Poster Note 64375

# Usability Study of a New HPLC, a New Tandem MS and a New Data Processing Software

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## Overview

**Purpose:** This usability study evaluated a new two-channel HPLC, a new Tandem MS and a data processing software for general clinical use, focusing on the areas of stability, robustness and time efficiency.

**Methods:** The testing methods included analyzing specimens of synthetic serum and urine spiked with standards and stable isotopic internal standards. The HPLC was operated with or without using on-line sample cleanup. The Tandem MS was operated using a selected reaction monitoring (SRM) method with polarity switching mode and with HESI ionization, or APCI ionization. The instruments in use were production equivalent units of Thermo Scientific™ Prelude MD™ HPLC and Thermo Scientific™ Endura MD ™ mass spectrometer. The data analysis was performed on Thermo Scientific™ ClinQuan MD™ production equivalent software.

**Results:** The RSD% (n=2,000) of retention time and concentration of Alprazolam in synthetic serum was observed at 0.85 and 1.49. The between instrument precision studies were conducted on 3 units using four example compounds in polarity switching mode (+/-) in synthetic urine and synthetic serum (n=120). HESI: Reserpine(+); Chloramphenicol (-). APCI: Testosterone (+); Estradiol (-). The corresponding %RSD's of concentrations are 3.87, 6.61, 3.48 and 8.08, respectively.

## Introduction

Stability, robustness and time efficiency are three major challenges in laboratories where a large number of samples are analyzed routinely. Here we report usability study of a new two channels HPLC, a new tandem mass spectrometer and a new data processing software to address these challenges. A total of 2,000 crashed synthetic serum samples spiked with Alprazolam and isotopic internal standard were analyzed continuously over 100 hours, with additional 44 QC samples. Instrument-to-instrument precision studies (3 units) are reported with four example compounds (Reserpine, Chloramphenicol, Testosterone, Estradiol) using both HESI probe and APCI probe in both positive and negative ionization modes.

## Methods

### Sample Preparation

Calibration Standards, Internal Standard (IS), QC sample and test sample were spiked with standards in crashed synthetic serum and synthetic urine as shown in Tables 1 to 3 below.

**TABLE 1. Concentrations of Robustness Study – Crashed Synthetic Serum**

| Various Samples | Concentrations (ng/mL) |
|---|---|
| Calibration Standard (Alprazolam) | 1, 2, 5, 10, 25, 50 and 100 |
| Internal Standard (Alprazolam-D5) | 5 |
| QC (Alprazolam) Mean (n=44) | 5.88 |
| Test Sample (Alprazolam) Mean (n=2,000) | 10.55 |

**TABLE 2. Concentrations of Precision Study – HESI Probe Polarity Switching – Crashed Synthetic Urine**

| Various Samples | Concentrations (ng/mL) |
|---|---|
| **HESI Positive** | |
| Calibration Standard (Reserpine) | 0.2, 0.5, 1, 2, 5 and 10 |
| Internal Standard (Reserpine-D9) | 2 |
| Test Sample (Reserpine) Mean (n=120) | 1.02 |
| **HESI Negative** | |
| Calibration Standard (Chloramphenicol) | 2, 5, 10, 20, 50 and 100 |
| Internal Standard (Chloramphenicol-D5) | 20 |
| Test Sample (Chloramphenicol) Mean (n=120) | 10.10 |

**TABLE 3. Concentrations of Precision Study – APCI Probe Polarity Switching – Crashed Synthetic Serum**

| Various Samples | Concentrations (ng/mL) |
|---|---|
| **APCI Positive** | |
| Calibration Standard (Testosterone) | 2, 5, 10, 20, 50 and 100 |
| Internal Standard (Testosterone-D3) | 20 |
| Test Sample (Testosterone) Mean (n=120) | 30.33 |
| **APCI Negative** | |
| Calibration Standard (Estradiol) | 4, 10, 20, 40, 100 and 200 |
| Internal Standard (Estradiol-D5) | 40 |
| Test Sample (Estradiol) Mean (n=120) | 61.01 |

### Liquid Chromatography

The Prelude MD HPLC consists of two separation channels, both of which include an on-line sample cleanup Thermo Scientific™ TurboFlow™ column for removing sample matrix, and an analytical column for compound separation. The instrument is capable of cross-channel sequencing for efficient use of the mass spectrometer time. The HPLC parameters for each channel (Channel 1 and Channel 2) were set as in Tables 4 to 6 below.

