

Incorporating SFC and SFC/MS into Problem Solving in Analytical Drug Development: The future is here and now.

Mark A. Olsen and Emily A. Kordwitz

Cephalon Inc.

CSC

383 Phoenixville Pike

Malvern

PA 19355

Outline

- **Introduction**
- **SFC Capabilities in Analytical Drug Development**
- **Using SFC/ESI/MS and SFC/APPI/MS and deuterated modifiers for structural elucidation**
- **Conclusions**
- **References**
- **Acknowledgements**
- **Questions**

Introduction

- **Analytical Drug Development**
- **Support all small molecule API development**
- **Responsible for support up to and including Phase 3**
- **Responsible for samples from:**
 - > **Medicinal Chemistry**
 - > **Process Chemistry**
 - > **Pharmaceutical Development**

Advantages I won't talk about

- **Speed**
- **Efficiency**
- **Ease of use**
- **Green**

Typical Development Applications

- **Chiral Method Development and Purity Evaluation.**
- **Achiral Method Development Impurity Isolation and Purification**
- **Structural Elucidation and Characterization of unknowns.**
 - > **Molecular Weight**
 - > **Structurally Significant Fragmentation**
 - > **Exchangeable Hydrogens**

Analytical Development SFC Capabilities

Berger Minigram



Worldwide Drug Development

Analytical Development SFC Capabilities

Jasco Analytical System



Worldwide Drug Development

Analytical Development SFC Capabilities

Jasco Prep System



Worldwide Drug Development

Now a little MS primer

- **Why is SFC uniquely compatible with MS?**
- **ESI (Electrospray Ionization)**
- **APPI (Atmospheric Pressure Photo Ionization)**
- **Exchangeable Hydrogens and Deuterated Modifiers**
- **Example of simple chiral separation**

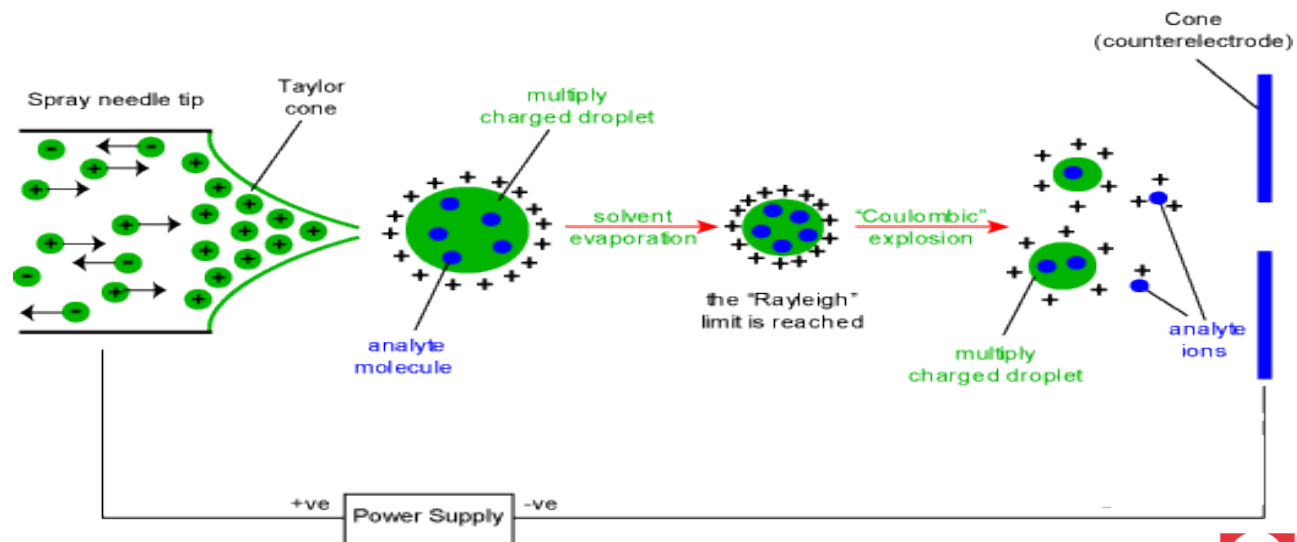
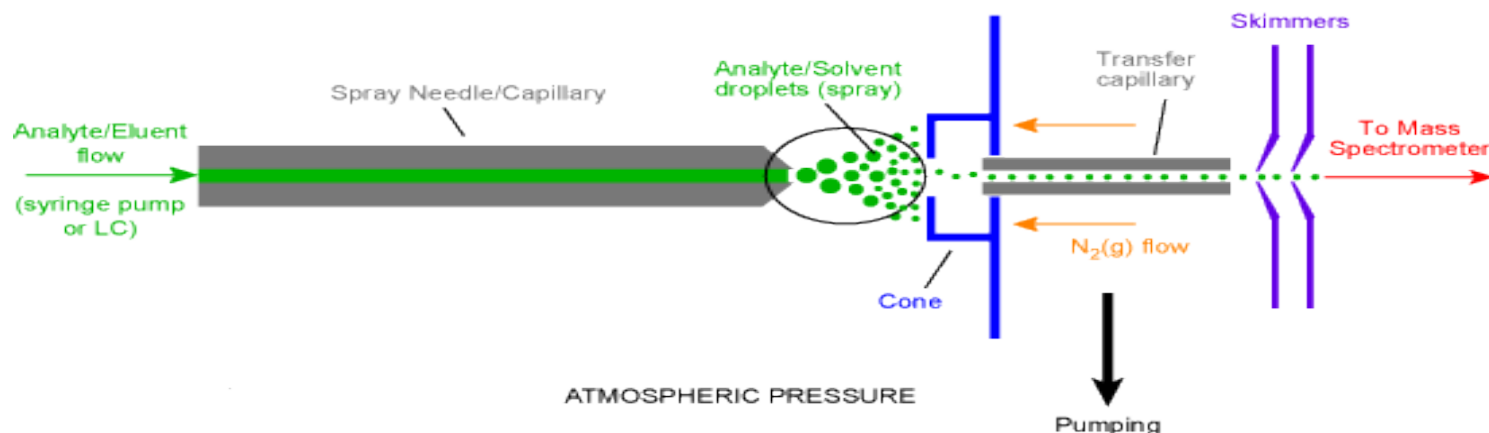
Analytical Development SFC Capabilities

