A Flexible LCMS Front End for High-Throughput Bioanalytical Analysis in GLP Environment

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Introduction

High throughput approaches for the quantitative analysis of drugs and related substances in complex biological matrices are critical to successful drug discovery and development programs. In an effort to capture efficiencies available through two existing technologies, a staggered parallel HPLC system was developed with Leap autosampler (CTC HTS PAL) equipped with trio valves and four independent HPLC pumps. Parallel LC-MS analysis is achieved by offset dual stream analysis with a time delay to allow staggering of the MS acquisition times.

The system is configured to carry out either of the two analytical modes:

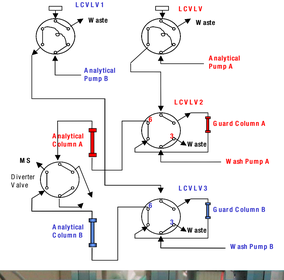
- Paralleled LC-MS analysis with or without guard column regeneration
- Paralleled on-line SPE followed by LC-MS analysis

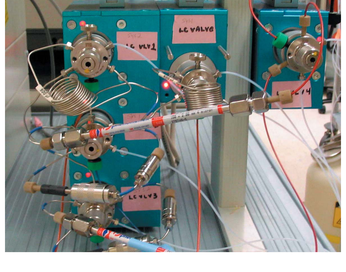
Timing and triggering for injections, analytical pumps, loading/washing pumps and data collection are controlled by the Cycle Composer software that runs custom macros. Switching between the two operational modes requires minimal re-plumbing. The system can also run either type of analysis in single stream mode. Two regulated clinical studies (conducted under GLP) were analyzed using the system and are presented as two examples. One study used the dual channel HPLC with guard column regeneration. The other used the dual channel on-line SPE.

Dual LCMS With Guard Column Regeneration

Guard Column Regeneration

- Extends the life time of guard column
- Minimizes the risk of guard column over pressure
- Eliminates late elutors that interfere with the analysis

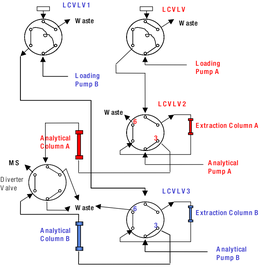


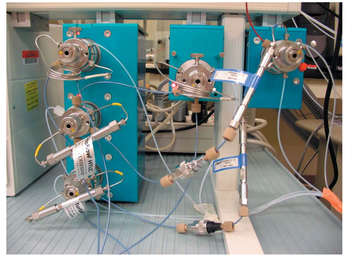


Dual On-line SPE Followed by LCMS

On-line SPE

- Simplifies the sample clean-up steps
- Eliminates the risk for cross-contamination
- High throughput



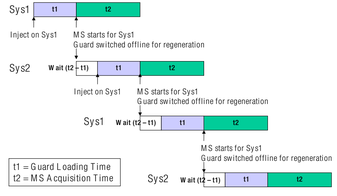


Configure the System Between Two Modes

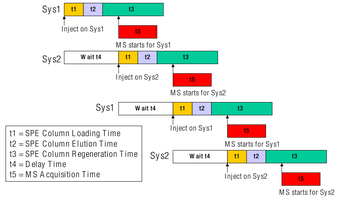
- Switch the loading/wash pump with analytical pump.
- Switch tubing connected in position 3 to 6 on LCVLV2 and LCVLV3.

- Load the corresponding macro in Cycle Composer

Dual HPLC Timing



Dual SPE Timing

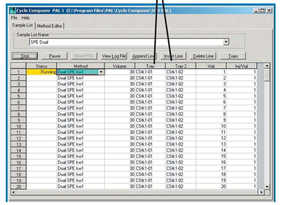


Cycle Composer Control

1 sample line for 2 sample injections

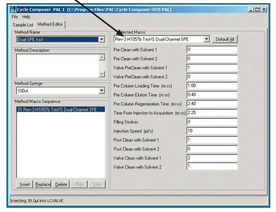
1 from Plate 1, 1 from Plate 2

Sample List



Custom Macro

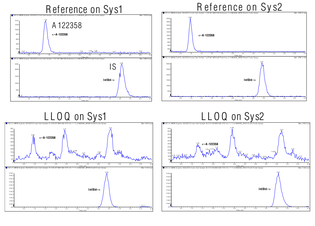
Method



Case I: Analysis of A-122358 (Paricalcitol) Using Dual HPLC for Clinical Study M04-693

Sample/ Extraction Volume		600 µL
Sample Extraction		Liquid-liquid extraction with 50/50 (v/v) hexane/ethyl acetate
Analytical Column		Synergi Max RP C12, 4µm, 80Å, 2.0x150mm, Phenomenex
	Flow Rate	0.3 mL/minute
	Mobile Phase	Methanol/Acetonitrile/0.5mM Ammonium Acetate pH unadjusted, 40/40/20 (v/v/v)
Guard Column		Synergi Max RP Security Guard C12, 2.0x4mm, Phenomenex
	Flow Rate	1.0 mL/minute
	Regeneration Solvent	THF/Methanol/Water, 40/40/20 (v/v/v)
Autosampler		CTC HTS PAL
	Injection Volume	50 µL
	Temperature	Room Temperature
	Needle/Valve Wash	Same as mobile phase
MS		API 4000
	Source	Turbo Ionspray
	Detection	MS/MS, positive ion
	Detection Channels	*m/z* 434.4 → 327.2 (A-122358); *m/z* 450.5 → 379.2 (IS)
Total Run Time	Single Stream	~ 11 min/ sample
	Dual Stream	~ 13 min/ 2 samples

