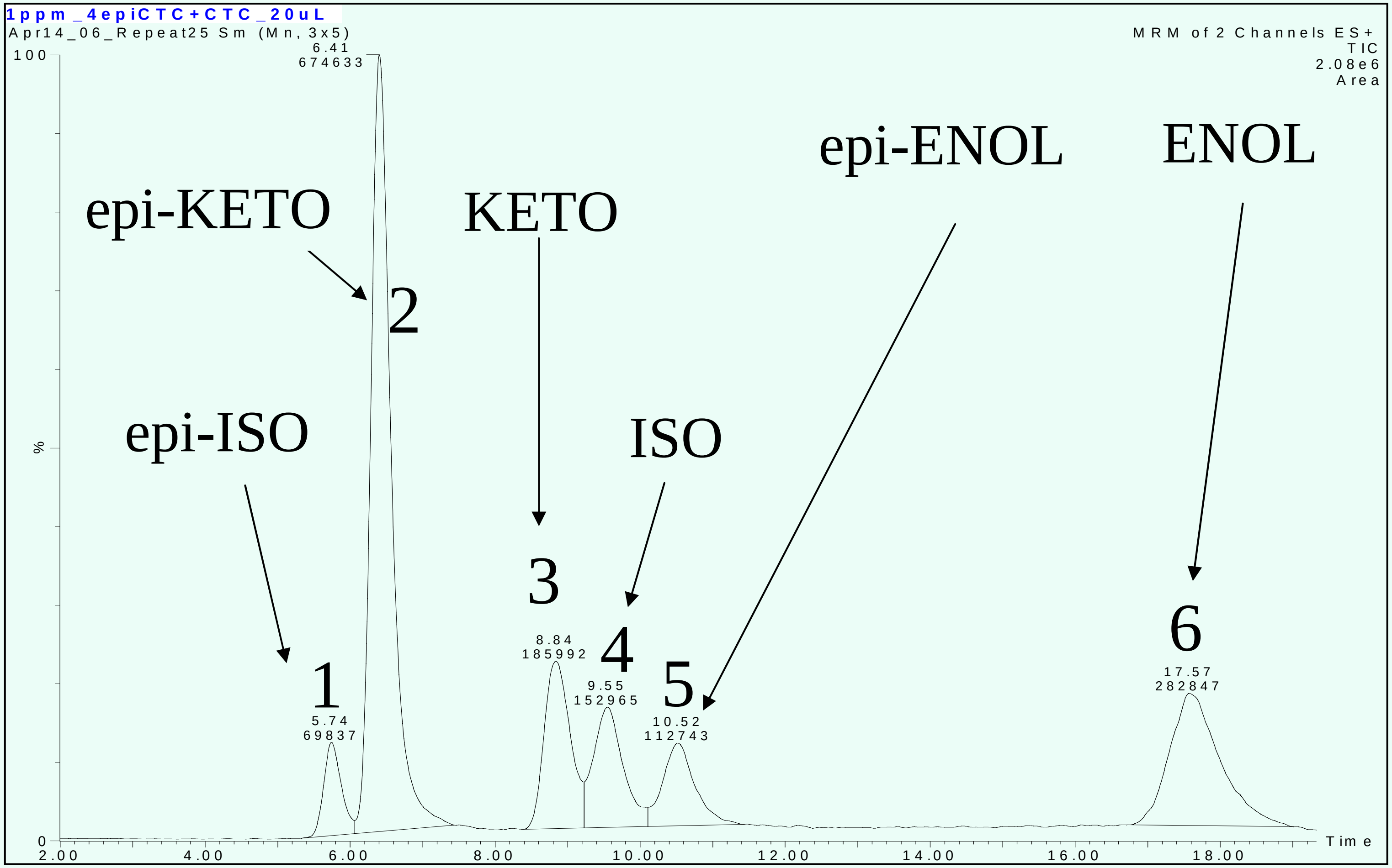
Chromatogram of Lincomycin



CTC-enol



Chromatogram of Chlortetracycline



CTC Forms (in H_2O)	Retention Time (min)
CTC-enol	16.0
Epi-CTC-enol	10.0
Iso-CTC-keto	8.7
CTC-keto	8.1
Epi-CTC-keto	6.1
Epi-iso-CTC-keto	5.4

Chlorotetracycline MS/MS

- Precursor Ion [M+H] (m/z 479)

- All forms of CTC: (m/z 479>462)

  - loss of [-NH_3]

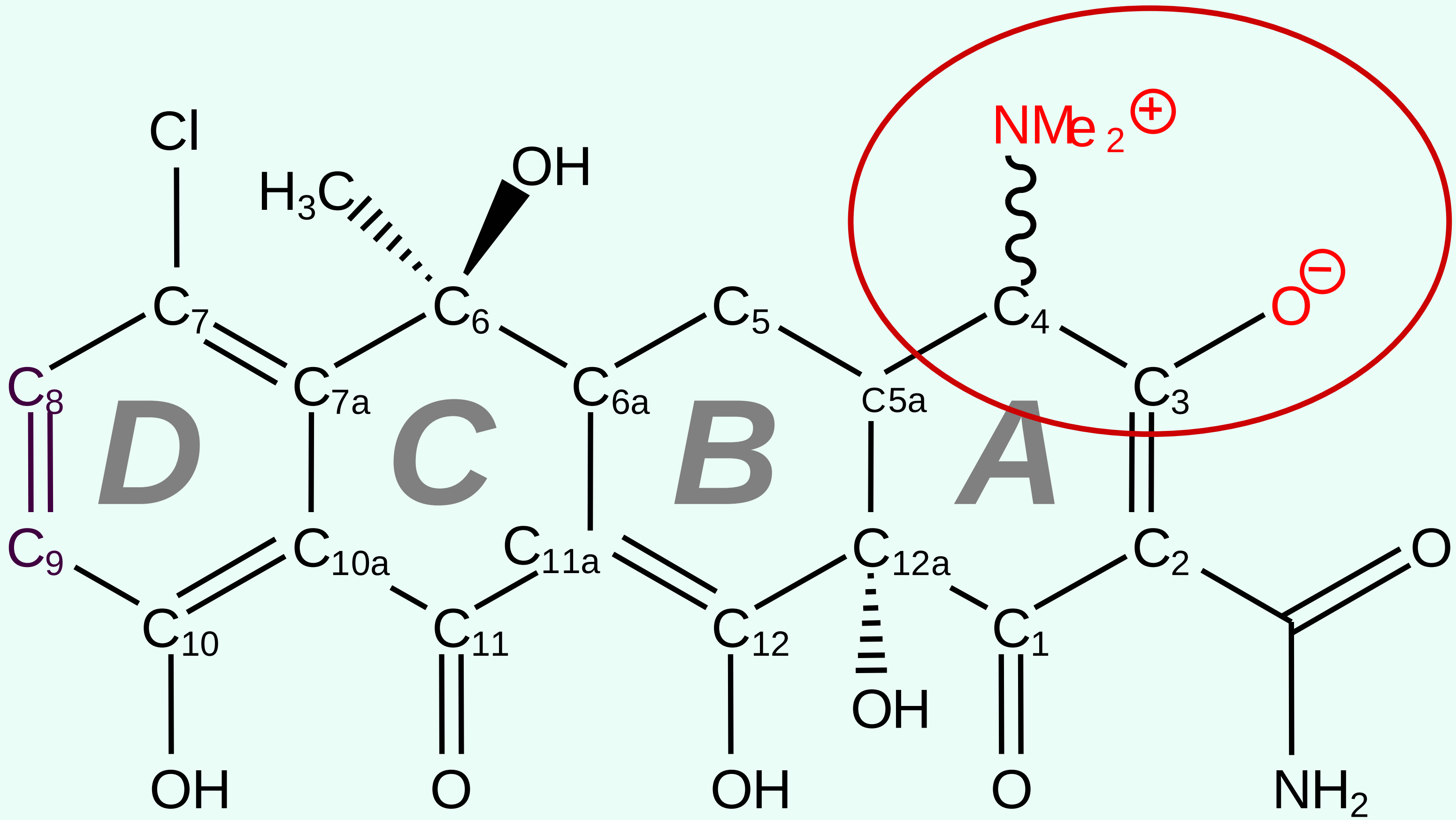
- Enol ONLY (m/z 479>444)

