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Electrospray LC/MS Response Differences from Equimolar Quantities of Drugs Using a Compact Mass Spectrometer Equipped with Atmospheric Pressure Ionization

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Overview

Purpose: To study the LC/MS ion current responses of an equimolar mixture of nine representative acidic and basic pharmaceutical drug compounds using the expression compact mass spectrometer operated with positive and negative

• electrospray (ESI)
• atmospheric pressure chemical ionization (APCI)

Methods: Sample injection into an analytical HPLC column followed by reversed-phase LC/MS analysis using atmospheric pressure ionization techniques.

Results: APCI and electrospray ionization appear to be complimentary with respect to maximum information for the targeted compounds.

▶ Introduction

The purpose of this study was to evaluate the positive and negative electrospray (ESI) and atmospheric pressure chemical ionization (APCI) LC/MS ion current response from a representative synthetic mixture of standard pharmaceutical compounds.

The selected drug compounds were prepared as an equimolar mixture and analyzed by an optimized gradient LC/MS procedure using a novel compact mass spectrometer (expression, Advion, Inc. Ithaca, NY). The total and extracted ion current chromatographic peaks were compared between electrospray and APCI in both the positive and negative ionization modes.

The results from this study shed some light on the relative merits of APCI vs. ESI as well as the importance of the optimal ionization polarity for the LC/MS determination of the selected acidic and basic small molecule drugs. We also note some differences in adduct formation between these two API techniques.

▶ Methods & Materials

Sample Preparation
• 10 micromoles of each of the nine drugs were weighed and placed in a scintillation vial to which 10 mL of DMSO were added, creating the 'Stock' solution.
• This 'Stock' solution was diluted to a '1 in 10' dilution intermediate solution where 1 mL of the 'Stock' was placed in a separate scintillation vial and diluted with 9 mL of 50/50 Methanol/Water.
• The above 'intermediate' solution was further diluted '1 in 10' again to the final diluted solution by diluting 1 mL of the intermediate solution with 9 mL of 50/50 Methanol/Water in a separate scintillation vial.

Instrumentation
• Advion expression Compact Mass Spectrometer equipped with a Phenomenex Luna C-18(2) 2.0 x 100 mm HPLC column packed with 3 micron particles.
• HPLC System: Shimadzu LC-10A binary pumps
• Gradient: 95/5 water/MeOH with 0.1% formic acid, isocratic for 1 min, then gradient elution over 5 min to 5/95 water/MeOH with 0.1% formic acid, isocratic for 1 min and then the gradient recycled over 1 min to initial LC conditions.
   – For ESI Negative mode formic acid was replaced by 0.1% NH4OH
   – For APCI Pos and Neg ionization the above additives were replaced by 10 mM NH4OAc.
• Flow rate maintained at 0.2 mL/min for each experiment.

Experimental for Ionization and HPLC

• Phenomenex 2.0 x 100 mm 3 micron C-18(2) Luna
   – Positive ESI LC/MS
      • 95/5 water/MeOH with 10mM, 0.1% formic acid, isocratic for 1 min, gradient to 5/95 over 11 min, isocratic for 1 min, then recycle.
   – Negative ESI LC/MS
      • 95/5 water/MeOH with 10mM, 0.1% NH4OH, isocratic for 1 min, gradient to 5/95 over 11 min, isocratic for 1 min, then recycle.
   – Positive and Negative APCI LC/MS
      • 95/5 water/MeOH with 10mM, 10mM NH4OAc, isocratic for 1 min, gradient to 5/95 over 11 min, isocratic for 1 min, then recycle.
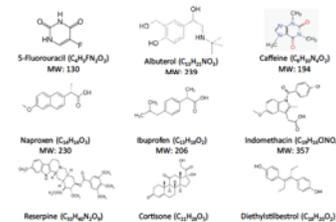
System Configuration
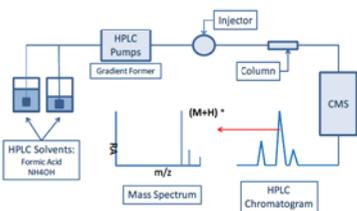
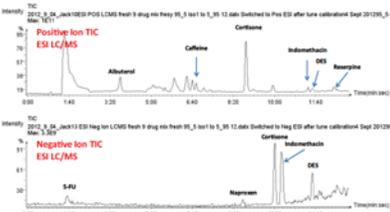## expression Compact Mass Spectrometer: Complete LC/MS System on a Laboratory Cart

66 cm/ 26 in.
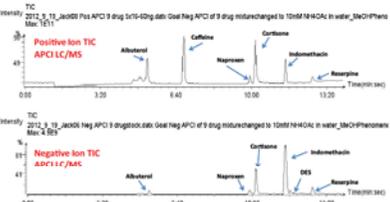28 cm/11 in.   56 cm/22 in.