**TABLE 4. HPLC Parameters for Robustness Study**

| HPLC | Parameters |
|---|---|
| TurboFlow Column | Thermo Scientific™ Cyclone-P™ (50x0.5mm) ; 25ºC |
| Analytical Column | Thermo Scientific™ Accucore™ aQ column (50x2.1mm, 2.6um) ; 30ºC |
| Loading MP A | 10 mM ammonium formate, 0.05% formic acid in water |
| Loading MP B | 10 mM ammonium formate, 0.05% formic acid in methanol |
| Loading MP C | Isopropanol:acetonitrile:acetone (45:45:10) |
| Eluting MP A | 0.05% formic acid in water |
| Eluting MP B | 0.05% formic acid in methanol |
| Injection Volume | 10 uL |

Note: MP = mobile phase

**TABLE 5. HPLC Parameters for Precision Study – HESI Probe**

| HPLC | Parameters |
|---|---|
| Analytical Column | Hypersil Gold (50 x 2.1mm, 3 µm); 30ºC |
| Eluting MP A | 0.05% formic acid in water |
| Eluting MP B | Methanol |
| Injection Volume | 10 uL |

**TABLE 6. HPLC Parameters for Precision Study – APCI Probe**

| HPLC | Parameters |
|---|---|
| Loading MP A | Water |
| Loading MP B | Methanol |
| Loading MP C | Isopropanol:acetonitrile:acetone (45:45:10) |
| Eluting MP A | Water |
| Eluting MPB | Methanol |
| Injection Volume | 20 uL |

Note: TurboFlow Column and Analytical Column are the same as Table 4

### Mass Spectrometry

The Endura MD MS is a tandem MS equipped with the neutral blocker that keeps ion optics cleaner, and the advanced electronics that maintains mass stability. Tandem MS was operated in selected reaction monitor (SRM) mode with heated electrospray ionization (HESI) probe or APCI probe in polarity switching mode using the following transition parameters in Table 7.

**TABLE 7. Tandem MS Parameters**

| Compound | Precursor (m/z) | Product (m/z) | Type | Probe /Polarity |
|---|---|---|---|---|
| Alprazolam | 309.22 | 274.11 | Confirming Ion | HESI/Pos |
| | 309.22 | 281.06 | Quantifying Ion | HESI/Pos |
| Alprazolam D-5 | 314.25 | 279.09 | Confirming Ion | HESI/Pos |
| | 314.25 | 286.08 | Quantifying Ion | HESI/Pos |
| Reserpine | 609.30 | 195.00 | Quantifying Ion | HESI/Pos |
| Reserpine D-9 | 618.30 | 204.00 | Quantifying Ion | HESI/Pos |
| Chloramphenicol | 321.00 | 152.00 | Quantifying Ion | HESI/Neg |
| | 321.00 | 257.00 | Confirming Ion | HESI/Neg |
| Chloramphenicol-D5 | 325.95 | 157.00 | Quantifying Ion | HESI/Neg |
| | 325.95 | 262.00 | Confirming Ion | HESI/Neg |
| Testosterone | 289.30 | 97.23 | Confirming Ion | APCI/Pos |
| | 289.30 | 109.20 | Quantifying Ion | APCI/Pos |
| Testosterone-D3 | 292.30 | 97.24 | Confirming Ion | APCI/Pos |
| | 292.30 | 109.22 | Quantifying Ion | APCI/Pos |
| Estradiol | 271.08 | 145.14 | Confirming Ion | APCI/Neg |
| | 271.08 | 183.13 | Quantifying Ion | APCI/Neg |
| Estradiol-D5 | 275.98 | 145.09 | Confirming Ion | APCI/Neg |
| | 275.98 | 187.15 | Quantifying Ion | APCI/Neg |

### Data Analysis

The test results of LC-MS analysis were processed using ClinQuan MD production equivalent software.

