Development and Validation of Methods for Compounds of Interest in Therapeutic Drug Monitoring on the New Prelude SPLC LC-MS/MS System

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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 the immunosuppressant drugs Sirolimus, Tacrolimus, Everolimus, and Cyclosporine A, and the chemotherapeutic drugs Busulfan, Docetaxel, Methotrexate and Imatinib were validated using a Prelude SPLC™ LC/MS/MS platform.

Introduction

Bioanalysis using LC-MS/MS can be difficult due to complex sample preparation and variability from sample handing. In addition, both immunosuppressant and chemotherapeutic drugs often have a narrow therapeutic range and require accurate monitoring to avoid toxic events from over dosing or lack of efficacy from under dosing. The use of liquid chromatography-tandem mass spectrometry (LC-MS/ MS) to quantify the immunosuppressant drugs cyclosporine A, serolimus, tacrolimus, and everolimus, and the chemotherapeutic drugs busulfan, methotrexate, imatinib, and docetaxel is common practice. We demonstrate the application of Prelude SPLC™ system in developing faster, more reproducible and lower solvent consuming methods for measuring immunosuppressant and chemotherapeutic drugs.

The Prelude SPLC 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 is reduced resulting in shorter run times and lower solvent costs for equivalent methods.

Methods

The immunosuppressants were prepared in human whole blood while the chemotherapeutics were prepared in human plasma. Online sample cleanup by turbo flow technology and analytical separation was performed on a new Prelude™ sample preparation liquid chromatography (SPLC) system. Detection of eluting analytes was performed with a TSQ Vantage™ triple stage quadrupole mass spectrometer, equipped with a heated electrospray ionization (HESI II) probe in positive ion mode using selected reaction monitoring (SRM). All methods were dual column methods using TurboFlow columns for online sample clean-up. The immunosuppressant used a Cyclone P 0.5 x 50 mm TurboFlow column and the chemotherapeutics used a C18 XL column. An Accucore C8 2.1 x 50 mm, 2.6µ column was the analytical column used for immunosuppressants while an Accucore C18 2.1 x 50 mm, 2.6µ column was used for busulfan, methotrexate, imatinib, and docetaxel. The mobile used in all experiments excluding docetaxol were 10mM ammonium formate, 0.05% formic acid in methanol and in water. Docetaxel used 0.1% formic acid in water and acetonitrile. Quantitation was calculated with Thermo Scientific LCquan™ software. The total run times were less than four minutes per sample. However, the Prelude SPLC is capable of multiplexing two HPLC channels to a single mass spectrometer reducing runs time by diverting the flow from one HPLC stream (when no compounds are eluting), while the second HPLC stream elutes into the mass spectrometer. The injections are off set in time such that only one HPLC channel is eluting compounds of interest at any given time. Therefore, the total sample run times are <2 minutes per sample when multiplexed. The methods consumed less than 3 mL of mobile phase per injection.

Results

The validated method ranges for this study were 1-50 ng/mL for Serolimus, Tacrolimus, and Everolimus, 10-2000 ng/mL for Cyclosporine A, 1-2000 ng/mL for busulfan, 10-2000 ng/mL for imatinib, 5-1000 ng/mL for docetaxel, and 10-750 ng/mL for methotrexate. Individual compounds were evaluated for both inter and intra-day accuracy and precision, recovery, carryover, specificity, bench top and auto sampler stability, and matrix effects. All the calibrators and controls were within ±15% of the expected concentration. The standard curves had correlation coefficients between 0.991 and 0.999. No stability issues were observed. Recoveries including matrix effects ranged from 90-110%. All compounds passed specificity (no interferences from blank matrix) and carryover criterion (<10% of LLOQ from blank following ULOQ). 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.

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 Busulfan. 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

- Validation of a wide range of immunosuppressant and chemotherapeutic drugs on a new online cleanup, PreludeTM sample preparation liquid chromatography mass spectrometer (SPLC-MS) has been demonstrated
- Prelude HPLC methods save both time and money compared to traditional HPLC systems. 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.

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
Busulfan	20 - 2000	0.995 – 0.998	89.4 – 93.5
Docetaxel	5 - 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

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
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

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
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

