The Utilization of Novel Platform in a LC-MS/MS Workflow for the Analysis of Vitamin D, Testosterone, Immunosuppressants, Chemotherapeutics and Cortisol

Dayana Argoti, Kerry Hassell, Sarah J. Fair, and Joseph Herman*

ThermoFisher Scientific, 101 Constitution Blvd., Franklin, MA 02038

Overview

Purpose: To demonstrate the validity of the Prelude Sample Preparation Liquid Chromatography (SPLC) system, a new LC/MS/MS platform that reduces solvent consumption, requires less maintenance, and is easier to use then traditional systems.

Methods: Prelude SPLC™, Turbulent Flow Chromatography, LC/MS/MS, Multiplexing

Results: Methods for 25-hydroxy-vitamin D2 and D3, testosterone, the immunosuppressant drugs Sirolimus, Tacrolimus, Everolimus, and Cyclosporine A, the chemotherapeutic drugs Busulfan, Docetaxel, Methotrexate and Imatinib, and cortisol were validated using a Prelude SPLC™ LC/MS/MS platform.

Introduction

A new LC system was specifically designed to reduce instrument maintenance, down time, and operating costs for high-throughput, LC/MS/MS applications which require sample clean-up prior to HPLC analysis. The Prelude SPLC System utilizes syringe pumps designed to deliver the volume of mobile phase required for each sample analysis with a single push of the piston. This pump design greatly reduces the wear and tear on pump seals and check valves, because the pistons in dual piston reciprocating pumps can move several hundred if not thousands of times per sample run. The majority of maintenance required on traditional HPLC pumps results from the wear of the seals and check valves; therefore, syringe pumps are more robust than traditional HPLC pumps. The Prelude SPLC System's also have extremely low dead volumes making rapid changes in mobile phases possible. The time required for many of the steps in a method to occur are reduced resulting in shorter run times and lower solvent costs for equivalent methods.

In order to prove the utility of the Prelude SPLC platform, several LC/MS methods that are currently used by clinical researchers were validated. The successful validation of such a wide range of analytes using the new platform demonstrates that the Prelude SPLC offers a viable alternative to existing LC/MS systems. Reduced system void volumes resulted in methods that had run times 20-30% shorter then their equivalent methods run on a conventional HPLC and produce a corresponding reduction in mobile phase consumption.

Methods

All samples were vortexed, mixed with internal standard solution and centrifuged. Supernatant was removed and transferred into sampling containers for LC-MS/MS analysis. On-line sample clean-up using a 0.5x50 mm ThermoScientific HTLC-C18 XL TurboFlow column was followed by chromatographic separations of 25-OH-D_2, 25-OH-D_3, immunosuppressants, chemotherapeutics, cortisol and testosterone using a 50x2.1mm, 2.6 µm particle size ThermoScientific Accucore PFP analytical column. The detector was a TSQ Vantage triple quadrupole mass spectrometer with HESI-II ionization probe in positive mode. Mobile phases were (A) 10 mM ammonium formate in water, (B) 10 mM ammonium formate in Methanol, and (C) 45/45/10 acetonitrile/isopropanol/acetone. All run times were 4 minutes or less and when mutiplexed the effective analysis time was reduced to 2 minutes per sample. The Immunosuppresants were run in spiked human whole blood with cell lysis and protein precipitation occurring at the same time as the addition of the internal standard. Testosterone analysis was performed in spiked testosterone depleted human plasma. Chemotherapeutics were run in spiked human plasma. Cortisol was run using synthetic urine but was validated against human urine samples containing known levels of Cortisol

Results

Accuracy and precision experiments were performed for system verification from three separate preparations on calibrators and controls on three different days . The interday and intraday accuracy and precision results were obtained for 25-OH-D_2 and 25-OH-D_3 at a concentration range of range of 2-100 ng/mL. The range for testosterone was 0.02-10 ng/mL. Immunosuppressants and chemotherapeutics were analyzed in ranges from 1-2000 ng/mL. The method range for cortisol was 3.62 - 362 ng/mL (0.1-10 nM). The method precision had RSD values were less than 15.0% for all compounds tested. Additionally, accuracy was ±15% of the theoretical value for all the methods. The correlation coefficient values for all the compounds ranged from 0.991 to 0.999, showing linearity throughout all concentrations and analytes. All the analytes passed carryover, benchtop stability, autosampler stability, and specificity criterion. Recoveries, including matrix effects, were all around 90% or higher. All the data is summarized in Tables 1 to 3. Figure 1 depicts representative standard curves for each compound tested. Representative chromatograms at the lower limit of quantitation (LLOQ) for each compound are shown in Figure 2.