  - loss of [-NH_3] + [-H_2O]

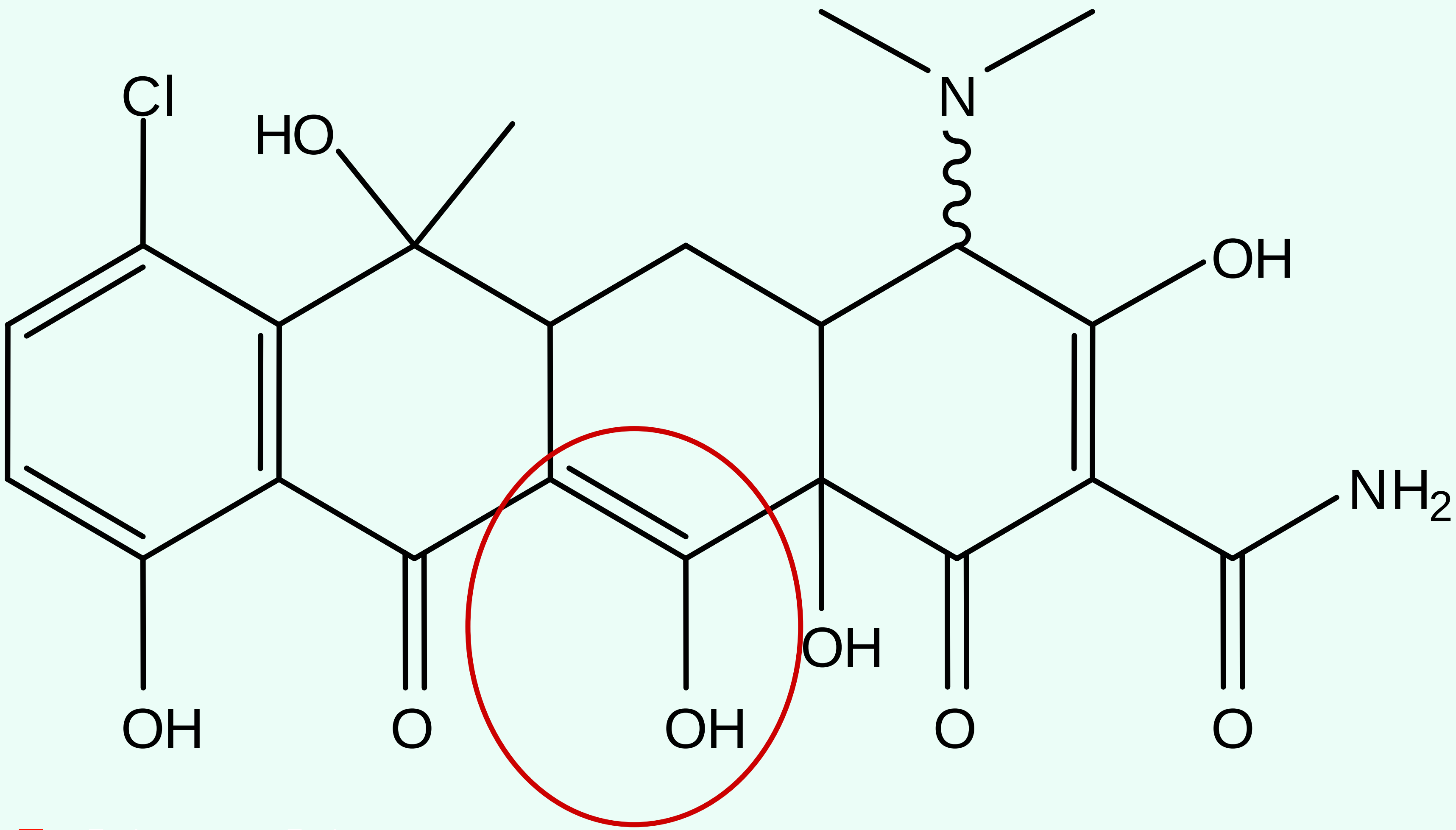
CTC Analytical Method

- **Zwitterion Formation**
- **C-4 Epimerization**
- **C-12 Enol/Keto Tautomerization**
- **C-6 Lactone Formation**

