Fast PK and Semi Quantitative Analysis of Metabolites Using High Resolution MS

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Introduction

It has become essential to eliminate poor candidates as early as possible in the drug development process. Unfavorable pharmacokinetic (PK) properties have frequently been the reasons for failure of new chemical entities (NCE) in the clinic. This realization led to the application of high throughput principles to the in vivo Lead Optimization process, wherein the NCE is first given to rodents to determine the PK profile in a rapid and limited study. This rapid primary screen is known as Fast PK and several critical decisions are based on this initial study.

Fast PK quantitative studies are traditionally performed using a technique known as selected reaction monitoring (SRM) on a triple quadrupole mass spectrometer. In the SRM mode, the ions specific to the NCE are focused onto the detection system, while all other non-specific ions are filtered away. As a result, only the NCE is quantified and its PK estimates determined, but any metabolite information contained in the sample is lost. With the understanding of this limitation of the SRM technique is the acknowledgement that potentially efficacious NCEs may be prematurely eliminated due to poor PK characteristics, when in fact more complete feedback from the same Fast PK study could provide the support for the decision to make a simple tweak of the chemical structure to improve bioavailability. This is where Orbitrap technology (OT) has the potential to revolutionize Lead Optimization, by providing metabolite information during the Fast PK analysis.

Experimental

Instrument Conditions

- Flow rate: 0.5mL/min (Agilent 1100)
- Gradient: Mobile phase A: Water (0.1% Formic acid) and (B) Acetonitrile (0.1% Formic acid). Gradient was 10%B, 1min, ramp to 90% B in 3min, hold 1min. Total run time 5.0min.
- Column: Atlantis® T3 C8, 2.1x50mm, 3.5um (Waters Corp., Milford, MA)
- Mass Spectrometer. TSQ Quantum Ultra™ and TSQ Quantum Exactive™ (Thermo Fisher Scientific, San Jose, CA)
- HESI-II positive ion (Spray Voltage 4.2kV, Vaporizer, 300C, Cap. Temp .50C, Sheath gas 65au, Aux Gas, 15au, Collision gas 1.5mTorr)
- Q1 at 1.0 FWHM for pre-cursor ion & Q3 at 0.7 FWHM for product ion (scan time 0.1s, scan width 0.002s)
- Exactive resolution: R=10K, 25K, 50K

Results

Figure 1 shows the comparison between bioanalysis results from a triple quadrople (TSQ Quantum Ultra, Thermo Fisher Scientific) and the Exactive Orbitrap (Thermo Fisher Scientific) at R=10K,25K, and 50K. A perfect correlation would indicate a slope of 1.000 and the quantitative performance is almost indistinguishable

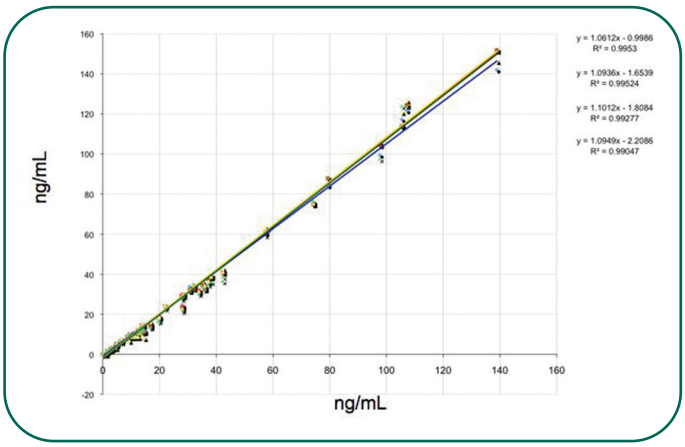

Figure 1

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between the two instrument platforms (see Figure 2) for discovery applications where the LLOQ is usually between 1-5ng/mL.

The key to identifying metabolites “on-the-fly” using high resolution mass spectrometry (HRMS) is utilizing the advantage provided by ‘mass defect’, which is the difference between the exact mass and the integer mass (which is equivalent to the number of protons and neutrons), as a result of mass deficiency. This mass deficiency is due to the fact that 12C is the only isotope with an integer exact mass of 12.00000 (ad infinitum) and used arbitrarily to set the atomic mass scale for all other elements. Since the common phase I and phase II metabolites have their own mass defects upon biotransformation they become additive (mathematically) to the precursor ion. Thus, their masses can be simply added or subtracted from the exact measured mass of the precursor ion and metabolites can be quickly identified and then quantified (see Figure 3). The quantitative analysis is performed against the parent NCE and since the response factors for the metabolites are not available at this early stage, the quantitative analysis is at best semi-quantitative or relative to the parent.