Structures of 9 Drugs in Mixture

5-Fluorouracil (C₄H₃FN₂O₂) MW: 130
Albuterol (C₁₃H₂₁NO₃) MW: 239
Caffeine (C₈H₁₀N₄O₂) MW: 194

Naproxen (C₁₄H₁₄O₃) MW: 230
Ibuprofen (C₁₃H₁₈O₂) MW: 206
Indomethacin (C₁₉H₁₆ClNO₄) MW: 357

Reserpine (C₃₃H₄₀N₂O₉) MW: 608
Cortisone (C₂₁H₂₈O₅) MW: 260
Diethylstilbestrol (C₁₈H₂₀O₂) MW: 268

▶ Results

ESI TIC's for Positive and Negative LC/MS

Positive Ion TIC ESI LC/MS

Negative Ion TIC ESI LC/MS

APCI TIC's for Positive and Negative LC/MS

Positive Ion TIC APCI LC/MS

Negative Ion TIC APCI LC/MS

Comparison of ESI and APCI LC/MS

ESI and APCI TIC'S for Positive Ion LC/MS

ESI Positive
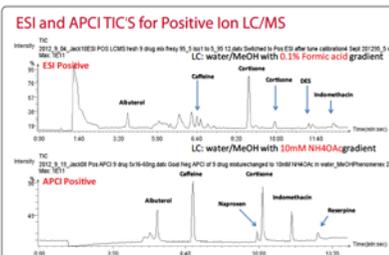
APCI Positive

ESI and APCI TIC's for Negative Ion LC/MS

ESI Negative
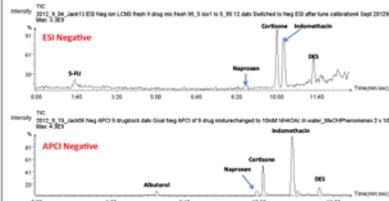
APCI Negative

ESI and APCI Positive Ion Cortisone Mass Spectra
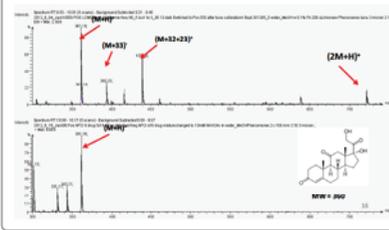
Summary Results

Detection by LC/MS

Drug	ESI Pos	ESI Neg	APCI Pos	APCI Neg
5-FU	No	Yes	No	No
Albuterol	Yes	No	Yes	Yes
Caffeine	Yes	No	Yes	Yes
Naproxen	No	Yes	Yes	Yes
Ibuprofen	No	No	No	No
Cortisone	Yes	Yes	Yes	Yes
Indomethacin	Yes	Yes	Yes	Yes
DES	Yes	Yes	No	Yes
Reserpine	Yes	Yes	Yes	Yes

▶ Summary

– No single ionization mode detected all nine compounds
– The response of the various compounds differs dramatically both within and between the different ionization modes.
– Positive and negative ionization benefit from different additives in mobile phase for analyte detection
– Ibuprofen was the only compound NOT detected by any mode
– Electrospray tends to produce adducts whereas APCI does not
– Changing the additive to affect ionization affects HPLC elution order
– Mass spectrometric detection greatly facilitates identifying each HPLC peak

Conclusions

• An equimolar mixture of nine dissimilar but common drugs produced different LC/MS chromatographic responses due to the breadth of acidic and basic nature of the analytes.
• A mixture of acidic and basic chemical compounds was separated and analyzed by LC/MS using a combination of positive and negative electrospray and atmospheric pressure chemical ionization techniques.
• An inexpensive compact single quadrupole mass spectrometer can provide routine LC/MS analysis with significantly more analytical information than conventional spectroscopic detectors.

References

[1]G. Rule, J. Henion, J Am Soc Mass Spectrom 10 (1999) 1322.
[2]K. Heinig, J. Henion, J Chromatogr B Biomed Sci Appl 732 (1999) 445.
[3]L.J. Jessome and D.A. Volmer, Ion suppression: a major concern in mass spectrometry. LCGC N. Am., 2006, 24,(5) 498-510.
[4]B. K. Matuszewski, M.L. Constanzer, and C.M. Chavez-Eng. Strategies for the assessment of matrix effects in quantitative bioanalytical methods based upon HPLC MS/MS. Anal. Chem., 2003, 75, 3019-3030.
[5]T. R. Covey, B.B. Schneider, H. Javaheri, J.C. Yves LeBlanc, J.J. Corr, and P. Kovarik, ESI, APCI and MALDI: A comparison of the central analytical figures of merit: sensitivity, reproducibility, and speed. Chapter 13 in Electrospray and MALDI Mass Spectrometry: Fundamentals, Instrumentation, Practicalities, and Biological Applications, Second Edition, Edited by Richard B. Cole, Copyright 2010, John Wiley & Sons, Inc.