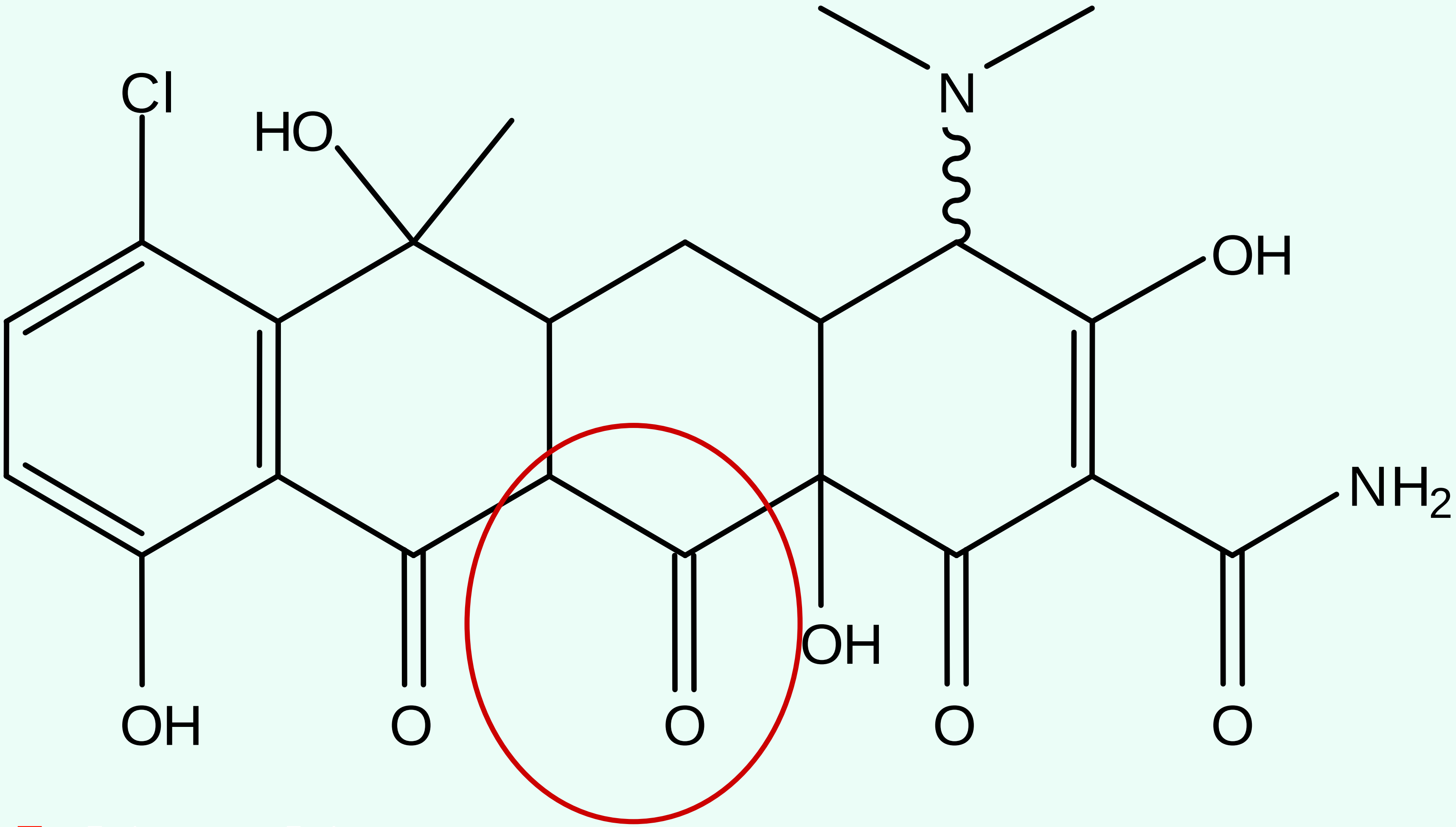
Zwitterion and Epimerization



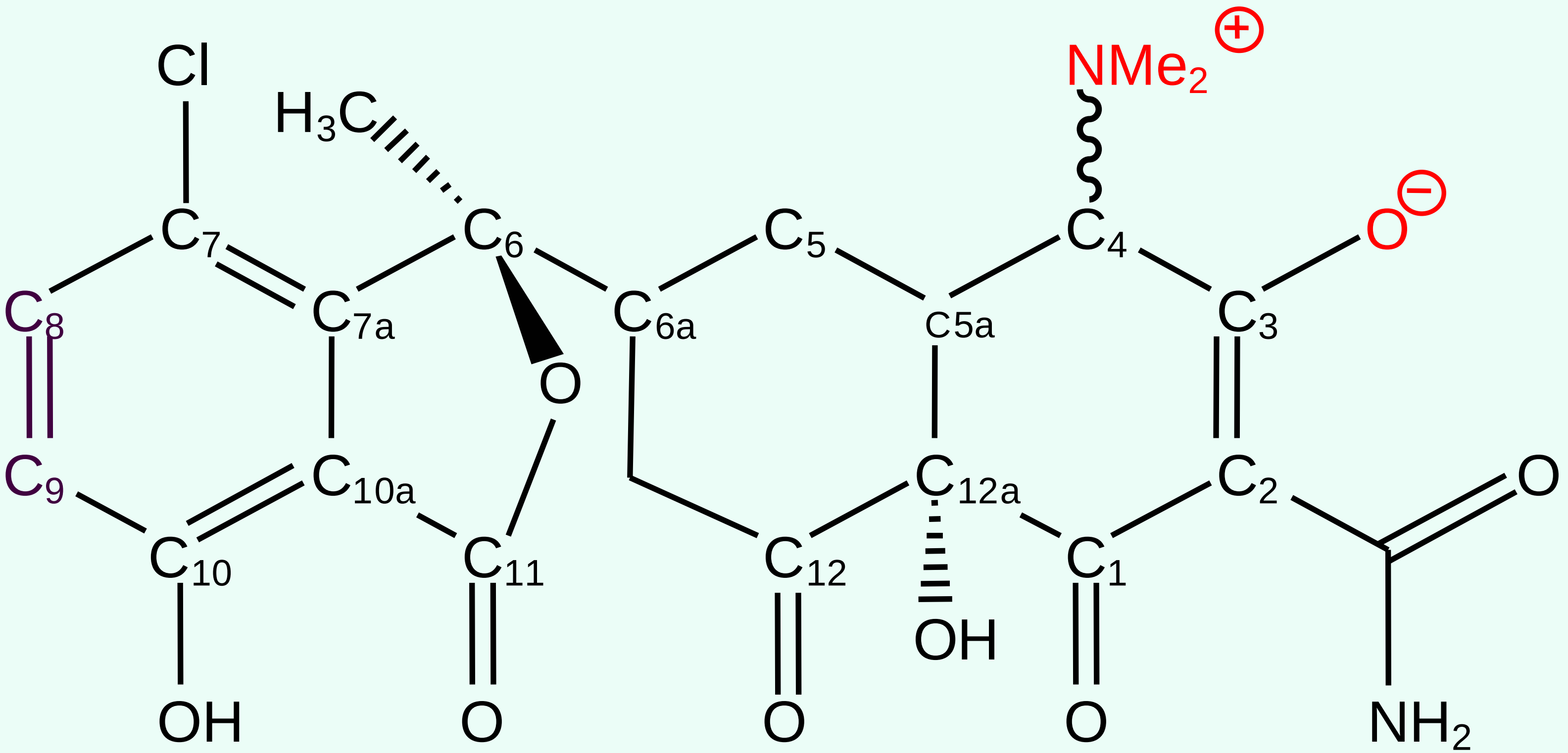
CTC-enol tautomer



CTC-keto tautomer

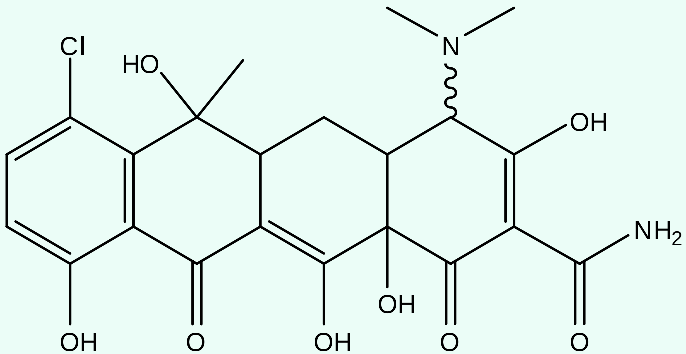


iso-CTC Lactone Formation



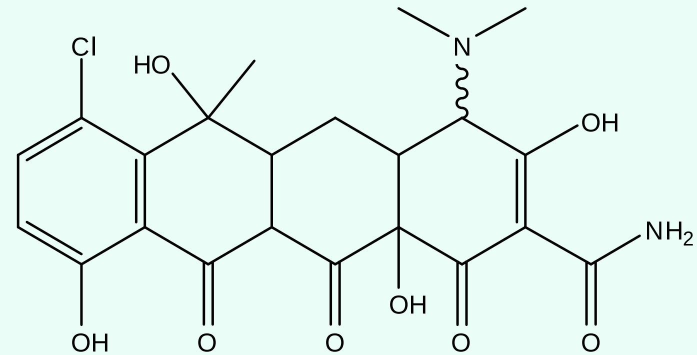
Complex Isomeric Distribution

CTC–*enol*



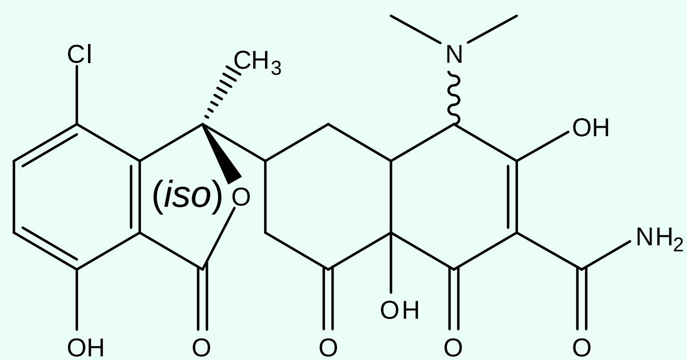
(enol)