Agilent QTOF MS

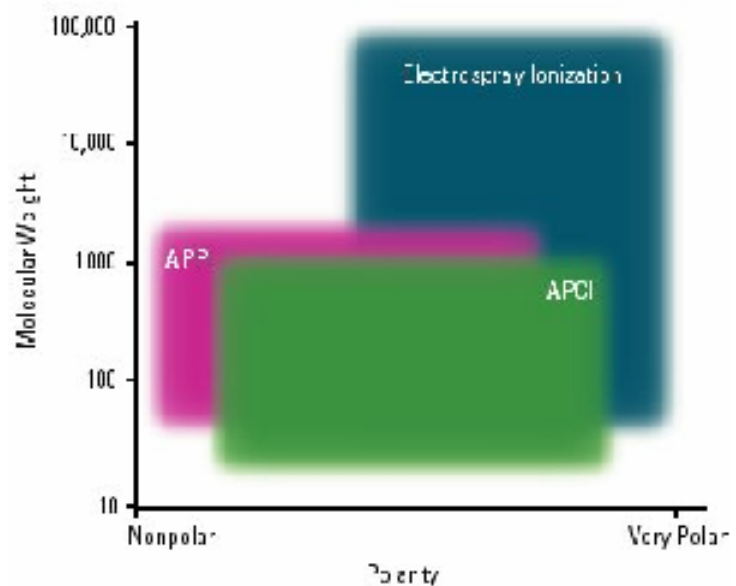
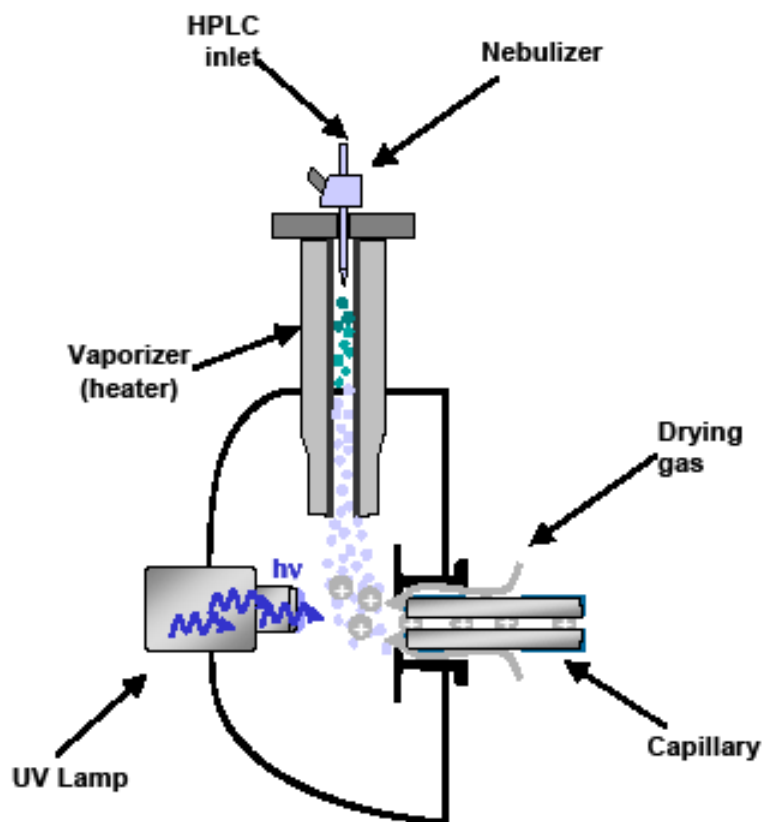


