Dextrorphan Metabolism Profiling by the Analysis of Dried Matrix Samples Collected from Liver Microsomes In-Vitro Fluid and Analyzed by MFLC-MS/MS

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Overview

Purpose – To demonstrate if the benefits of Dried Matrix Spotting (DMS) techniques could be used to halt the CYP2D6 metabolism of Dextrorphan to Dextrorphan.

Methods – Time course of Dextrorphan in activated human liver microsomal incubations collected with DMS and extracted with methanol, followed by MFLC-MS/MS (ABSciex 5500® QTRAP coupled with an eksigent™ microLC 200 System).

Results – DMS can be used to perform the metabolic profiling of Dextrorphan. MFLC provided nearly a five-fold increase in Dextromethorphan response when compared to conventional HPLC.

Introduction

Dextrorphan is a safe, readily accessible, antitussive that has a well characterized metabolic pathway in humans. The CYP2D6 mediated O-demethylation to Dextrorphan is well characterized. Typical activated human liver microsomal incubations (HLMs) Dextromethorphan metabolism experiments require the addition of large volumes of organic solvents or acid in order to stop the CYP2D6 transformation to Dextrorphan. Experiments were conducted in order to determine if the benefits of Dried Matrix Spotting (DMS) techniques could be used to halt the CYP2D6 mediated activity. In order to develop a more sensitive method, microflow liquid chromatography coupled with a mass spectrometer (MFLC-MS/MS) was utilized for the determination of Dextromethorphan and Dextrorphan in the HLMs in-vitro fluid.

The Dextromethorphan HLMs in-vitro solution was incubated at 37°C for 0, 15, 30, 45, and 60 minutes prior to either spotting on the DMS card or aliquoting into a tube. Once placed into the tube, acetonitrile was immediately added to stop the Dextromethorphan conversion to Dextrorphan.

Methods

DMS Extraction

- Dextromethorphan and Dextrorphan extracted from 37°C activated HLMs in-vitro fluid DMS
- Card type: FTA DMPK-C (GE Healthcare) containing Alturas Analytics CID #1 for visual spot verification (see Figure 1)
- Sample volume: 25 µL
- Punch diameter: 6 mm
- Internal standard: 20 µL of Propranolol added to spot
- Solvent: Methanol

Conventional Precipitation Extraction

- Dextromethorphan and Dextrorphan extracted from 37°C activated HLMs in-vitro fluid DMS
- Sample volume: 25 µL
- Internal standard: 20 µL of Propranolol added to sample aliquot
- Solvent: Acetonitrile

MFLC

- eksigent™ microLC 200 System
- Shimadzu LC-20AD using acetonitrile and water with 1% formic acid
- Flow rate: 45 µL/minute
- Column: ProntoSIL (MAC-MOD) 120-3-C18-EPS 3 µm, 50 mm x 0.5 mm
- Column temperature: 50°C

Conventional HPLC

- Shimadzu LC-20AD using acetonitrile and water with 1% formic acid
- Flow rate: 700 µL/minute
- Column: ProntoSIL (MAC-MOD) 120-3-C18-EPS 3 µm, 50 mm x 2.0 mm
- Column temperature: 50°C

MS

- AB SCIEX QTRAP® 5500 operating in MRM mode
- ESI
- Positive ion mode
- MRM transitions:
  - Dextromethorphan: 272.0 → 147.0
  - Dextrorphan: 258.0 → 133.0
  - Propranolol: 280.3 → 116.2

Results

- DMS can be used to stop the Dextromethorphan conversion to Dextrorphan

Table 1: Dextromethorphan Signal Comparison of Conventional HPLC and MFLC

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>Shimadzu HPLC 700 µL/Min</th>
<th>Eksigent MFLC 45 µL/Min</th>
<th>MFLC Signal Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Height</td>
<td>0.9 E4</td>
<td>4.2 E4</td>
<td>4.7 X</td>
</tr>
<tr>
<td>Peak Area</td>
<td>2.1 E4</td>
<td>8.6 E4</td>
<td>4.1 X</td>
</tr>
</tbody>
</table>

Table 2: Dextromethorphan In-vitro Transformation Comparison of Acetonitrile Crash and DMS

<table>
<thead>
<tr>
<th>Incubation Time Prior to Spotting or Acetonitrile Crash (min)</th>
<th>Acetonitrile Crash Difference from T=0 (%)</th>
<th>DMS Dextromethorphan Difference from T=0 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-23.3</td>
<td>-25.7</td>
</tr>
<tr>
<td>30</td>
<td>-54.0</td>
<td>-52.1</td>
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<tr>
<td>45</td>
<td>-74.0</td>
<td>-73.1</td>
</tr>
<tr>
<td>60</td>
<td>-82.8</td>
<td>-80.3</td>
</tr>
</tbody>
</table>

Conclusions

- DMS can be utilized to perform in-vitro metabolite profiling experiments instead of the traditional organic crash methods.
- The MFLC provided nearly a five-fold increase in Dextromethorphan response when compared to conventional HPLC.
- DMS may facilitate long term storage of samples and minimize potential liquid solubility issues.

References