Electron Capture Negative APCI LC/MS/MS Analysis of Prostaglandins, Estradiol and Vitamin D$_2$/D$_3$

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MPL 368

ABSTRACT

Cationic classes of compounds are traditionally very difficult to ionize or tend to show low sensitivity in LC/MS/MS techniques. Electron capture negative APCI is a technique that has provided a path to increased sensitivity for these tough to ionize compounds.

Using an API-4000™ and a modified source housing, this study compares the analysis of several compounds under conventional negative APCI and electron capture negative APCI.

RESULTS

ABSTRACT

Figure 1. Prostaglandin Structures

4.35 2.26 5.67 0.58

INTRODUCTION

In atmospheric pressure chemical ionization (APCI), the cosine discharge can provide a source of electrons in the gas phase. This can be an advantage for some compounds that can undergo electron capture and also for those that are ionized by electron capture. Negative ionization can be particularly useful for compounds such as the prostaglandin (PG) group. These derivatized compounds generate negative ions through the loss of the PFB group, which leads to an increase in the overall MS response. This is an advantage for many ‘tough to ionize’ compounds that cannot be analyzed by LC/MSMS techniques. Typically, compounds the prostaglandins, are analyzed by GC/MS which require extensive sample preparation and long analysis times. 6-keto-PGF$_1$ and 2,3-dinor-6-keto-PGF$_1$ are two components that are present in biological tissues and fluids in picomolar to low nanomolar levels. Quantitation of these prostaglandins in a complex biological matrix, such as urine, is challenging. This study compares the analysis of several prostaglandins, including 2,3-dinor-PGF$_1$, thromboxane B$_2$, and derivatization methods. The derivatization of these compounds was also evaluated as well as 6-keto-PGF$_1$ and 2,3-dinor-6-keto-PGF$_1$ from urine samples. 3-estradiol and vitamin D$_2$ derivative were also tested for sensitivity.

MATERIALS AND METHODS

Prostaglandin stock solutions were prepared (Cayman Chemical), as well as the derivatized version (with pentafluorobenzyl bromide) (see Figure 1 and 2). Under optimal LC/MSMS conditions for both reversed phase and normal phase chromatographic conditions, the performance was evaluated on flow injection data from equivalent concentrations. The most abundant single negative transition for each prostaglandin compound was used. Under conventional negative APCI mode, typical needle currents of 3 to 6 amps were employed. For analysis of the PFB derivatives, an external power supply (Spellman) provided current up to 30 amps. The API-4000™ source housing was modified to allow for maximum needle position adjustment for maximum needle position adjustment (see Figure 3).

Conventional -ve APCI:

- standard solutions were prepared by serial dilution in 50/50 ACN/H$_2$O
- Mobile Phase; 50/50 ACN/H$_2$O @ 1.0 mL/min
- 5 L FIA
- 5 L injection, 5 pg on column

Electron Capture -ve APCI:

- 5 L FIA
- Mobile Phase; 50/50 ACN/H$_2$O @ 1.0 mL/min
- 5 L injection, 5 pg on column
- 30 µL FIA
- 5 L injection, 5 pg on column

On-column analysis for 6-keto-PGF$_1$ and 2,3-dinor-6-keto-PGF$_1$ from urine samples employed gradient mode reverse phase chromatography @ 300 µL/min with a post column addition of 50/50 ACN/H$_2$O @ 1.0 mL/min. The corresponding derivatized PFB derivatives were monitored.

RESULTS

ABSTRACT

Figure 2. Example of PFB Derivatization

<table>
<thead>
<tr>
<th>Peak Int. (Subt.)</th>
<th>Area (counts)</th>
<th>1 Spike urine samples with D$_3$/D$_4$ compounds</th>
<th>2 Solid Phase Extraction</th>
<th>3 Incubation with methoxyamine HCl</th>
<th>4 TLC</th>
<th>4 Reconstitution and injection</th>
<th>5.23 8.36 10.45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S/N = 26.6</td>
</tr>
</tbody>
</table>

Figure 3. Source Housing Modification

A slight modification to the API-4000™ source housing included a more gentle cosine discharge (i.e., 5.0 L injection) using the same amount of power supply. A slight modification to the API-4000™ source housing included a more gentle cosine discharge (i.e., 5.0 L injection) using the same amount of power supply (see Figure 3). The average sensitivity increase for compounds that have been a challenge to us under conventional means. For compounds such as prostaglandins, estradiol and vitamin D$_2$/D$_3$ derivatives, that have traditionally been analyzed by GC/MS techniques, electron capture LC/MS/MS is rapidly approaching the sensitivity levels required to compete. While the sensitivity is getting closer, the sample preparation and analysis times are much less with the LC/MS/MS technique. For GC/MS analysis, a 2-day sample preparation is required, along with a 30-minute analysis time. A factor of 2-3 in both sample preparation and analysis time is realized with LC/MS/MS.

CONCLUSIONS

Electron capture negative APCI has provided at least an order of magnitude level of sensitivity increase for compounds that have been a challenge to us under conventional means. For compounds such as prostaglandins, estradiol and vitamin D$_2$/D$_3$ derivatives, that have traditionally been analyzed by GC/MS techniques, electron capture LC/MS/MS is rapidly approaching the required sensitivity levels required to compete. While the sensitivity is getting closer, the sample preparation and analysis times are much less with the LC/MS/MS technique. For GC/MS analysis, a 2-day sample preparation is required, along with a 30-minute analysis time. A factor of 2-3 in both sample preparation and analysis time is realized with LC/MS/MS.

REFERENCES


TRADEMARKS/LICENSING

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