Worldwide Drug Development

Electrospray Ionization (ESI)



Atmospheric Pressure Photoionization (APPI)



Courtesy of "Agilent Technologies. Photomate APPI Tutorial"

A little more about APPI:

- Ionization via APPI is dependent on relative ionization potentials.
- The krypton lamp used in this APPI source typically has energies of 10 eV or 10.6 eV.
- If the solvent's ionization potential is higher than the lamp photon energy, the solvent absorbs a photon but does not ionize, and the energy gained by the solvent gradually disperses. Some solvents that have large ionization potentials are: water (12.6 eV), acetonitrile (12.2 eV) and methanol (10.8 eV).
- If the ionization potential of the solvent is almost equal to or lower than the lamp photon energy, then the solvent will ionize. The ionized solvent thus becomes a charge carrier that can ionize the analyte molecules.
- Some solvents that have small ionization potentials are isopropyl alcohol (10.2 eV), hexane (10.1 eV) and isooctane (9.86 eV).
- Solvent dimers, which have an abundance of 1-100 ppm, have an ionization potential of about 1 eV less than the solvent monomer. Therefore, solvent dimers can aid in ionizing the analyte molecules. For example, the dimer of methanol has an ionization potential of about 9.74 eV, which is usually low enough to ionize analyte molecules (3).

Solution phase chemistry and exchangeable hydrogens

Exchangeable groups:

RCOOH, R-SH, R-OH, -NRH, -NH₂

Deuterated modifiers:

CH₃OD, CH₃CH₂OD, CH₃CH₂CH₂OD, etc

When compounds experience a molar excess of deuterated modifier they exchange.

RCOOD, R-SD, R-OD, -NRD, -ND₂

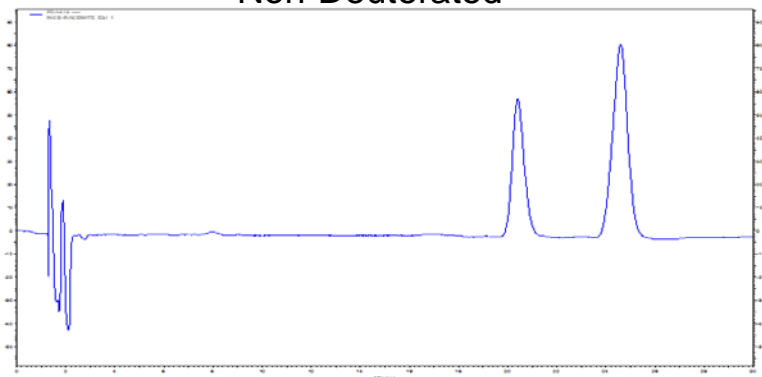
An accurate count of exchangeable hydrogens can be obtained.

Example:

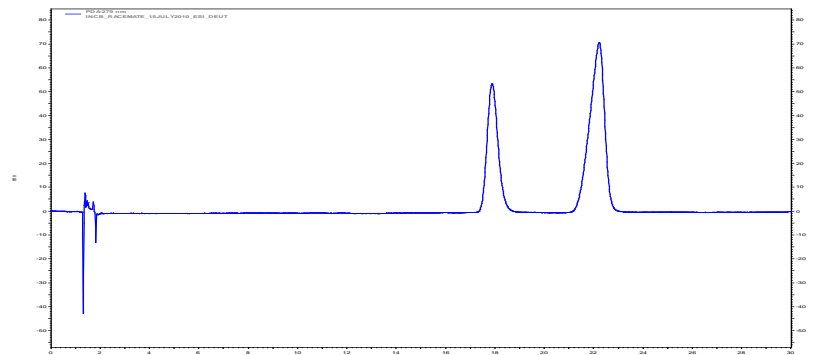
A racemic mixture of an experimental compound was separated by chiral SFC using both a non-deuterated and deuterated modifier. The number of exchangeable hydrogens was determined using both SFC/ESI/MS and SFC/APPI/MS.

