Comparison of MFLC-MS/MS Validation Data of Methotrexate in Human Plasma to Conventional HPLC-MS/MS

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Overview

- **Purpose** – To determine if MFLC (Microflow Liquid Chromatography) coupled with an MS system can generate bioanalytical validation data that exceeds FDA criteria and is comparable to conventional HPLC-MS/MS systems.
- **Methods** – Validation of Methotrexate in human plasma utilizing MFLC-MS/MS (Eksigent ekspert™microLC 200 System coupled with an ABSciex 5500® QTRAP) and HPLC-MS/MS (Shimadzu LC-10AD coupled with an ABSciex 5500® QTRAP).
- **Results** – The MFLC-MS/MS system provides bioanalytical validation data that passes FDA criteria and is comparable to the HPLC-MS/MS validation data.

Introduction

Micro-flow liquid chromatography coupled with a mass spectrometer (MFLC-MS/MS) has many advantages (sensitivity increase, less solvent consumption and reduced source contamination). These advantages have led to the manufacture of specialized LC pumps that accurately deliver ≤100 µL/min of solvent and LC columns with an inside diameter of ≤1 mm. In order to determine if the MFLC-MS/MS system can provide bioanalytical data that exceeds the criteria required by the FDA, a validation (determination of Methotrexate in human plasma) was performed. A validation was also performed using the conventional HPLC-MS/MS for comparison purposes.

Here we report data generated for the determination of Methotrexate from human plasma by MFLC-MS/MS and HPLC-MS/MS.

Methods

Extraction

- 50 µL of human plasma sample
- 25 µL of the internal standard Methotrexate-D_3
- Acetonitrile added for protein precipitation
- Supernatant transferred and water 1% formic acid added

MFLC

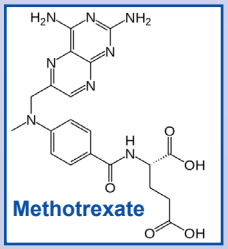
- Eksigent ekspert™microLC 200 System gradient MFLC using acetonitrile and water with 1% formic acid
- Flow rate: 35 µL/minute
- Column: ProntoSIL 120-3-C18, 0.5 x 50 mm (MAC-MOD)
- Column temperature: 50°C

Conventional HPLC

- Shimadzu LC-10AD using acetonitrile and water with 1% formic acid
- Flow rate: 700 µL/minute
- Column: ProntoSIL 120-3-C18, 2.0 x 50 mm (MAC-MOD)
- Column temperature: 50°C

MS

- ABSciex 5500® QTRAP operating in MRM mode
- ESI
- Positive ion mode
- MRM transitions:
  Methotrexate: 455.4 → 308.1
  Methotrexate-D_3: 458.4 → 311.5



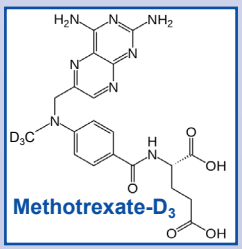


Table 1: Validation Data Comparison of HPLC and MFLC Systems

	Shimadzu HPLC 700 µL/min	Eksigent MFLC 35 µL/min
***Intraday A/P (%)**	107±11.8	93.9±12.9
***Interday A/P (%)**	112±13.8	106±13.4
Matrix Factor	1.02	1.07

* All data includes the LLOQ and ULOQ values

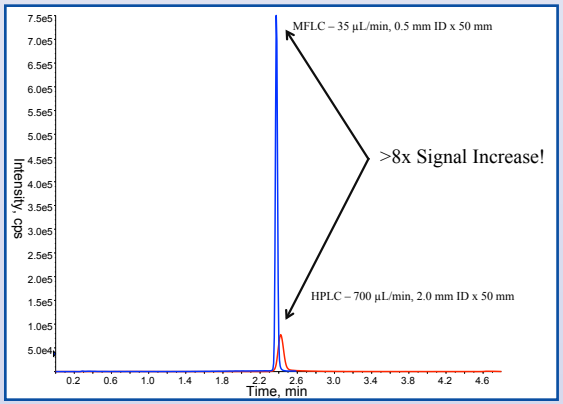


Figure 1. MFLC-MS/MS and HPLC-MS/MS chromatograms from the analysis of a 2.0 µL injection of Methotrexate extracted from human plasma.

Conclusions

- The MFLC-MS/MS system can be used to perform bioanalytical validations.
- The validation data produced by the MFLC-MS/MS system is equivalent to the data generated from the conventional HPLC-MS/MS system.
- Only ~125 mL of solvent was needed to perform the MFLC-MS/MS validation compared to ~2,500 mL of solvent required for the HPLC-MS/MS validation.
- MFLC-MS/MS system is less susceptible to contamination than the HPLC-MS/MS system.

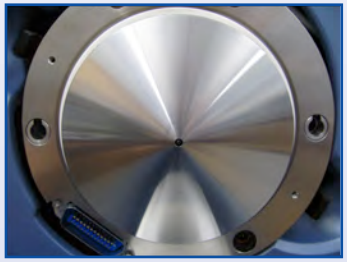


Figure 2. QTRAP® 5500 interface plate after ~400 extracted human plasma injections from the Eksigent system – no switching valve.

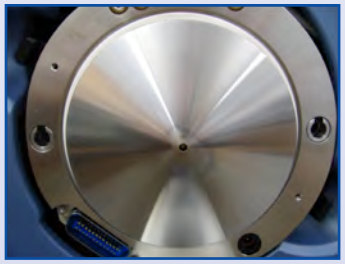


Figure 3. QTRAP® 5500 interface plate after ~150 extracted human plasma injections from the Shimadzu conventional HPLC system – no switching valve.

Figure 4. AB SCIEX QTRAP® 5500 Coupled with an Eksigent ekspert™ microLC 200 MFLC System.