

**TECHNIQUES FOR UPLC-UV/MS METHOD DEVELOPMENT OF A PHARMACEUTICAL PURITY ANALYSIS**

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**Introduction**

Improper profiling of pharmaceutical drug solutions or dosage form impurities requires significant chromatography and sample preparation expertise. In today’s drug development pace, LC/MS is often used as a standard impurity and degradant detection tool. LC/MS requires components to develop systems to analyze a specific drug or its impurities. Quality control and regulatory specifications are typically used to select detection limits and resolution conditions. Method development pace of today’s pharmaceutical pipeline.

**Experimental**

**Approach**

The ideal approach would be to have authentic impurity standards and prepare standard solutions for the method development. In many situations, impurity standards are not readily available and formal degradants of the drug substance in an appropriate starting point is generated for degradation from heating or reaction. This is typically a mixture of this starting material, and final products of the drug substance that were applied. Depending on the drug substance, it is typically required in a mixture of several steps from initial degradation to product isolation.

**Column Scouting**

The different column choices should be run under identical detection conditions. The major impurities are labeled A-J. The scouting gradient used with 200/500 nanomolar concentrations (g/ml were performed). Initial results from the phase I selection chemistry provide the best conditions. However, the HPLC CI was chosen due to the overall phase I and phase II conditions and the resolution of the methods. In this initial scouting, the UPLC–MS/MS (with the HPLC–MS/MS) was used as the validation method of choice and optimized under the tested conditions.

**Manipulating Selectivity**

Exploring various temperature, 

**Temperature Scouting**

When compared to the column scouting results, selectivity is entirely manipulated. In a temperature scouting, different temperatures for method optimization after choosing a column and pH and at which all other development options have been established.

**Table: Selection of temperature parameters**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Column: XTerra® MS C18</th>
<th>pH</th>
<th>Mobile Phase</th>
<th>Flow Rate</th>
<th>Injection Volume</th>
<th>UPLC Conditions</th>
<th>MS Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>4.0</td>
<td>64.0</td>
<td>F</td>
<td>14.0</td>
<td>30.0</td>
<td>300µL</td>
<td>500µL</td>
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</tbody>
</table>

**Method Development Tools**

**Single Column**

A single column API was configured with the MS for peak tracking and method optimization. A positive/ negative ion mode was used to create the master ion of the parent compound. To monitor the peak integrity and stability, the method was used with a low mass limit to equal energy. 0.025/0.025 peak height should remain constant.

**Method Optimization Utilizing Chromatography Simulation Software**

UPLC bridged hybrid chemistry to that of the closest comparative available HPLC column was used for successful predictions. Co-eluted peaks of each injection using the MS data can lead to more successful predictions. 0.0025in peek tubing would be ideal.

**Method Evaluation**

Chromatography simulation software can expedite much of the initial scouting and optimization using chromatography simulation software to achieve the same resolution between any critical pair in HPLC or UPLC resulted in a **"Win" scenario#2:** As seen in the example below, increasing temperature resulted in increased peak intensity and allows for faster flow rates resulting in faster run time.

**References**


**DISCUSSION AND CONCLUSIONS**

One subject that was well addressed was the choice of temperature and increasing temperature resulted in increased peak intensity and allows for faster flow rates resulting in faster run time. There were other observations when performing the UPLC approach. Peaks are very numerous which may require high sampling rates. Sensitivity could also be achieved with specific peaks present.

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