SFC Chromatograms

SFC Chromatogram 1
Non-Deuterated



SFC Chromatogram 2
Deuterated



Conditions

Column: ChiralPak AD-H (4.6 x 250 mm), 5u particle
 Pump A: Carbon Dioxide
 Pump B (Modifier): 2-Propanol/0.1%DEA or
 2-Propanol-OD/0.1%DEA
 Mobile Phase: Isocratic 85% Carbon Dioxide and 15% 2-
 Propanol/0.1%DEA
 Total Run Time: 30 min
 Flow Rate: 2.5 mL/min
 Column Temperature: 35°C
 Injection Volume: 10 µL
 UV Wavelength: 220 nm

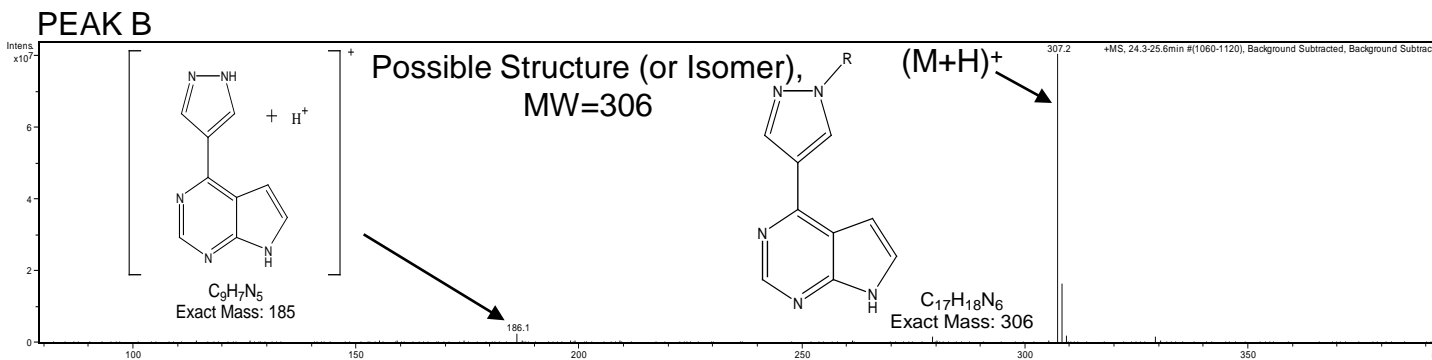
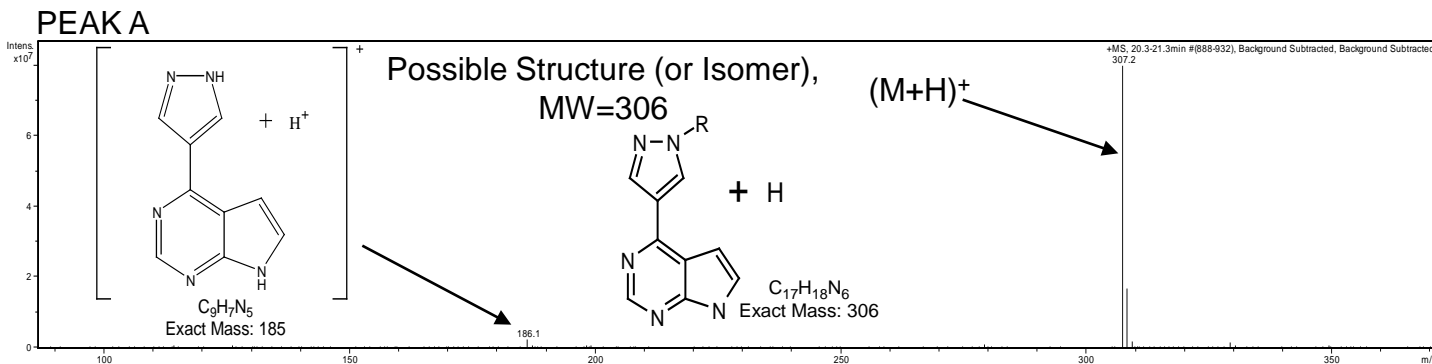
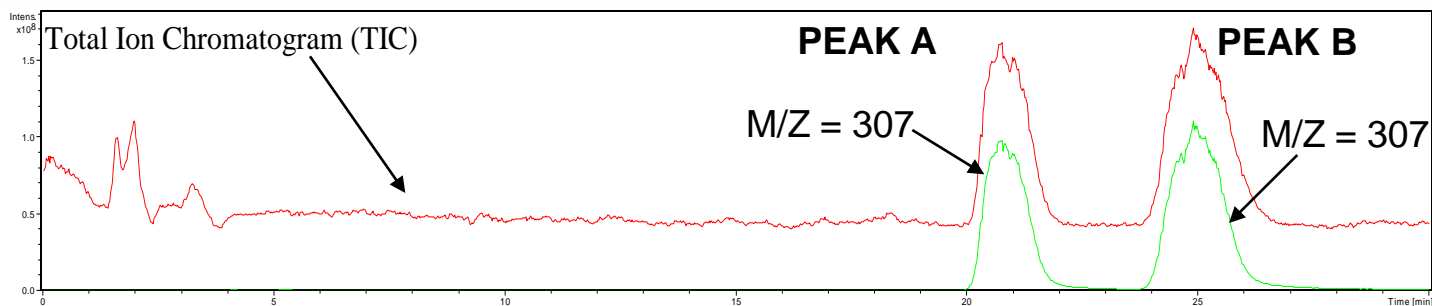
General ESI Conditions