CTC-*keto*

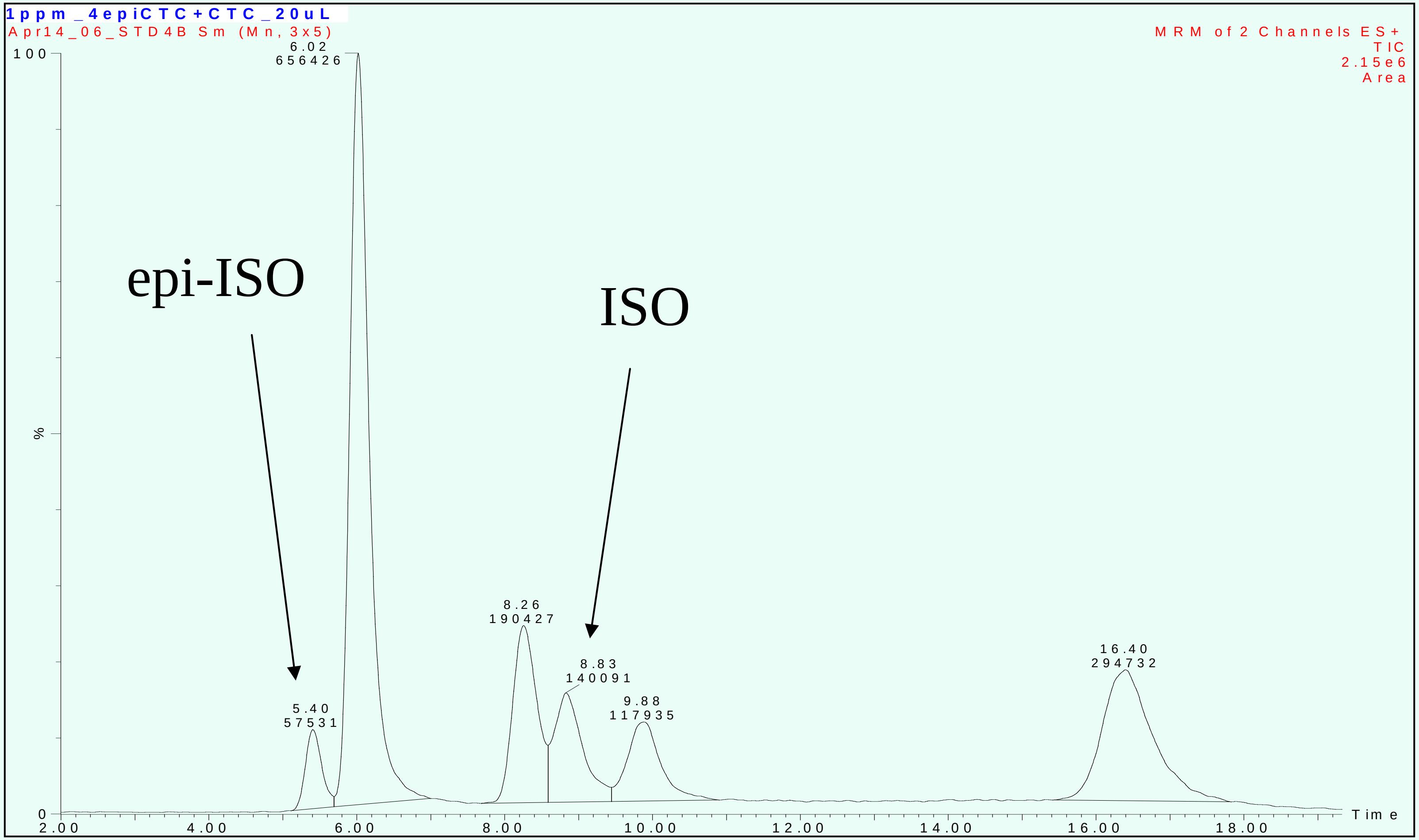


(keto)

iso-CTC



1ppm CTC+4-epiCTC (de-ionized H_2O)



1 2 3 4 5 6

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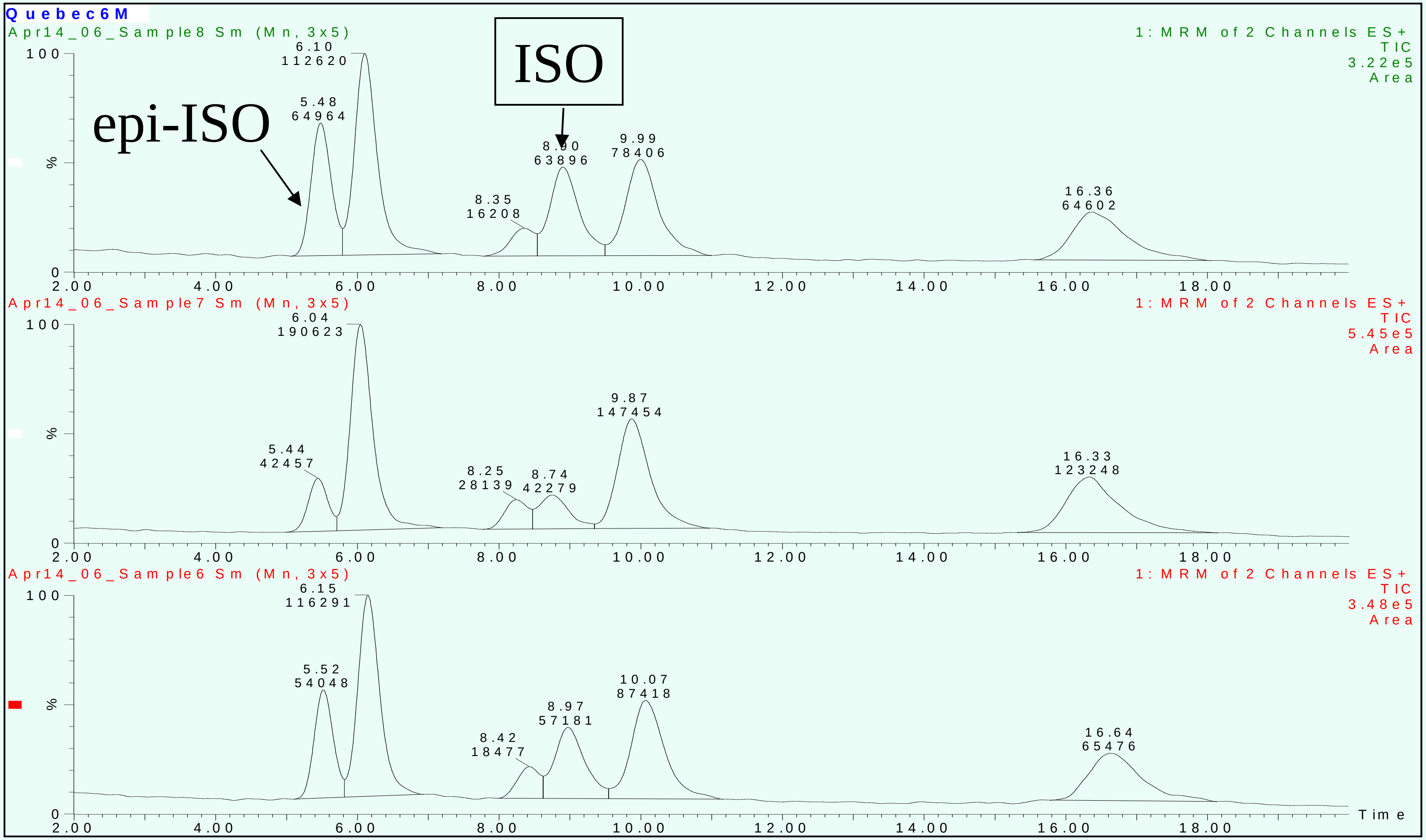
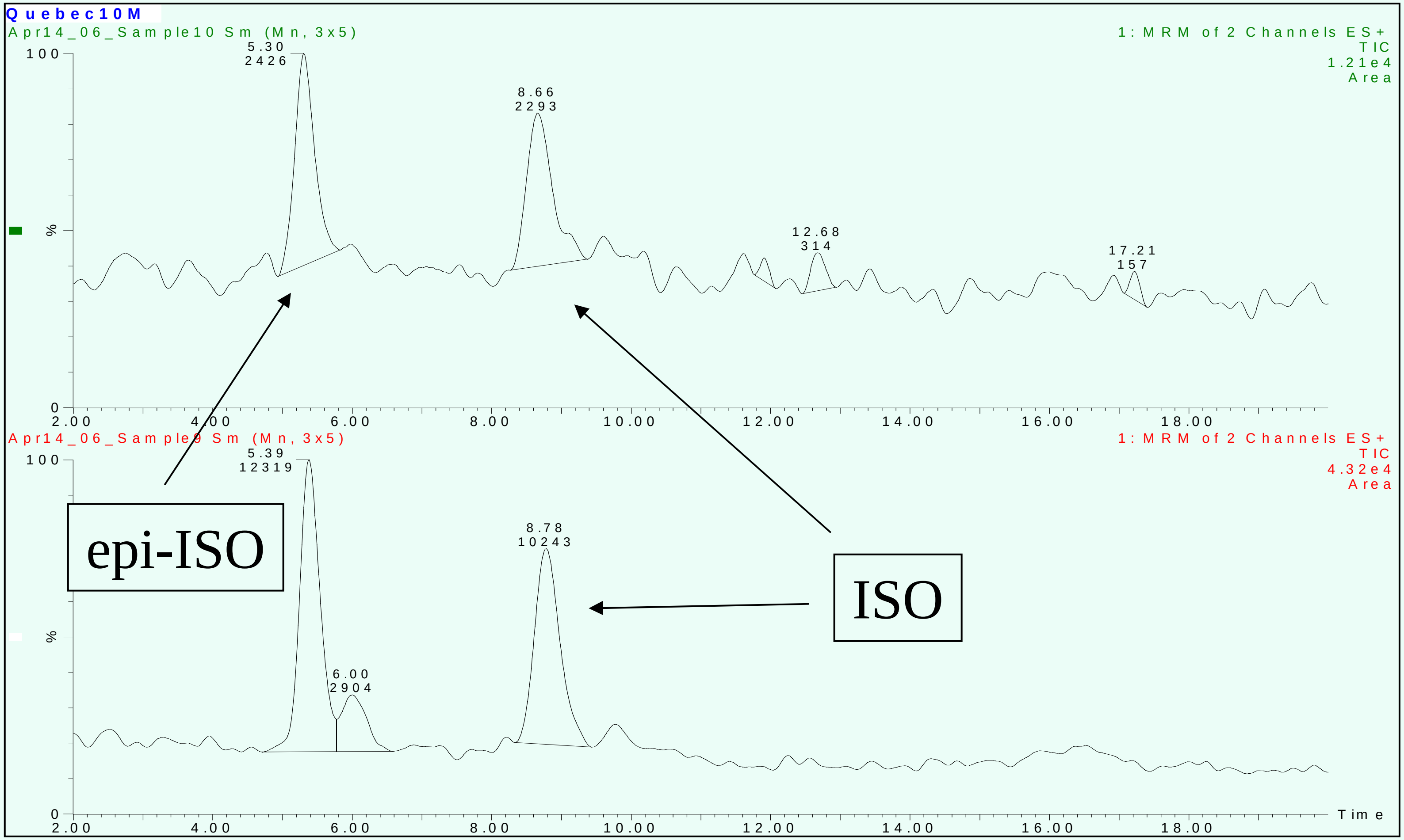
Watershed Samples pH<1 (H_2SO_4)