## Results

### Improving Productivity; Maximizing MS Utilization and Throughput

This HPLC includes automation of online sample cleanup using TurboFlow Technology, and removes tedious hand-on manual steps. The use of two HPLC channel separations minimizes MS idle time. With a single channel HPLC instrument, the MS has idle time not acquiring data, whilst waiting for chromatographic elution. This multichannel optimization technology removes idle time, increases utilization time of tandem MS, and doubles the sample throughput.

A total of 2,000 crashed synthetic samples spiked with Alprazolam standard and isotopic internal standard were analyzed continuously for 100 hours, with an additional 44 QC samples. Retention time measurements showed a precision of 0.85%RSD as shown in Figure 1.

### Tandem MS Performance: Stability, Precision and Robustness

This Tandem MS provides excellent stability of quantitative measurement. The %RSD of concentration measurement of these 2,000 injections was 1.49 as shown in Figure 2.

**FIGURE 1. Retention Time Plot of 2,000 injections from two HPLC channels ; Average Retention Time = 0.731 min, %RSD = 0.85**

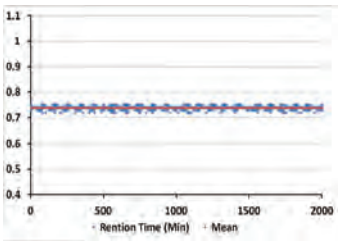
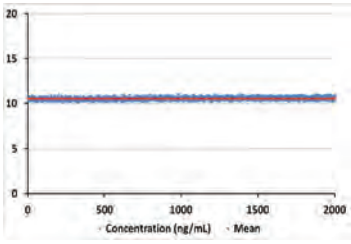



**FIGURE 2. Concentration Plot of 2,000 injections; Average Concentration = 10.55 ng/mL, %RSD = 1.49**




### Stability of Mass Ion Ratios

The ion ratio of confirming ion to quantifying ion demonstrated an excellent stability over 2,000 injections as shown in the Table 8 below.

**TABLE 8. Stability of Mass Ion Ratios**

| Compound | Ion Ratio | Average | %RSD |
|---|---|---|---|
| Alprazolam | m/z 274.11 to m/z 281.06 | 0.1915 | 1.37 |
| Alprazolam-D5 IS | m/z 279.09 to m/z 286.08 | 0.2167 | 2.54 |

### Precision Study

The precision studies of different ionization modes on HESI probe and APCI probe were conducted using four example compounds in polarity switching mode (+/-) in synthetic urine and synthetic serum (n=120, 5 replicates, 4 runs, 2 channels, 3 units). The %RSD's are shown in Table 9.

**TABLE 9. Precision Study – Between Instruments (3 units)**

| Compound | Ionization | Polarity | Replicates (N) | Mean (ng/mL) | %RSD |
|---|---|---|---|---|---|
| Reserpine | HESI | Positive | 120 | 1.02 | 3.87 |
| Chloramphenicol | HESI | Negative | 120 | 10.10 | 6.61 |
| Testosterone | APCI | Positive | 120 | 30.33 | 3.48 |
| Estradiol | APCI | Negative | 120 | 61.01 | 8.08 |

### Data Analysis

The data processing software includes three levels of user permissions for technicians, supervisors, and lab directors as shown in Figure 3 below. This software has built-in flexibility in assigning roles and responsibilities, and audit trail function is provided for streamlining record keeping.

**FIGURE 3. ClinQuan MD software streamlines workflows, addresses CLIA-required roles and responsibilities, maintains records, helps ensure data integrity.**


## Conclusion

This usability study demonstrated the performance of robustness and precision of these three Class I medical devices for general clinical use.

The analytical performance of Prelude MD HPLC and Endura MD MS was demonstrated in this study of 2,000 injections, showing stability, robustness, and time-efficiency for the LC-Tandem MS analysis with a continuous run time of 100 hours. ClinQuan MD data processing software streamlines workflow and helps ensure data integrity.

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Printed in the United States.





PN64375-EN 0415S