Dry Temperature: 350 °C
 Dry Gas: 12 L/min
 Nebulizer: 60 psi
 Total Run Time: 30 min
 UV Wavelength: 220 nm

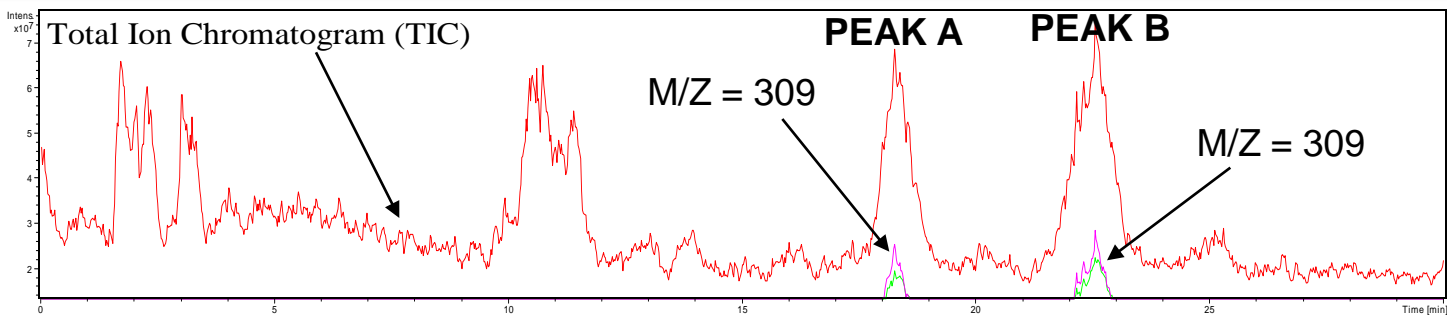
Conditions APPI Conditions

Dry Temperature: 350 °C
 Vaporizer Temperature: 275 °C
 Dry Gas: 12 L/min
 Nebulizer: 60 psi
 Total Run Time: 30 min
 UV Wavelength: 220 nm

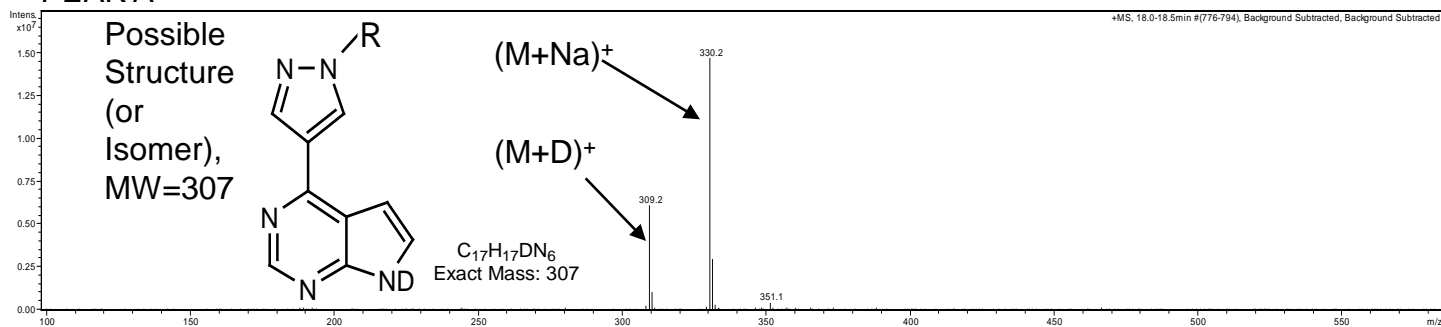
Racemate (SFC/ESI/MS)



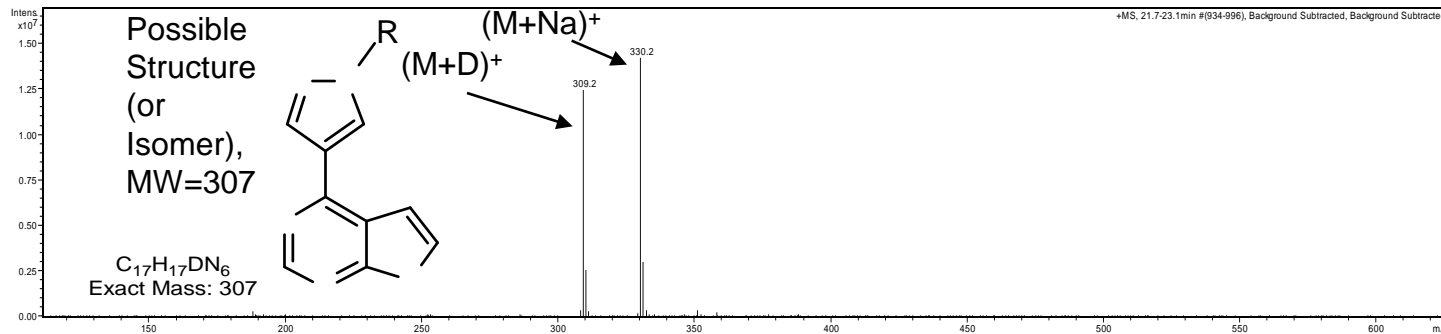
Racemate Deuterated (SFC/ESI/MS)



