Dextromethorphan 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 Dextromethorphan to Dextrorphan.
- **Methods** – Time course of Dextromethorphan in activated human liver microsomal incubations collected with DMS and extracted with methanol, followed by MFLC-MS/MS (ABSciex 5500® QTRAP coupled with an ekspert™ microLC 200 System).
- **Results** – DMS can be used to perform the metabolic profiling of Dextromethorphan. MFLC provided nearly a five-fold increase in Dextromethorphan response when compared to conventional HPLC.

Introduction

Dextromethorphan 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[1]. 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, micro-flow 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 37ºC for 0, 15, 30, 45, and 60 minutes prior to either spotting on the DMS card or aliquotting 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

- ekspert™ microLC 200 System Gradient MFLC 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: 260.3 → 116.2

Table 1: Dextromethorphan Signal Comparison of Conventional HPLC and MFLC

	Shimadzu HPLC 700 µL/min	Eksigent MFLC 45 µL/min	MFLC Signal Gain
Peak Height	0.9 E4	4.2 E4	4.7 X
Peak Area	2.1 E4	8.6 E4	4.1 X

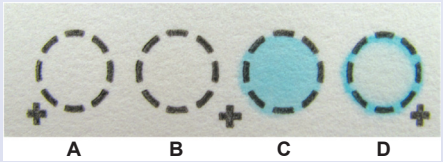


Figure 1: FTA DMPK-C Card

(A) No color-indicating dye or sample spot,
(B) No color-indicating dye with sample spot,
(C) Color-indicating dye with no sample spot and
(D) Color-indicating dye with sample spot.

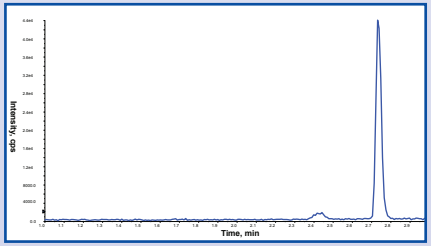


Figure 2: MFLC-MS/MS Dextromethorphan Chromatogram from the Analysis of HLMs In-vitro Fluid at T=0

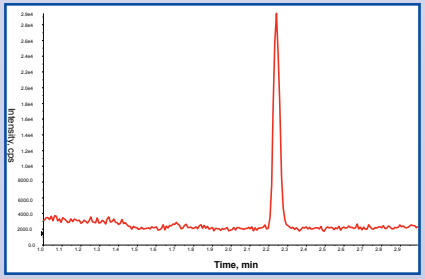


Figure 3: MFLC-MS/MS Dextrorphan Chromatogram from the Analysis of HLMs In-vitro Fluid at T=60 minutes

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

Incubation Time Prior to Spotting or Acetonitrile Crash (min)	Acetonitrile Crash Dextromethorphan Difference from T=0 (%)	DMS Dextromethorphan Difference from T=0 (%)
15	-23.3	-25.7
30	-54.0	-52.1
45	-74.0	-73.1
60	-82.8	-80.3

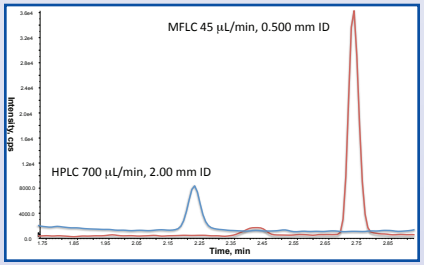
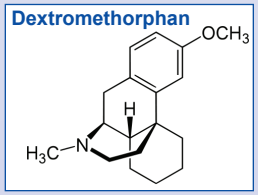
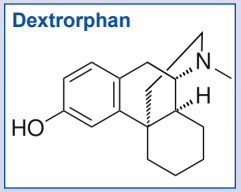
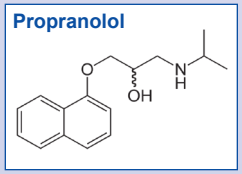


Figure 4: HPLC-MS/MS and MFLC-MS/MS Dextromethorphan chromatograms from the analysis of a 5.0 µL injection of HLMs In-vitro Fluid at T=0







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

1. Van, et. al. J. Pharm Sci. 2009 Feb;98(2):763-71