TABLE 1. Method Range, Linearity and Recovery

Compound Name	Method Range (ng/mL)	Linearity (r^2)	Recovery
Cyclosporin A	10 – 2000	0.992 – 0.998	87.3 – 93.9
Sirolimus	1 – 50	0.998 – 0.999	86.9 – 93.9
Everolimus	1 – 50	0.992 – 0.998	88.5 – 95.2
Tacrolimus	1 – 50	0.998 – 0.999	87.3 – 97.9
Testosterone	0.020 -10.0	0.994 – 0.999	99.9 – 103.5
Cortisol	3.62 - 362	0.997 – 0.999	88.3 – 114.1
Busulfan	20 - 2000	0.995 – 0.998	89.4 – 93.5
Docetaxel	10 - 1000	0.993 – 0.999	96.6 – 102.1
Imibitib	10 - 2000	0.991 – 0.998	92.0 – 110.2
Methotrexate	10 - 750	0.992 – 0.998	102 – 111.8
25-hydroxy Vit D2	2.0 - 100	0.992 – 0.998	92.2 – 94.5
25-hydroxy Vit D3	2.0 - 100	0.992 – 0.996	95.0 - 98.9

TABLE 2. Intraday Accuracy and Precision

Compound Name	Intraday Accuracy Range (% Difference from Theoretical)			Intraday Precession Range (%RSD)		
	Low QC	Mid QC	High QC	Low QC	Mid QC	High QC
Cyclosporin A	2.38 – 12.4	3.61 – 10.9	2.11 – 9.72	1.7 – 4.2	1.1 – 2.9	1.4 – 2.7
Sirolimus	1.78 – 16.5	2.33 – 14.9	0.11 – 13.6	7.5 -10.6	1.8 – 2.8	4.7 – 7.6
Everolimus	1.98 – 18.9	2.66 – 13.4	0.81 – 10.2	5.4 – 8.3	1.7 – 3.5	1.6 – 4.1
Tacrolimus	1.09 – 13.3	0.87 – 5.32	0.34 – 8.38	4.8 – 6.0	1.3 – 2.6	1.4 – 2.3
Testosterone	0.18 – 11.4	0.15 – 5.24	1.63 – 4.84	3.4 – 3.6	1.5 – 2.6	0.8 – 1.2
Cortisol	1.6 - 9.3	0.76 – 12.0	0.03 – 15.1	4.0 – 6.3	2.3 – 3.9	2.6 – 5.1
Busulfan	0.56 – 16.5	0.17 – 8.17	0.22 – 5.83	1.1 – 10.9	1.8 – 3.3	1.6 – 4.2
Docetaxel	0.37 – 11.9	0.14 – 5.61	0.26 – 6.98	1.6 – 9.4	1.1 – 3.7	0.9 – 3.4
Imatinib	1.0 - 9.5	0.3 – 9.8	0.0 – 11.7	1.0 – 1.9	1.1 – 7.4	1.3 – 6.2
Methotrexate	0.13 – 18.5	0.12 – 9.74	0.10 – 10.5	3.3 – 7.5	0.6 – 5.9	2.8 – 7.8
25-hydroxy Vit D2	0.5 – 14.8	0.09 – 12.5	0.3 – 11.2	5.0 – 11.5	2.9 – 6.6	1.9 – 5.1
25-hydroxy Vit D3	1.0 – 17.8	0.3 – 12.9	0.9 – 13.3	6.3 – 6.8	2.3 – 3.9	2.0 – 3.2

TABLE 3. Interday Accuracy and Precision

Compound Name	Interday Accuracy (% Difference from Theoretical)			Interday Precession (%RSD)		
	Low QC	Mid QC	High QC	Low QC	Mid QC	High QC
Cyclosporin A	2.00	0.75	3.06	12.2	9.7	12.2
Sirolimus	2.00	4.00	3.75	7.8	8.1	1.8
Everolimus	2.35	3.11	2.98	9.7	5.4	4.6
Tacrolimus	1.67	0.50	3.75	5.1	3.2	2.9
Testosterone	5.00	0.32	3.12	3.5	1.3	0.15
Cortisol	1.10	1.72	3.50	3.3	3.8	2.7
Busulfan	4.76	0.35	3.85	5.6	5.4	3.9
Docetaxel	2.66	1.51	1.28	4.2	4.4	3.1
Imatinib	11.0	1.33	3.74	4.0	2.0	5.9
Methotrexate	2.33	2.80	0.48	5.5	2.8	7.5
25-hydroxy Vit D2	4.83	2.52	2.87	3.9	4.0	4.8
25-hydroxy Vit D3	5.33	2.53	0.00	3.4	3.1	3.9

FIGURE 1. Standard Curves for Each Compound Tested Using a Prelude SLPC™ LC/MS/MS System