1 2 3 4 5 6

2ppb CTC spike (de-ionized H_2O)



1 4

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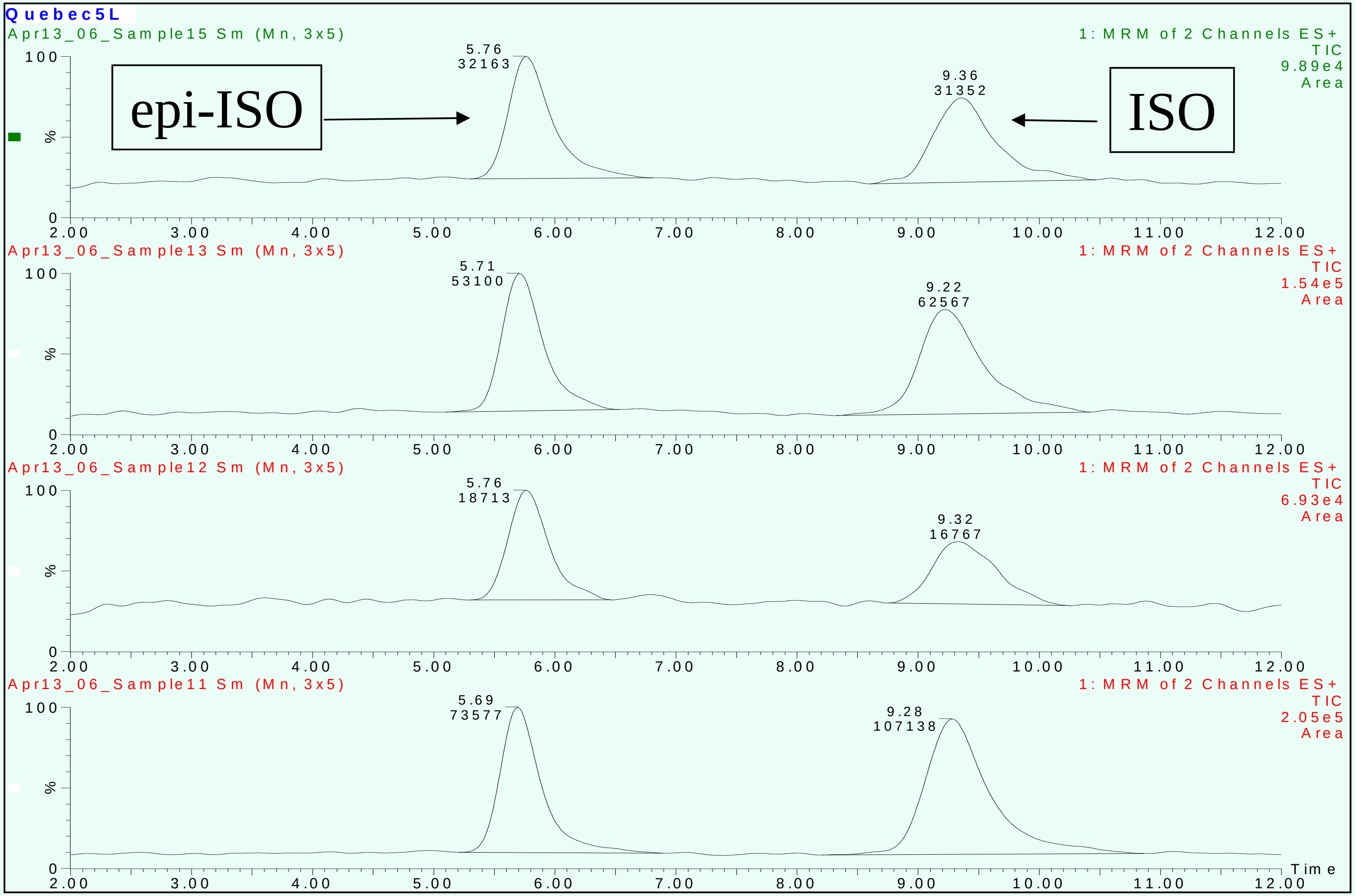
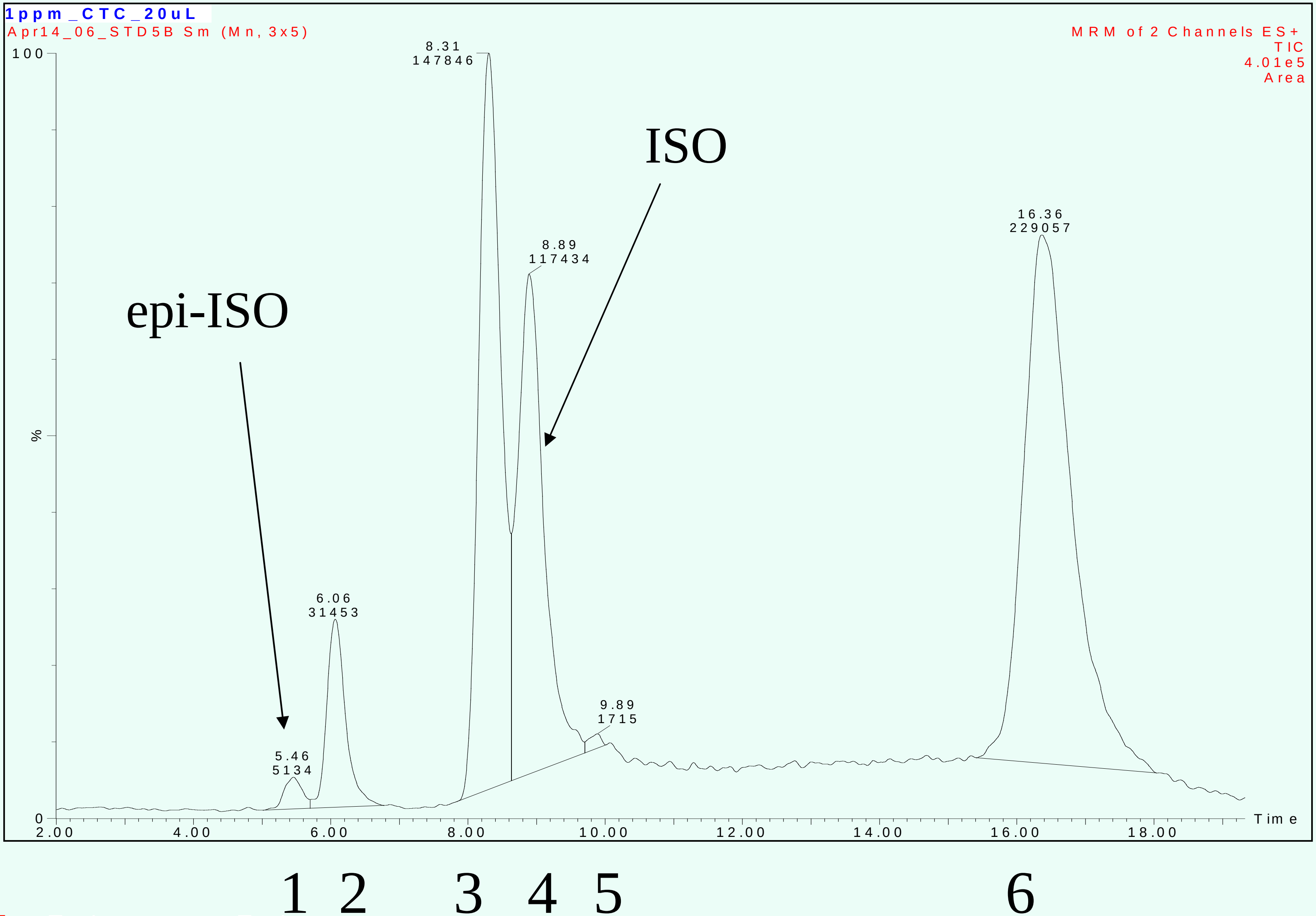
2ppb CTC spike (Watershed Samples)



