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Automated Rapid Injection (ARI) for High Throughput LC-MS/MS

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Overview

- An LC-MS method with an isocratic flow and in-line filter only was used to achieve a less than 12 second injection-to-injection throughput. A previously established 1-minute LC-MS method with a ballistic gradient on a C18 column was used as a baseline comparison. The new method allows for a 5-fold increase in sample productivity.
- A 96-well plate containing 8 individual compounds treated with liver microsomes with 6 time points (with duplicates) over the course of an hour was used to assess the two methods. The drug depletion curves and systemic clearance (Clsys) values calculated from the results were used as evaluation metrics.

1. Introduction

Hit-to-Lead processes for Pharma and Biopharma laboratories are tedious, but through the advancement of hardware performance and automated data processing, results for the next pharmaceutical revelation are only a few mouse-clicks away. LCMS screening is more illuminating than ever with the Automated Rapid Injection (ARI) system to achieve dynamic results from complete studies in any plate format in a fraction of the time. Realize 12 second (apex to apex) flow injection analysis utilizing the flagship LC-40 system, coupled with the industry's fastest polarity switching and scanning speed LCMS 8060 Triple Quad for the earliest detection to IP workflow. The Shimadzu ARI is complete with automated reports, optional raw data outputs, and algorithm-based data processing to hit metrics faster with more confidence.

2. Methods

- **LC Methods**

Both methods used a Shimadzu LC-40XR UHPLC coupled with the Shimadzu LCMS-8060 Triple Quadruple MS for positive and negative mode MRM analysis utilizing 5ul injection volumes from 96-DW plate format (other 96-well and 384-well configurations possible). For the ARI method, full plate injections appear in a single data file for automated data processing with i-PeakFinder peak integration algorithm and Lab Solutions data processing software. For the 1min method, the traditional individual data files were created for each sample.

Method Parameters	Column Method	Automated Rapid Injection (ARI) Method
MPA	Water w/ 0.1%Formic Acid	Water w/ 0.1%Formic Acid
MPB	Acetonitrile w/ 0.1% Formic Acid	Acetonitrile w/ 0.1% Formic Acid
Flow (mL/min)	0.6	1.5 (50/50 Split)
Solvent Program	gradient elution (see Fig 1.)	Isocratic A%:25, B%:75
Column	Phenomenex Kinetex XB-C18 2.1X30mm, 2.6u	Phenomenex SecurityGuard ULTRA (AJ0-9000)
Inj-to-inj time(Sec)	60	<12
MRM Transitions	Individual Cmp MRM method	8 Cmp MRMs in method
Injection Vol. (uL)	5	5

Table 1. Comparison of LC parameters from Column and ARI methods

- **Mass Spec Method**

For both methods, the same interface parameters were used on the LC-MS 8060. Interface Voltage: 5KV, Neb Gas:3L/min, Heating Gas: 10L/min, Interface Temp: 350C, DL Temp: 300C, Heat Block Temp: 450C, Dry Gas: 10L/min. ESI probe position+5mm. All optimum interface parameters and MRM parameters were determined and stored via database by Lab Solutions Connect. MRM parameters for each compound are described in Table 2.

- **Data processing**

Chromatographic data were collected and analyzed by LabSolutions software. LC-MS data were taken by LabSolutions . The resultant peak area ratio versus time data was fitted to a non-linear regression using GraphPad Prism version 9 (GraphPad Software, LLC, San Diego, CA).

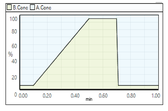

Figure 1: LC gradient profile for 1min method

3. Results

Automated Rapid Injection (ARI) LCMSMS XIC Chromatogram

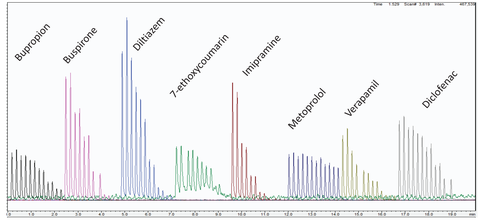

Figure 2: XICs in a single chromatogram for all 6 time pts (x2) for all 8 Compounds (Total 96 injections in 19min). Per color group going left to right, T0r1/r2, T5r1/r2, T10r1/r2, T20r1/r2, T40r1/r2, T60r1/r2.

Compound Name	m/z(1)	m/z(2)	Collision Energy (V)	Ion Mode
Bupropion	240.1178	184.1	-10	Pos
Buspirone	386.2577	122.05	-30	Pos
Diltiazem	415.1678	178.05	-25	Pos
7-ethoxycoumarin	191.0678	163.1	-15	Pos
Imipramine	281.1977	86.1	-15	Pos
Metoprolol	268.1878	116.1	-20	Pos
Verapamil	455.2878	165.1	-30	Pos
Diclofenac	294.0122	250.1	10	Neg

Table 2: Target MRM Transitions used to generate Area values used for calculations. Additional MRMs (not shown) were monitored for confirmation.