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

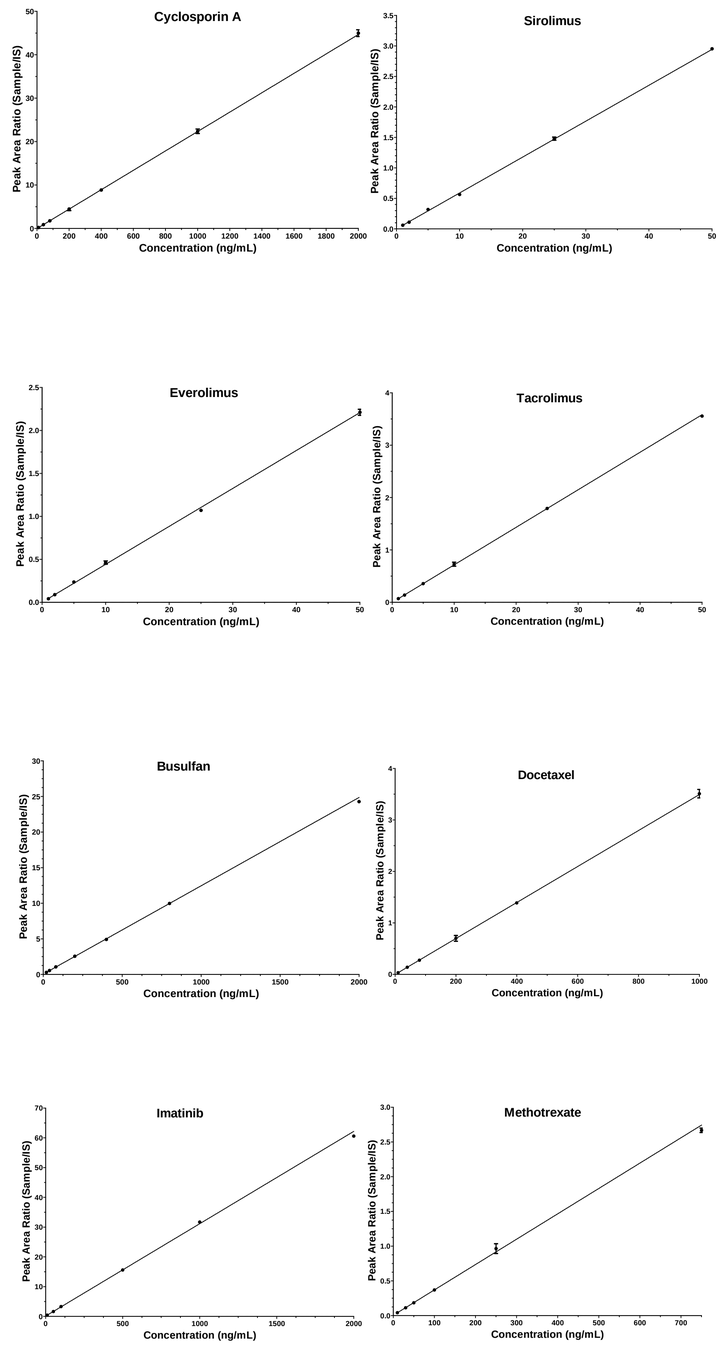

FIGURE 2. Representative Chromatograms at the LOQ for Each Compound Tested Using a Prelude SLPC™ LC/MS/MS System

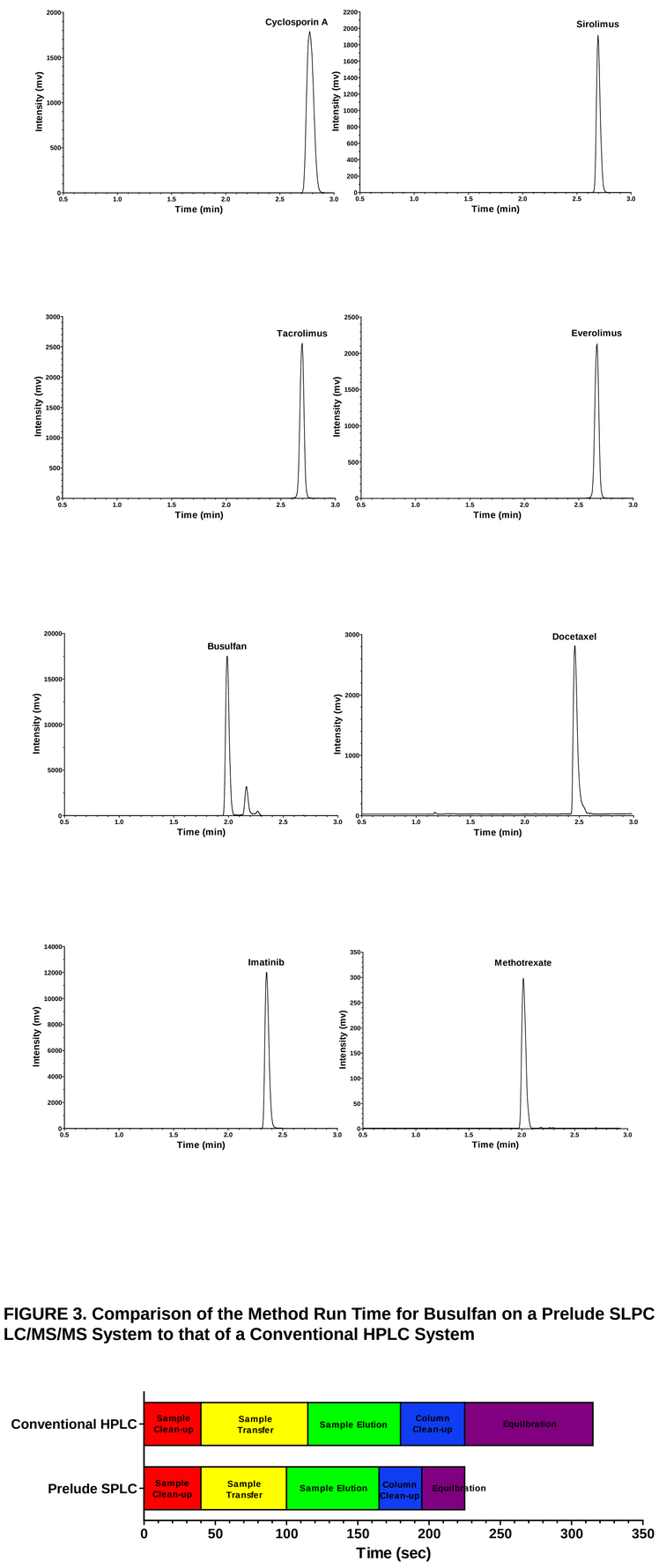

FIGURE 3. Comparison of the Method Run Time for Busulfan on a Prelude SLPC LC/MS/MS System to that of a Conventional HPLC System

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