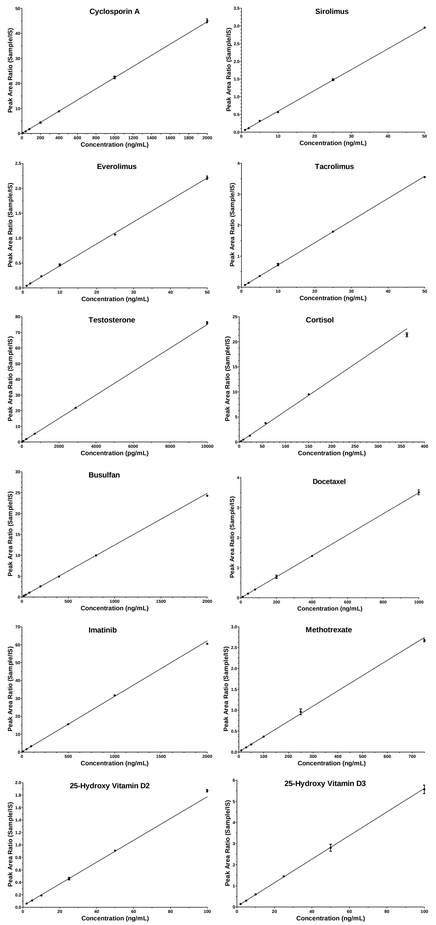


FIGURE 2. Representative Chromatograms at the LOQ for Each Compound Tested Using a Prelude SLPC™ LC/MS/MS System

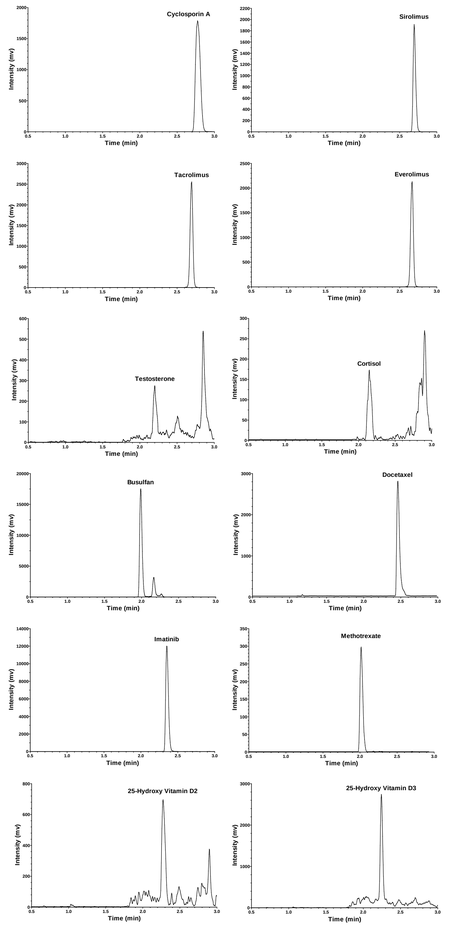
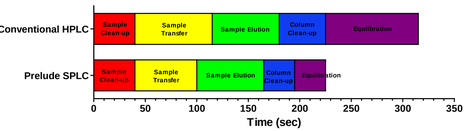


FIGURE 3. Comparison of the Method Run Time for Vitamin D on a Prelude SLPC LC/MS/MS System to that of a Conventional HPLC System



The improvement in run times resulting from the lower void volumes of the Prelude SPLC System verses a conventional HPLC is illustrated in Figure 3 for vitamin D. The same mobile phases and columns were used for the comparison. When using on-line clean-up the duration of certain steps cannot be changed because they are dependent on the chromatographic separation needed. The duration of others steps in the process are related to how long it takes for solvent changes to reach the column. The sample clean-up and sample elution steps are dependent on the chromatography and; therefore, the time for those steps remain the same. However, the transfer, column cleaning and re-equilibration steps can be reduced. On a conventional HPLC the transfer step was 75 sec vs. 60 seconds on the Prelude SPLC. The column clean-up and equilibration steps were reduced from 150 to 60 seconds. The result is a reduction in run time of 29% (5:15 minutes to 3:45 minutes). A shorter run time also reduced solvent consumption by 33%.

Conclusion

- A large number of compounds, with logP values ranging from -1 to 5, have been validated on a new LC/MS/MS platform demonstrating the viability of the Prelude SPLC System for compounds of interest to clinical researchers..
- The Prelude SPLC System's lower void volume results in sample run times that are 20-30% shorter. The reduced run time results in reduced cost due to lower consumption of mobile phases and less waste disposal.
- The Prelude SPLC uses a single syringe fill per sample, which removes the need for pulse dampeners, reduces the mechanical wear and tear on pump parts such as pump seal and active check valves, and does not need proportioning valves. The result is far less required maintenance, reducing operating cost and down time.