Evaluation of a New HPLC, a New Tandem MS and a New Data Processing Software for General Clinical Use

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Overview

Purpose: This 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 prototype (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™ prototype (production equivalent) software.

Results: A total of 500 crashed synthetic serum samples spiked with Alprazolam and isotopically-labeled internal standard were analyzed continuously for 24 hours, with an additional 11 QC samples inserted at intervals during the same 24 hours. Cross-channel %RSD's (n=500) of retention time and concentration was observed at 0.85, 1.39, respectively. The precision studies of different ionization modes with HESI and APCI were conducted using four example compounds with polarity switching mode (+/-) in synthetic urine and synthetic serum (n=40, 5 replicates, 4 runs, 2 channels). HESI: Reserpine(+); Chloramphenicol (-). APCI: Testosterone (+); Estradiol (-). The corresponding %RSD's of concentrations are 1.74, 4.48, 1.84 and 7.20, respectively. This study demonstrated the performance of robustness and precision of these three Class I medical devices for general clinical use.

Introduction

There is an increasing interest in using LC-tandem MS in clinical laboratories because users can develop their own tests, use a smaller amount of samples, and measure several analytes in a single run.

Starting from the 1950's, breath samples had been analyzed by MS, followed by body fluids and other complex biological matrices.

Various innovative mass spectrometry technologies have been developed for the detection of a wide range of compounds, including parent compounds and metabolites. Mass spectrometers coupled in series (tandem MS) provide definitive identification and quantitation of target compounds using specific transitions from precursor ions to product ions. Different sample cleanup techniques and chromatographic separations are used prior to delivering compounds into the mass spectrometer.

In addition to choosing appropriate technologies for analysis of target compounds, clinical laboratories often encounter challenges with sample clean-up (pre-analytical phase) and data processing (post-analytical phase) when facing the demand to provide test results in a timely manner with increasing numbers of incoming samples.

In this study, we evaluated three Class I medical devices to address these challenges: Prelude MD (two channel HPLC), Endura MD (tandem MS) and ClinQuan MD (data processing software). The HPLC instrument consists of two separate channels, both of which include an online sample cleanup Thermo Scientific™ TurboFlow™ column for removing sample matrix, and an analytical column for chromatographic separation. This HPLC is capable of cross-channel sequencing by only introducing the portion of the chromatogram of target compounds into the mass spectrometer therefore doubling the sample throughput. The mass spectrometer is a triple-stage quadrupole tandem MS, providing up to 500 SRM transitions per second for compound identification and quantitation. The data processing software includes three levels of user permissions for technicians, supervisors, and lab directors. This software has built-in flexibility in assigning roles and responsibilities, and an audit trail function is provided for streamlining record keeping.

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

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=11)	5.82
Test Sample (Alprazolam) Mean (n=500)	10.48

TABLE 2. Concentrations of Precision Study – HESI Probe Polarity Switching

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=40)	1.05
HESI Negative	
Calibration Standard (Chloramphenicol)	2, 5, 10, 20, 50 and 100
Internal Standard (Chloramphenicol-D5)	20
Test Sample (Chloramphenicol) Mean (n=40)	10.31

TABLE 3. Concentrations of Precision Study – APCI Probe Polarity Switching

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=40)	30.53
APCI Negative	
Calibration Standard (Estradiol)	4, 10, 20, 40, 100 and 200
Internal Standard (Estradiol-D5)	40
Test Sample (Estradiol) Mean (n=40)	61.11

Liquid Chromatography

The Prelude MD HPLC consists of two separation channels, both of which include an on-line sample cleanup 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 Mobile Phase A	10 mM ammonium formate, 0.05% formic acid in water
Loading Mobile Phase B	10 mM ammonium formate, 0.05% formic acid in methanol
Loading Mobile Phase C	Isopropanol:acetonitrile:acetone (45:45:10)
Eluting Mobile Phase A	0.05% formic acid in water
Eluting Mobile Phase B	0.05% formic acid in methanol
Injection Volume	10 uL

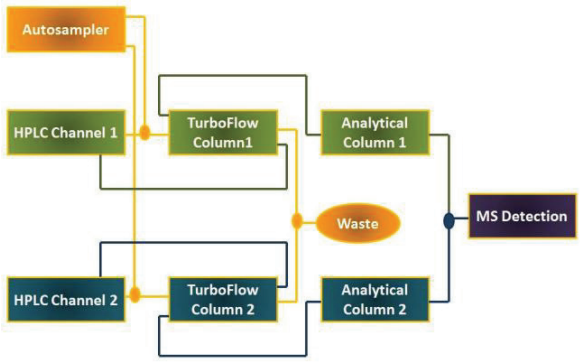
TABLE 5. HPLC Parameters for Precision Study – HESI Probe

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

TABLE 6. HPLC Parameters for Precision Study – APCI Probe

HPLC	Parameters
TurboFlow Column	Thermo Scientific™ Cyclone-P™ (50x0.5mm) ; 25ºC
Analytical Column	Thermo Scientific™ Accucore™ aQ column (50x2.1mm, 2.6um) ; 30ºC
Loading Mobile Phase A	Water
Loading Mobile Phase B	Methanol
Loading Mobile Phase C	Isopropanol:acetonitrile:acetone (45:45:10)
Eluting Mobile Phase A	Water
Eluting Mobile Phase B	Methanol
Injection Volume	20 uL

FIGURE 1. Cross channel sequencing for two parallel HPLC separations with series MS detection increase MS utilization



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 prototype software.