Drug in Vitro Metabolism Assay Description

•Test compounds (0.5uM) were incubated with pooled liver microsomes from male Sprague Dawley rats (0.5mg/mL total protein) at 37 ° C in the presence of an NADPH-generating system containing 50 mM, pH 7.4 potassium phosphate buffer, 3 mM magnesium chloride, 1 mM EDTA, 1 mM NADP, 5 mM glusose-6-phosphate, and 1 Unit/mL glucose-6-phosphate dehydrogenase. All concentrations were relative to the final incubation volume of 125uL. Incubations were conducted at 37° C for 0, 5, 10, 20, 40 and 60 minutes in a water bath and terminated by rapid mixing with 150 uL of ice-cold acetonitrile containing internal standard, and precipitated proteins were removed by centrifugation prior to LC-MS/MS analysis. Aliquots of the resulting supernatant fractions were diluted (20-fold) with water and analyzed by LC-MS/MS, monitoring for depletion of parent compound.

Compound Name	Clsys (mL/min/Kg) 1min Method	Clsys (mL/min/Kg) ARI Method
Bupropion	37.8	36.5
Buspirone	54	53.8
Diltiazem	52.7	52.6
7-ethoxycoumarin	42	44.9*
Imipramine	60.6	61
Metoprolol	17.2	15.8
Verapamil	46.9	53.8*
Diclofenac	37.8	36.5

Table 3: Systemic Clearance values calculated using established 1min method and Automated Rapid Injection (ARI) Method

*Some compounds showed signs of degradation between analysis on the 1min method and ARI method since approx. 1month had elapsed.

Drug Depletion Curves

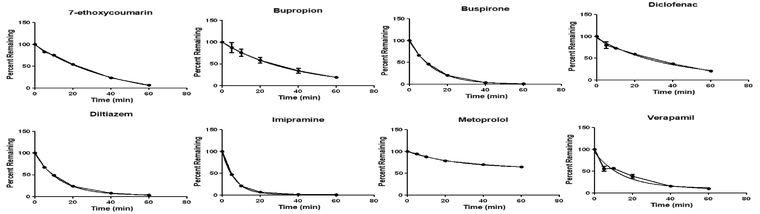

Figure 3: Drug depletion curves using data from **1min method**

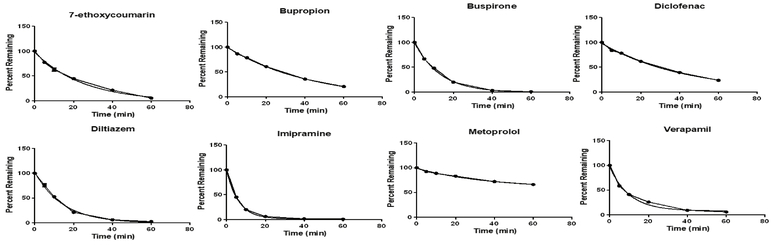

Figure 4: Drug depletion curves using data from **Automated Rapid Injection (ARI) method**

%RSD for 10 injections	T00r1	T00r2	T05r1	T05r2	T10r1	T10r2	T20r1	T20r2	T40r1	T40r2	T60r1	T60r2
Diclofenac	4.3	3.5	4.5	3.7	4.3	4.3	7.0	5.5	7.1	5.9	5.5	8.7
Verapamil *	3.7	6.5	6.2	10.3	4.5	8.7	5.7	5.8	8.2	14.6	7.2	10.3
Bupropion	9.9	7.5	5.6	5.7	6.6	6.7	6.1	7.2	6.3	5.8	8.0	7.8
Buspirone	4.4	3.7	4.0	5.8	4.6	3.3	3.3	5.5	7.3	5.6	12.0	13.4
Diltiazem	3.3	3.1	2.9	2.4	3.5	2.6	3.3	3.0	5.5	7.2	2.9	7.5
Imipramine	2.0	2.5	2.2	2.0	2.6	3.3	6.7	5.6	12.0	7.2	12.8	13.6
7-ethoxycoumarin*	11.0	14.4	11.7	12.0	12.6	13.8	16.3	14.5	16.0	24.0	41.1	40.6
Metoprolol	2.0	3.2	4.4	2.8	3.8	2.5	4.1	3.0	4.6	3.0	5.1	3.4

*Some compounds showed signs of degradation between analysis on the 1min method and ARI method since approx. 1month had elapsed.

Table 4: %RSDs for the average of each sample Area after injecting full 96-well plate 10X consecutively using the Automated Rapid Injection (ARI) Method.

4. Conclusions

- The Automated Rapid Injection system performed as well as the established 1min Chromatographic method for determining Drug Depletion Curves and Systemic Clearance values, but increased the productivity 5-fold by reducing injection-to-injection from 1min down to <12 seconds. The robustness of the ARI system was also proven by largely maintaining an average area %RSD <10% for each sample after 10 consecutive plate injections.
- Additional work (not shown here) was also done with pooling 6 or more compounds at a given time point. While overall areas were reduced, the drug depletion curves for the pooled compounds were similar to the individual compound injections. This work shows a potential for a 30X increase in productivity. Preliminary work also has been started to reduce sample injection volume to <0.5uL and inj-to-inj time to <6 seconds.
- By eliminating the LC Column separation and sample analysis time, the average Pharma In Vitro screening group can significantly increase productivity and reduce cost of operation on each Automated Rapid Injection (ARI) LCMS system which can also easily perform traditional LC-MS chromatographic analysis when needed.