PEAK A



PEAK B

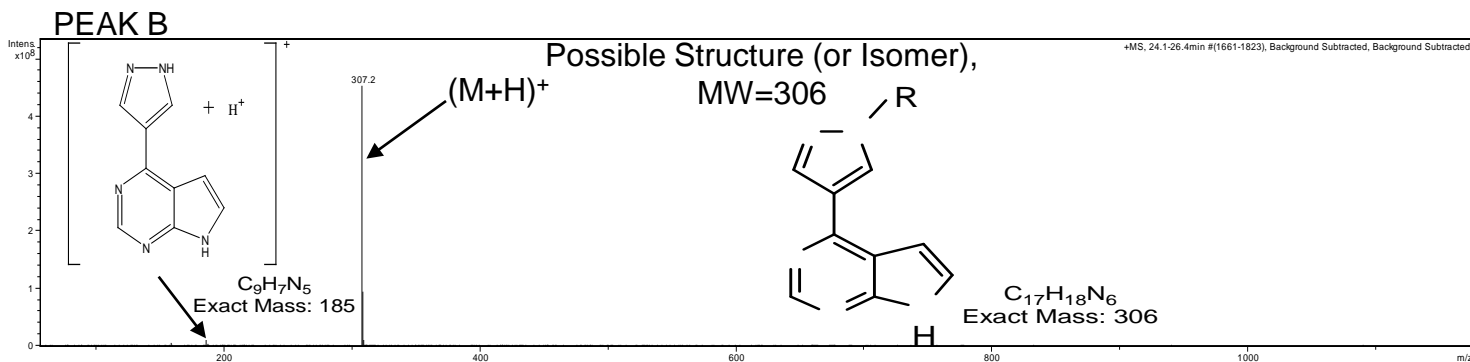
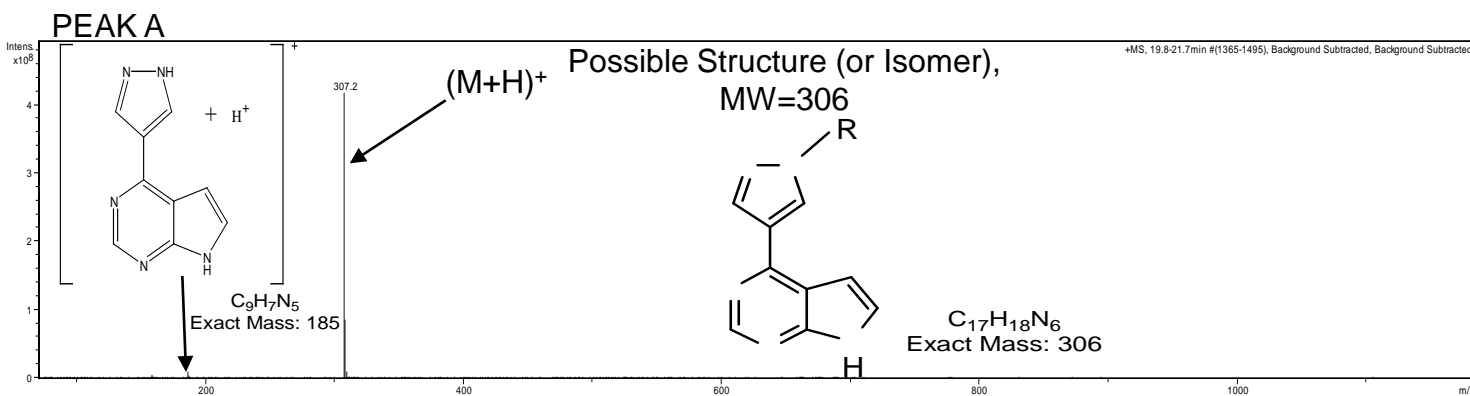
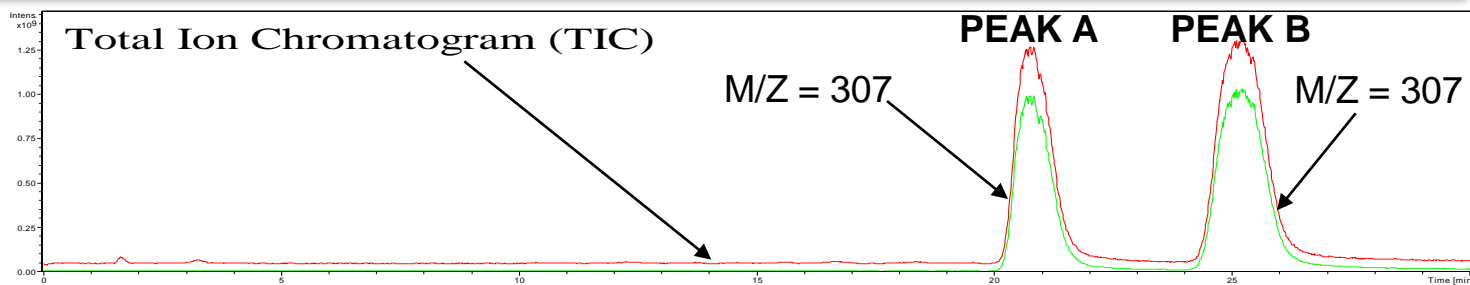