Results

Robustness Study

HPLC Solvent Consumption

This HPLC instrument uses a low amount of solvents for each injection, and Table 8 below shows the solvent consumption for each injection in this study.

TABLE 8. Low Solvent Consumption

Solvent	Usage per Injection
Loading Mobile Phase A	2.83 mL
Loading Mobile Phase B	1.40 mL
Loading Mobile Phase C	1.00 mL
Eluting Mobile Phase A	1.87 mL
Eluting Mobile Phase B	0.53 mL

Improving Productivity; Maximizing MS Utilization and Throughput

This HPLC instrument includes automation of online sample cleanup using TurboFlow Technology, and therefore removing tedious hands-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. Figure 1 depicts the schematic process of cross channel sequencing of two parallel HPLC separations. This multichannel optimization technology removes idle time, increase utilization time of tandem MS, and doubles the sample throughput.

A total of 500 crashed synthetic samples spiked with Alprazolam standard and isotopic internal standard were analyzed continuously for 24 hours, with an additional 11 QC samples. Retention time measurements showed a precision of 0.85% RSD as shown in Figure 2. All QC values were within 2 standard deviations.

Tandem MS Performance: Stability, Precision and Robustness

This Tandem MS provides excellent stability for quantitative measurements. For 500 injections measuring concentrations, 1.39% RSD was achieved as shown in Figure 3. All QC values were within 2 standard deviations.

FIGURE 2. Retention Time Plot of 500 injections from two HPLC channels ; Average Retention Time = 0.74 min, %RSD = 0.85%

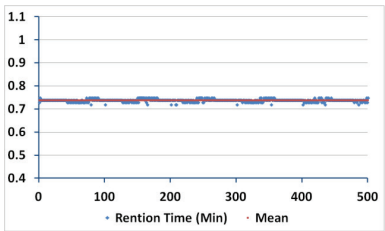
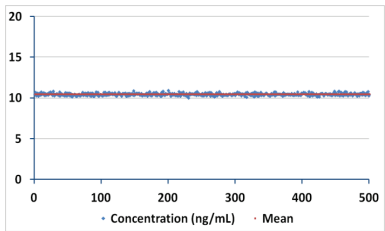


FIGURE 3. Concentration Plot of 500 injections; Average Concentration = 10.48 ng/mL, %RSD = 1.39%



Stability of Mass Ion Ratios

The ion ratio of confirming ion to quantifying ion demonstrated an excellent stability over 500 injections as shown in the Table 9 below.

TABLE 9. Stability of Mass Ion Ratios

Compound	Ion Ratio	Average	%RSD
Alprazolam	m/z 274.11 to m/z 281.06	0.1918	1.31
Alprazolam-D5 IS	m/z 279.09 to m/z 286.08	0.2166	2.43

Precision Study

The precision studies of different ionization modes on HESI probe and APCI probe were conducted using four example compounds with polarity switching (+/-) in synthetic urine and synthetic serum (n=40, 5 replicates, 4 runs, 2 channels). HESI: Reserpine(+); Chloramphenicol (-). APCI: Testosterone (+); Estradiol (-). The corresponding %RSD's of concentrations are 1.74, 4.48, 1.84 and 7.20, respectively., as shown in Table 10.

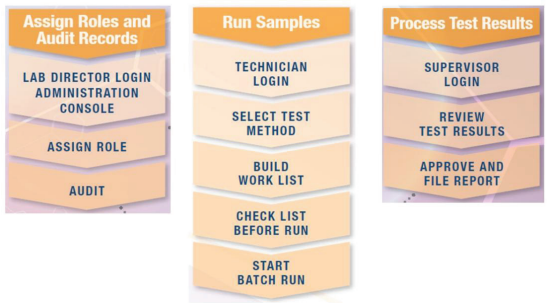
TABLE 10. Precision Study

Compound	Ionization	Polarity	Replicates (N)	Mean (ng/mL)	%RSD
Reserpine	HESI	Positive	40	1.05	1.74
Chloramphenicol	HESI	Negative	40	10.31	4.48
Testosterone	APCI	Positive	40	30.53	1.84
Estradiol	APCI	Negative	40	61.11	7.20

Data Analysis

The data processing software includes three levels of user permissions for technicians, supervisors, and lab directors as shown in Figure 4 below. This software has built-in flexibility in assigning roles and responsibilities, and audit trail function is provided for streamlining record keeping.

FIGURE 4. ClinQuan MD software streamlines workflows, addresses CLIA-required roles and responsibilities, maintains records, helps ensure data integrity

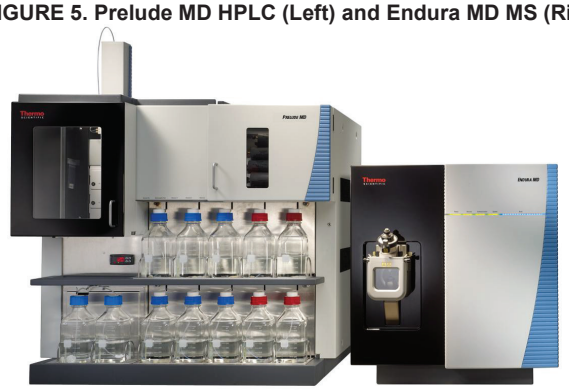


Conclusion

This 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 500 injections, showing stability, robustness, and time-efficiency for the LC-Tandem MS analysis with a continuous run time of 24 hours. ClinQuan MD data processing software streamlines workflow and helps ensure data integrity.

FIGURE 5. Prelude MD HPLC (Left) and Endura MD MS (Right)



For in vitro diagnostic use.

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