Ion Chromatograms



Validation Results

	Dynamic Range		Overall Recovery	
	LLOQ	ULOQ	A-122358	IS
Mean	0.01022 ng/mL	2.03724 ng/mL	122.9%	55.2%
CV %	14.1	4.9		
Bias%	1.1	-1.2		

STD Statistics from M04-693

	Calculated Concentration (ng/mL)								
	STD LVL 1	STD LVL 2	STD LVL 3	STD LVL 4	STD LVL 5	STD LVL 6	STD LVL 7	STD LVL 8	STD LVL 9
	0.01023	0.02045	0.03110	0.06221	0.10368	0.20736	0.41472	0.82944	1.01376
Mean	0.01021	0.02037	0.03142	0.06240	0.10408	0.20608	0.40992	0.83176	1.01748
S.D.	0.00044	0.00154	0.00200	0.00387	0.00454	0.01028	0.01820	0.03447	0.05020
%CV	4.3	7.6	6.4	6.2	4.4	5.0	4.4	4.1	4.9
%Bias	-0.2	-0.4	1.0	0.3	0.4	-0.6	-1.2	0.3	0.4
n	74	71	75	75	76	77	76	77	77

QC Statistics from M04-693

	Calculated Concentration (ng/mL) and % Bias		
Run	QC LOW	QC MID	QC HIGH
Date	0.02950	0.11064	0.80214
Mean	0.02973	0.11082	0.79614
S.D.	0.00383	0.00973	0.07085
%CV	12.9	8.8	8.9
%Bias	0.8	0.2	-0.7
n	302	301	302

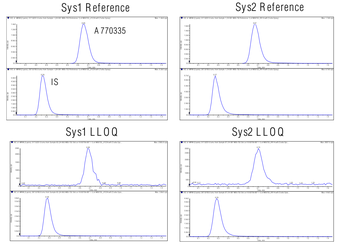
Sample Throughput

- Samples analyzed between 25-Aug-2004 and 26-Oct-2004
- Total number of samples 6875

Case II: Analysis of A-770335 (Fenofibric Acid) Using Dual SPE for Clinical Study M05-732

Sample/ Extraction Volume		50 µL
Sample Extraction		On-line solid phase extraction
	Extraction Column	Cohesive Technologies Turbo HTLC C18, 1.0 x 50 mm
	Flow Rate	4.0 mL/minute
	Loading/Re-equilibration Solution	2 mM Acetic Acid
	Washing Solution	ACN
Analytical Column		Waters Symmetry Shield RP18, 5 µm, 2.1 x 50 mm
	Flow Rate	0.30 mL/minute
	Mobile Phase	1:2 (v/v) ACN:H_2O (4.7 mM ammonium, 6 mM acetate)
Guard Column		Regis Technologies, SPS Guard, ODS, 5 µm, 100 Å, 1 cm x 3.0 mm
Autosampler		CTC HTS PAL
	Injection Volume	30 µL
	Temperature	Room temperature
	Needle/Valve Wash 1	50 mM Acetic Acid
	Needle/Valve Wash 2	ACN
MS		API 3000
	Source	Turbo Ionspray
	Detection	MS/MS, negative ion
	Detection Channels	*m/z* 317.0 → 231.0 (A-770335); *m/z* 267.0 → 195.0 (IS)
Total Run Time	Single Stream	~ 4.5 min/ sample
	Dual Stream	~ 5.0 min/ 2 samples

Ion Chromatograms



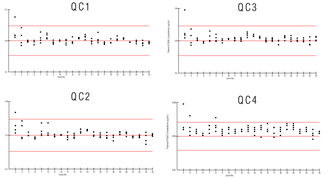
Validation Results

Dynamic Range			Recovery		
	LLOQ	ULOQ		A-770335	IS
Mean	0.016 µg/mL	5.486 µg/mL	Matrix Effect*	63.3%	NA
CV %	5.9	6.0	Extraction Efficiency	99.8%	97.0%
Bias%	3.8	3.4	* Signal loss due to matrix ion suppression		

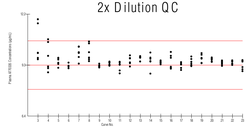
STD Statistics from M05-732

Levels	Mean	S.D.	%CV	%Bias	n
STD1	5.346	0.111	2.1	0.2	22
STD2	4.313	0.063	1.5	1.1	22
STD3	2.183	0.033	1.5	2.3	22
STD4	1.089	0.020	1.8	2.1	22
STD5	0.533	0.008	1.5	-0.1	22
STD6	0.264	0.004	1.5	-1.0	22
STD7	0.129	0.002	1.6	-3.2	22
STD8	0.065	0.002	3.1	-2.5	22
STD9	0.033	0.001	3.0	-1.0	22
STD10	0.017	0.000	0.0	2.0	22

QC Graphs from M05-732



2x Dilution QC



Sample Throughput

- Samples analyzed within 10 working days
- Total number of samples 2880

Conclusion

On-line SPE has been proven to significantly increase analyst productivity. Reduced sample preparation time allows the analyst to spend more time on batch documentation and laboratory maintenance. Utilization of the dual systems uses mass spectrometer dead time for analysis of parallel data without sacrificing chromatographic separation.

The custom configured Leap dual systems have been proven to be flexible and reliable. It provides a high-throughput platform for all of our current bioanalytical needs.

Future Direction

Cycle Composer runs as a separate software application on data acquisition computer. Macros controlling the LC are run concurrently with AB Sciex Analyst. We are working closely with Leap Technology to integrate the LC with the MS acquisition software platform (Analyst and Xcalibur) by using Leap Shell. After completion of the project, only one integrated LCMS method and one sample list will be needed.

Acknowledgement

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