Summary:

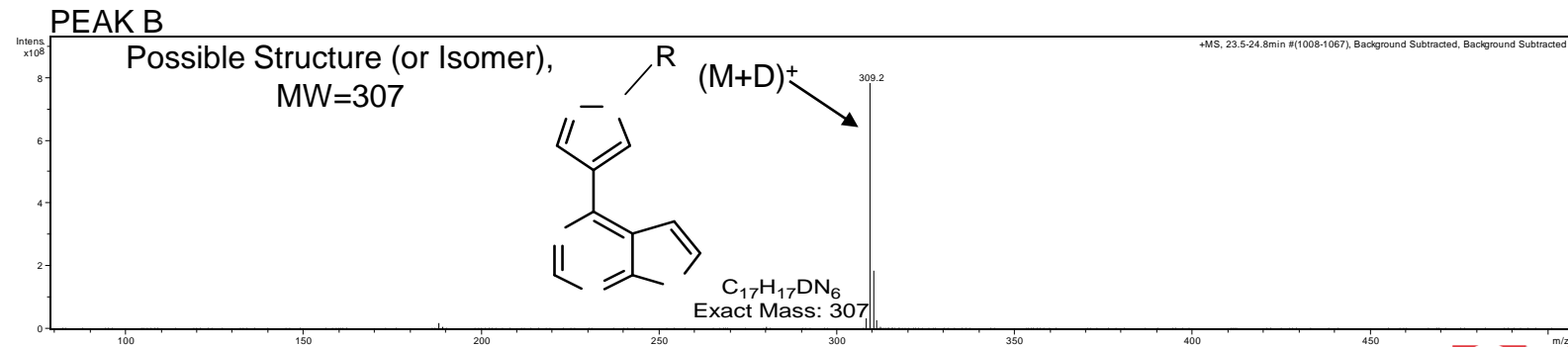
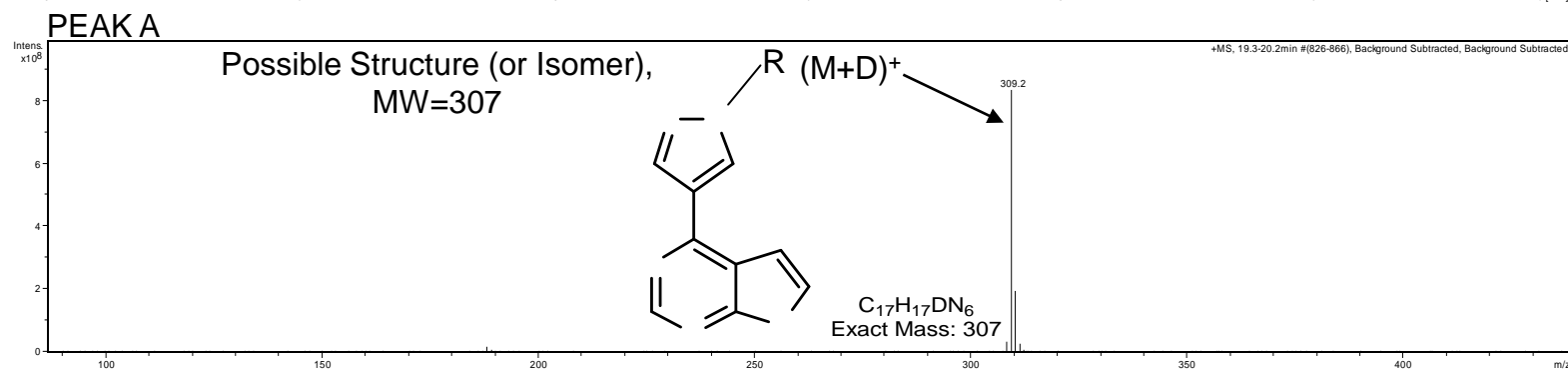
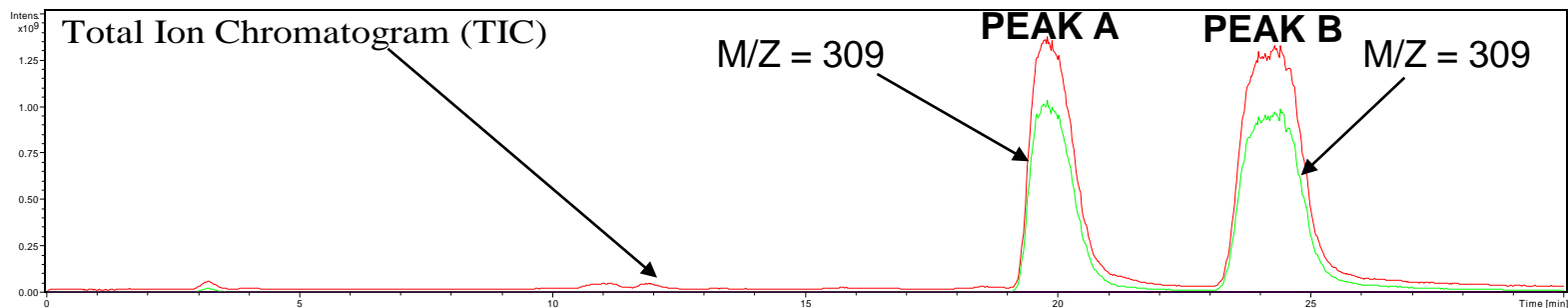
SFC/ESI/MS clearly shows a molecular weight of 306 Da for each enantiomer (PEAK A and PEAK B).