1

4

1ppm CTC Standard (de-ionized H_2O)



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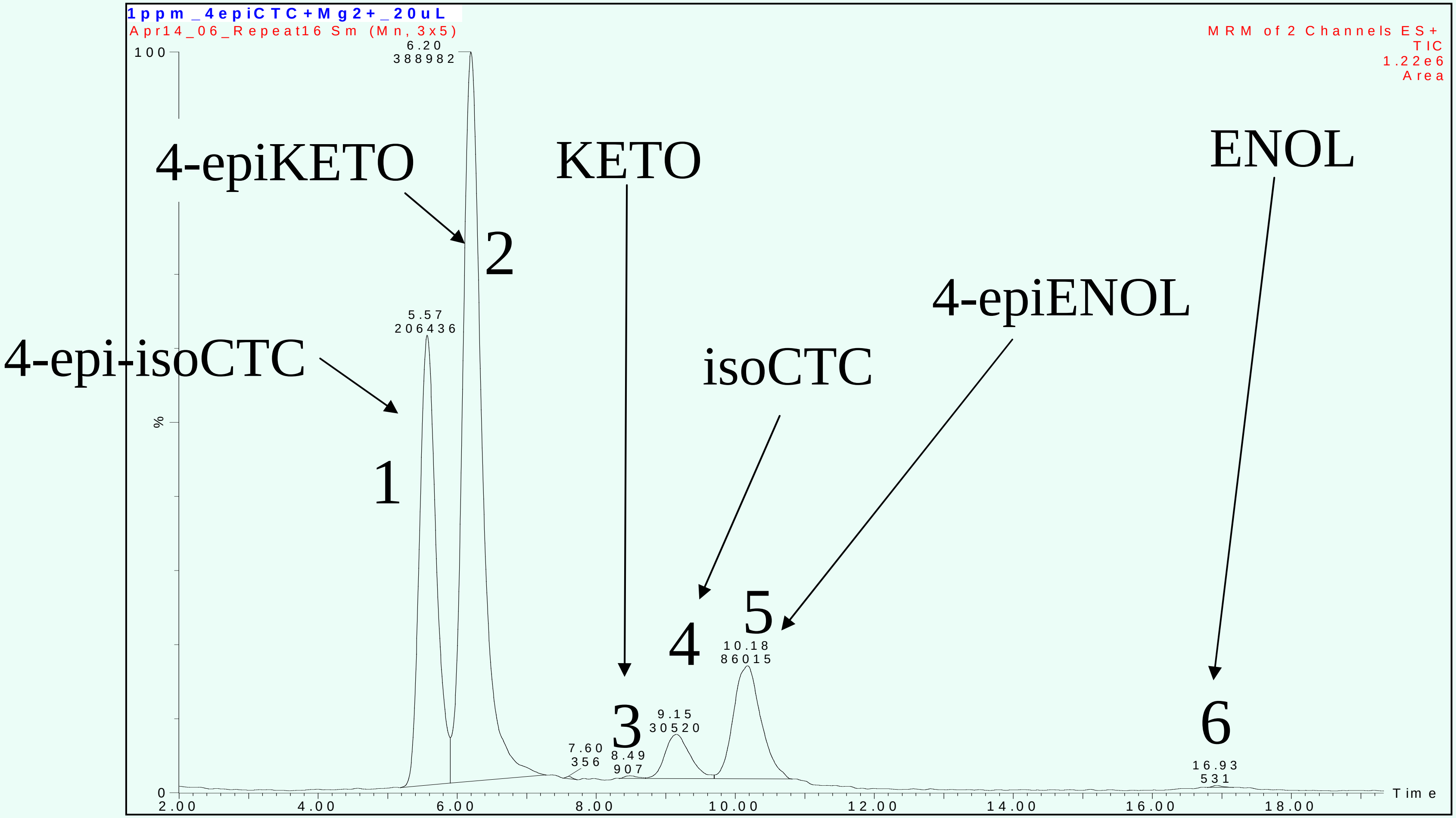
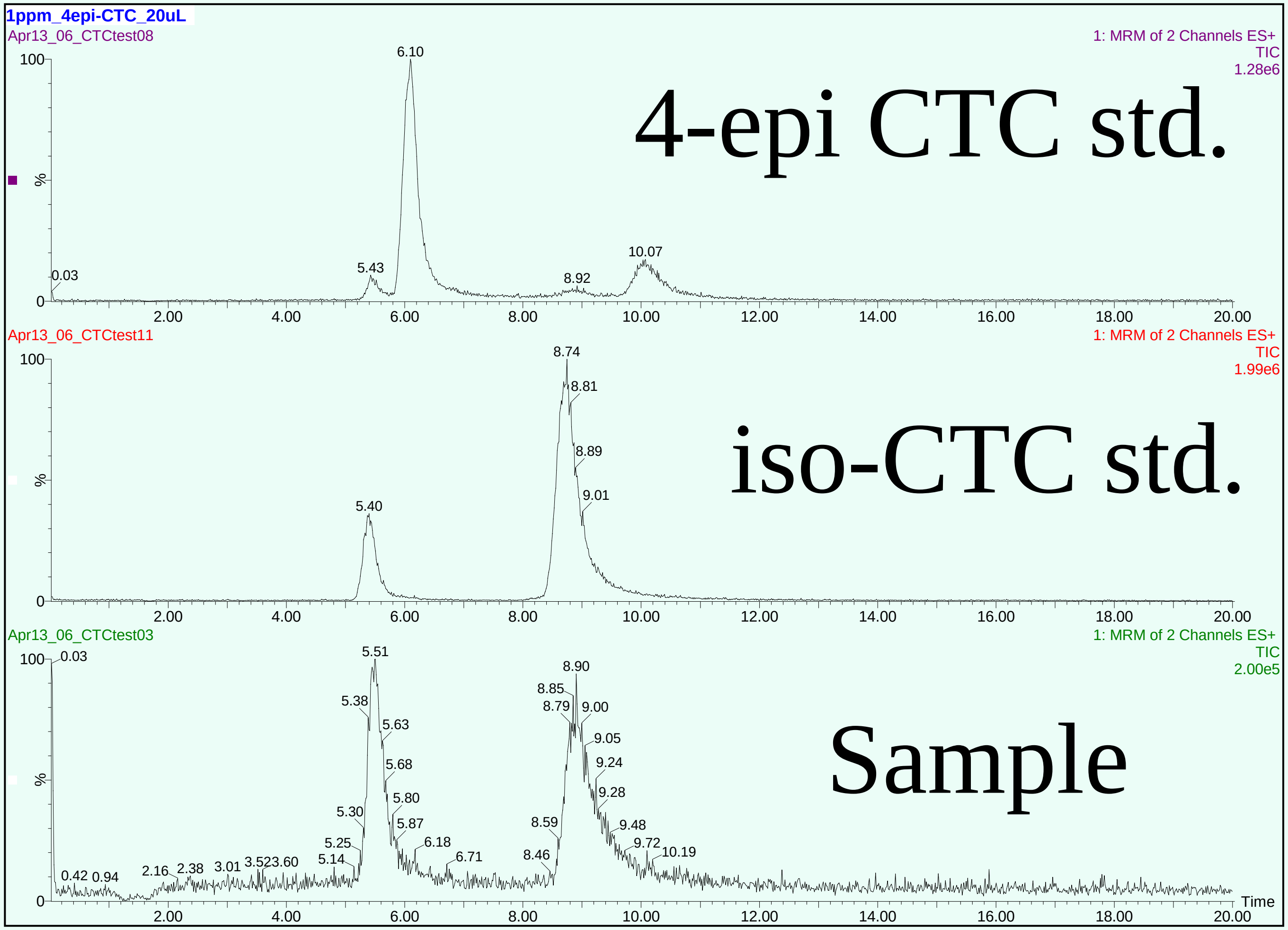
Effect of adding Mg^{2+}