[Target] (ng/mL)	File Name	Peak Area Ratio	[Calculated] (ng/mL)	Standard Deviation	Precision % CV	Accuracy, % Difference
1	901tw104.q0	0.02692	0.960			-4.0
1	901tw180.q0	0.02804	1.01			1.0
Mean	n=2	0.02748	0.985	0.04	3.59	-1.5
2.5	901tw105.q0	0.06219	2.62			4.8
2.5	901tw181.q0	0.06013	2.52			0.8
Mean	n=2	0.06116	2.57	0.07	2.75	2.8
5	901tw106.q0	0.10925	4.83			-3.4
5	901tw182.q0	0.11873	5.27			5.4
Mean	n=2	0.11399	5.05	0.31	6.16	1.0
10	901tw107.q0	0.21703	9.89			-1.1
10	901tw183.q0	0.23368	10.7			7.0
Mean	n=2	0.22535	10.3	0.57	5.56	3.0
25	901tw108.q0	0.51663	24.0			-4.0
25	901tw184.q0	0.52057	24.1			-3.6
Mean	n=2	0.51860	24.1	0.07	0.29	-3.6
50	901tw109.q0	1.07242	50.1			0.2
50	901tw185.q0	1.01675	47.4			-5.2
Mean	n=2	1.04459	48.8	1.91	3.91	-2.4
100	901tw110.q0	2.11565	99.0			-1.0
100	901tw186.q0	2.21199	104			4.0
Mean	n=2	2.16382	102	3.54	3.47	2.0
250	901tw111.q0	5.12692	240			-4.0
250	901tw187.q0	5.23770	246			-1.6
Mean	n=2	5.18231	243	4.24	1.75	-2.8
500	901tw112.q0	10.41716	489			-2.2
500	901tw188.q0	10.15797	477			-4.6
Mean	n=2	10.28756	483	8.49	1.76	-3.4
1000	901tw113.q0	21.05740	989			-1.1
1000	901tw189.q0	21.72499	1020			2.0
Mean	n=2	21.39119	1000	21.92	2.19	0.0

[Target] (ng/mL)	File Name	Peak Area Ratio	[Calculated] (ng/mL)	Standard Deviation	Precision % CV	Accuracy, % Difference
1	901tw104.q0	0.00048	1.03			3.0
1	901tw180.q0	0.00046	1.02			2.0
Mean	n=2	0.00047	1.03	0.01	0.69	3.0
2.5	901tw105.q0	0.00261	2.60			4.0
2.5	901tw181.q0	0.00218	2.28			-8.8
Mean	n=2	0.00240	2.44	0.23	9.27	-2.4
5	901tw106.q0	0.00633	5.32			6.4
5	901tw182.q0	0.00507	4.40			-12.0
Mean	n=2	0.00570	4.86	0.65	13.39	-2.8
10	901tw107.q0	0.01207	9.54			-4.6
10	901tw183.q0	0.01169	9.26			-7.4
Mean	n=2	0.01188	9.40	0.20	2.11	-6.0
25	901tw108.q0	0.03317	25.0			0.0
25	901tw184.q0	0.03213	24.2			-3.2
Mean	n=2	0.03265	24.6	0.57	2.30	-1.6
50	901tw109.q0	0.06478	48.2			-3.6
50	901tw185.q0	0.06676	49.6			-0.8
Mean	n=2	0.06577	48.9	0.99	2.02	-2.2
100	901tw110.q0	0.13689	101			1.0
100	901tw186.q0	0.13314	98.2			-1.8
Mean	n=2	0.13501	99.6	1.98	1.99	-0.4
250	901tw111.q0	0.33427	245			-2.0
250	901tw187.q0	0.35246	258			3.2
Mean	n=2	0.34337	252	9.19	3.65	0.8
500	901tw112.q0	0.74347	540			8.0
500	901tw188.q0	0.71715	521			4.2
Mean	n=2	0.73031	531	13.44	2.53	6.2
1000	901tw113.q0	1.45577	1040			4.0
1000	901tw189.q0	1.48043	1060			6.0
Mean	n=2	1.46810	1050	14.14	1.35	5.0

Figure 2

This is an exciting new application of HRMS for drug discovery applications, where metabolism information can be provided to the synthetic chemists as well as the pharmacologist to further improve the viability of the NCE under study.

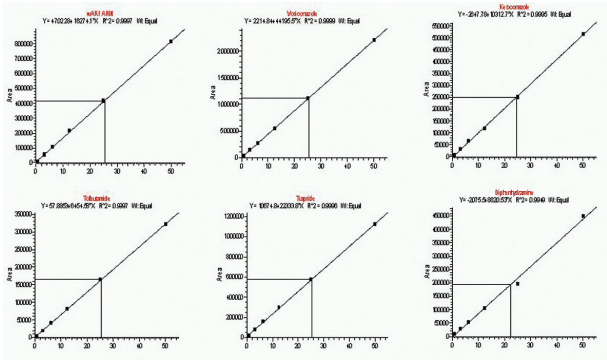

Figure 3

Conclusion

Much like a triple quadrupole, OT technology can enhance the Lead Optimization process by providing PK data for the NCE, but with the added capability to provide simultaneous, semi-quantitative information on metabolites present in the incurred sample. This allows informed decisions to be made further upstream in the discovery process, thereby reducing the cost of new drug development.

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