The proposed structures are confirmed by the (M+H)⁺ ion at m/z = 307 Da and the observed fragment ion (186 Da) in each mass spectrum. The deuterium exchange experiment confirms that the structure contains one exchangeable hydrogen [(M+D)⁺ = 309 Da]. Note the low signal to noise ratio in the total ion chromatogram (TIC).

Racemate (SFC/APPI/MS)



Racemate Deuterated (SFC/APPI/MS)



Summary:

Ionization of the enantiomers (Peaks A and B) by APPI gives a molecular weight of 306 Da for each enantiomer. The proposed structures are confirmed by the (M+H)⁺ ion at m/z = 307 Da and the observed fragment ion (186 Da) in each mass spectrum. The deuterium exchange experiment confirms that the structure contains one exchangeable hydrogen [(M+D)⁺ = 309 Da].

Note the much higher signal to noise ratio in the APPI total ion chromatograms (TIC) relative to the ESI chromatograms.

Conclusions:

- SFC is easily interfaced with MS.
- SFC/ESI/MS and SFC/APPI/MS have been shown to be useful for routine structural analysis in a chemical development environment.
- SFC/APPI/MS appears to be more sensitive than SFC/ESI/MS because of low or no charge competition and less ion suppression.
- The number of exchangeable hydrogens, an essential piece of structural information, can be determined readily using deuterated modifiers.
- Genius is one tenth inspiration and nine tenths perspiration - Thomas Edison

References

- (1) Bierbaum, Veronica M., Robert K. Boyd, et al. *What Is Mass Spectrometry?* Santa Fe, NM: American Society for Mass Spectrometry, 1995. Print.
- (2) Berger, Terry A. *Packed Column SFC*. Newark: Royal Society of Chemistry, 1995. Print.
- (3) Syage, J.A., L.C. Short, and S.S. Cai. "APPI: The Second Source for LC-MS." *Chromatography Online - Liquid & Gas Separations. Peer-reviewed Research for Chromatographers & Chemists*. Chromatography Online, 1 Mar. 2008. Web. 22 July 2010.
<<http://chromatographyonline.findanalytichem.com/lcgc/article/articleDetail.jsp?id=504702>>.
- (4) Balogh, Michael P. "Ionization Revisited." *Chromatography Online - Liquid & Gas Separations. Peer-reviewed Research for Chromatographers & Chemists*. Chromatography Online, 1 Dec. 2006. Web. 22 July 2010.
<http://chromatographyonline.findanalytichem.com/lcgc/Hyphenated/Ionization-Revisited/ArticleStandard/Article/detail/393926>>.
- (5) Gates, Paul. "Electrospray Ionisation." *School of Chemistry - Bristol University - UK*. Bristol University, 20 Jan. 2004. Web. 22 July 2010.
<<http://www.chm.bris.ac.uk/ms/theory/esi-ionisation.html>>.
- (6) Agilent Technologies. Photomate APPI Tutorial.
- (7) Poster Presentation 59th ASMS Conference on Mass Spectrometry and Allied Topics Denver, Colorado

Acknowledgements

- Kristin Krupa and Catherine DeBrosse
- Christopher Neville and Gregory Gilmartin
- Analytical and Process Chemistry Groups

Thank you

Questions?



Worldwide Drug Development