Environmental Sample



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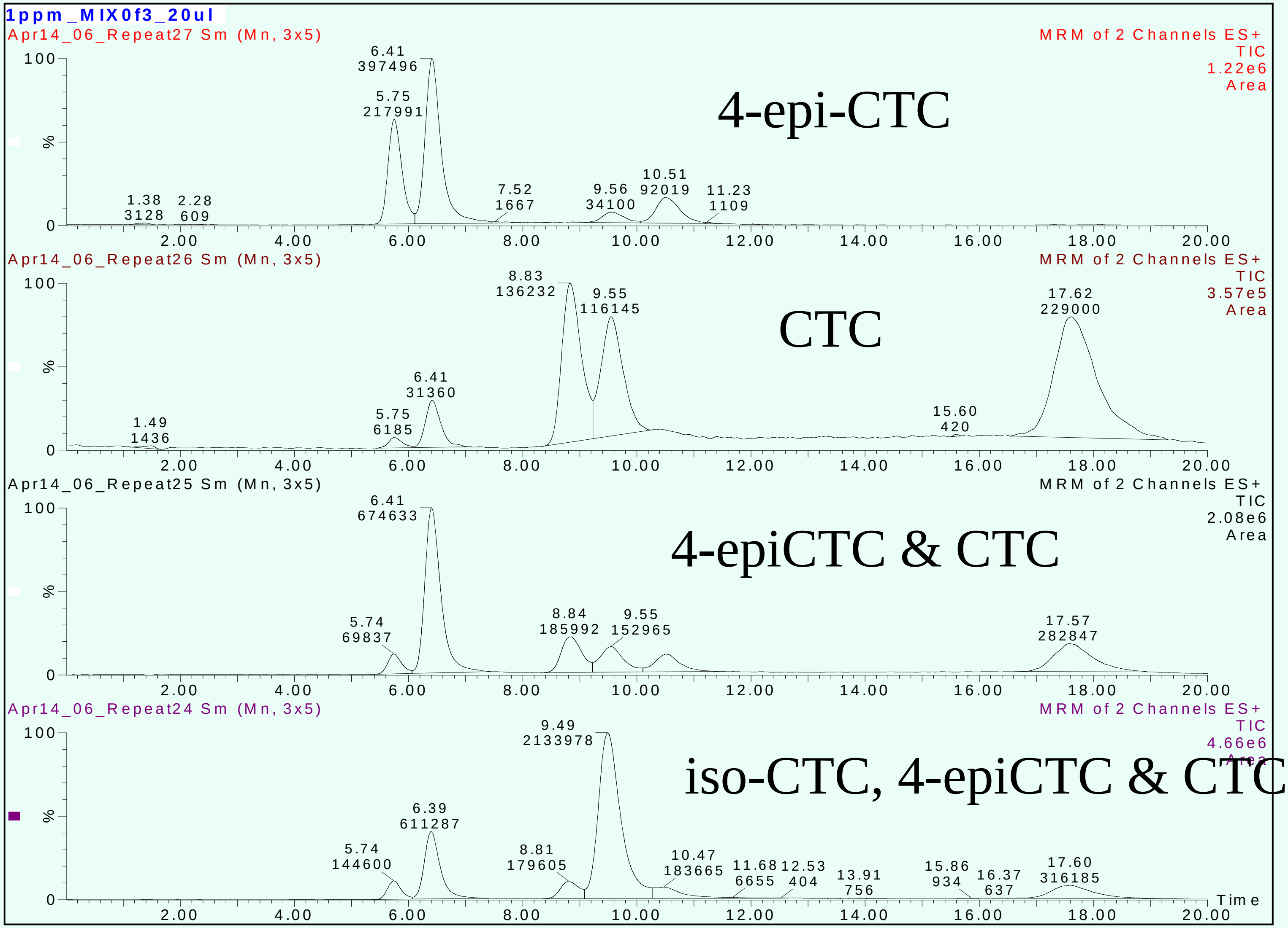
1ppm Standards



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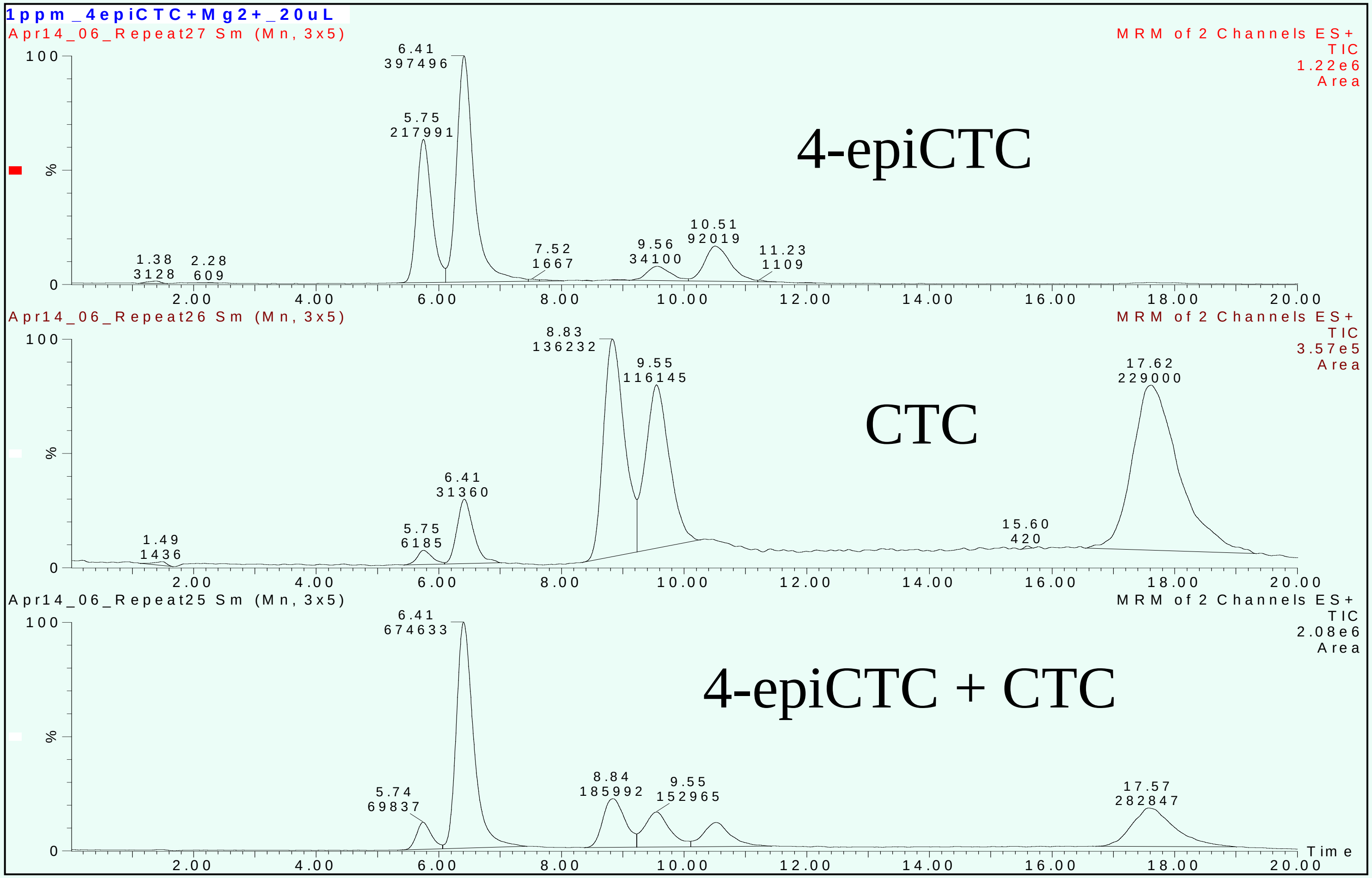
Standards in dd. H2O



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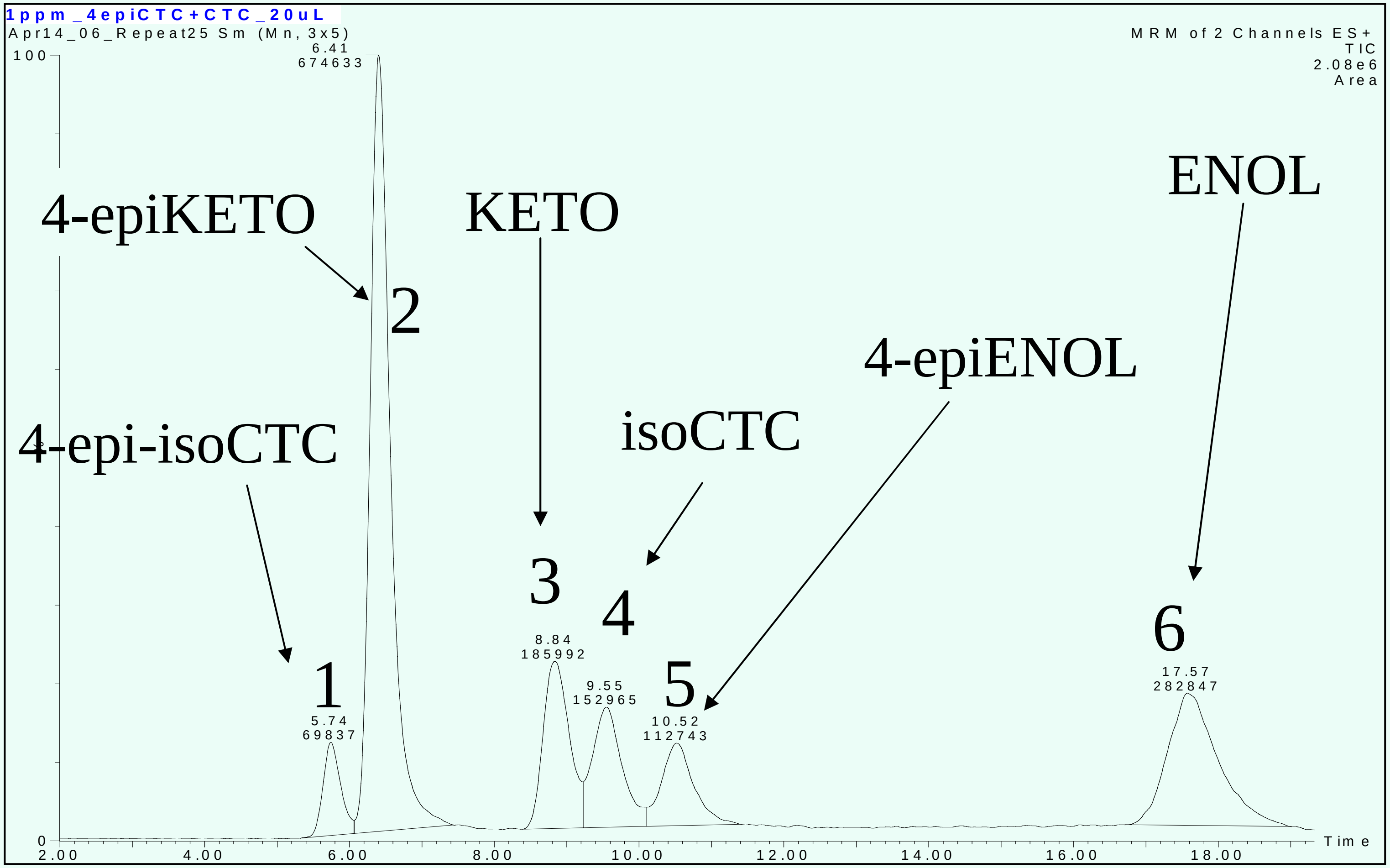
Six Chlortetracycline Isomers



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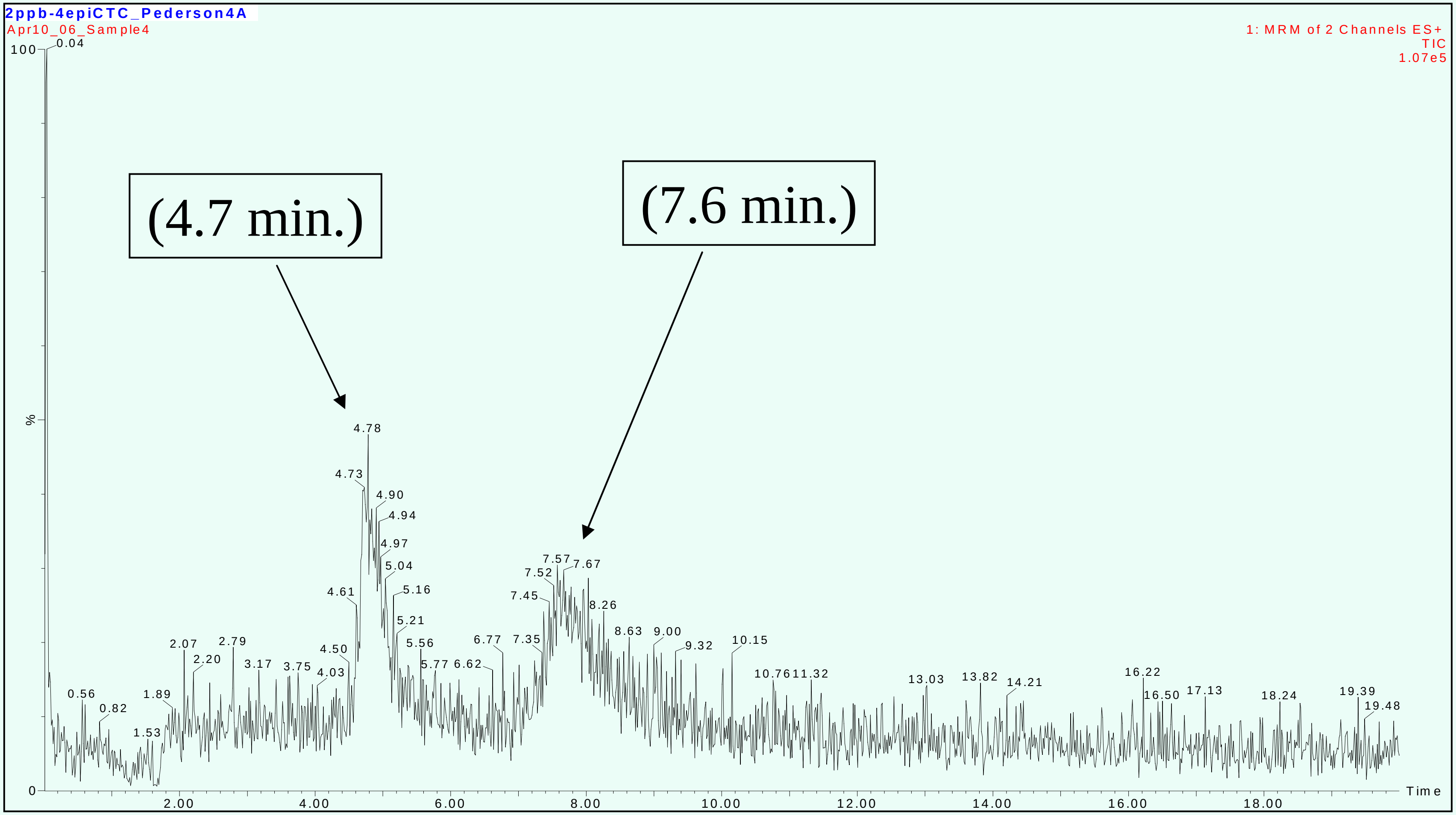
CTC Treated Wetland Sample



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Conclusions

- isomerization complicates the determination of CTC in environmental samples
- Temperature, pH, Mg^{2+} conc. influence CTC isomeric distribution
- Iso-CTC is the most frequently detected form of CTC found in environmental samples

Acknowledgements

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David Gallén, Jennifer Holm, Marley Waiser, Sandra Kuchta