HIGH THROUGHPUT AND FULLY AUTOMATED SAMPLE PREPARATION FOR QUANTITATIVE BIOANALYSIS USING LC/MS/MS

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Purpose

Bioanalytical functions in the pharmaceutical industry face continuous pressure to shorten development timelines and minimize sample volumes requirements. The automation of bioanalysis samples in 384 well plates offer several known benefits, including reduced samples volume, increased throughput, improved accuracy/precision, and lower material cost.

Introduction

Bioanalysis is a crucial aspect of drug discovery and development, involving the precise measurement of drugs and their metabolites in biological samples. The manual tasks linked to these procedures demand substantial labor, inconsistency, error and time investment. However, employing robotic liquid handlers for simultaneous sample processing in a 384 wells setup has considerably reduced the duration analysts need to dedicate to sample preparation within the laboratory as well as improve accuracy, reproducibility and robustness of the assay. We employed the Bravo automated liquid handling platform to carry out the preparation of calibration curves, quality control (QC) and extraction of pharmacokinetic samples. The study included 16 different compounds, for which a 12-points calibration curve was generated. Additionally, three sets of QCs samples at high, mid, and low levels were analyzed in duplicate. Dextromethorphan, bupropion, imipramine, propranolol, ketoconazole, atenolol, raloxifene, doxepin, haloperidol, piroxicam, warfarin, tolbutamide, verapamil, sulindac, ranitidine and difelikefalin were selected as model analyst.

Methods

Sample preparation:

To a 10 µL of samples, 90 µL of acetonitrile containing an internal standard was added to precipitate the proteins in the sample. The extracts were filtered and centrifuged. The supernatant was transferred to 384 wells plate for LC-MS/MS.

Liquid Chromatography

- Pump: Binary pumps LC-30AD
- Autosampler: SIL-30ACMP
- Analytical column: ACQUITY UPLC HSS T3 column, 100 Å, 1.8 mM, 2.1 mm X 50 mm
- Column Temperature: 50 ℃
- Mobile Phase A: 0.1% formic acid in water
- Mobile Phase B: 0.1 % formic acid in acetonitrile
- Flow rate: 0.6 mL/min
- Injection volume: 4 mL

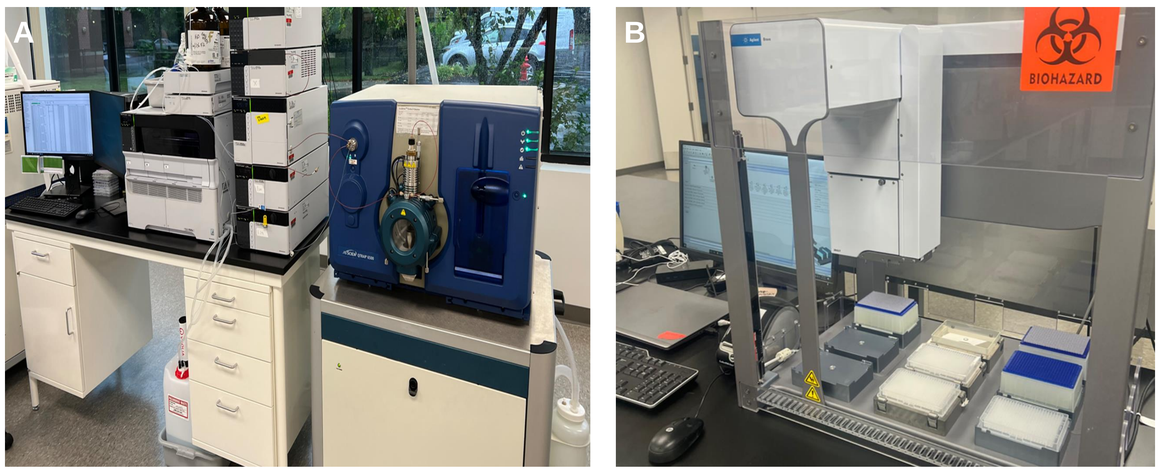

Figure 1: A) 6500 Qtrap and B) Bravo automated liquid handling platform

Mass spectrometry

- MS system: AB Sciex Triple Quad 6500
- Condition: ESI (+) MS/MS

Compound Name	Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)
Ranitidine	315.1	176	50	25
Sulindac	357	233	50	75
Verapamil	455.2	150	1	50
Warfarin	309	163.1	60	20
Tolbutamide	271.1	91.1	30	35
Piroxicam	332.08	95.0	60	25
Haloperidol	376.1	165.0	70	35
Doxepin	280.2	106.9	110	25
Raloxifen	474.6	112.1	280	35
Atenolol	267.1	190	60	25
Ketoconazole	531.1	489.1	120	45
Propranolol	260.1	183.1	70	25
Imipramine	281.2	86.0	80	35
Bupropion	240.3	184.0	70	15
Dextromethorphan	272.5	215.1	120	35
Diclofenac	296.1	215	50	25
Sildenafil_(IS)	475.3	58.1	155	55
Metoprolol	268.1	116	90	25

Table 1: MRM condition

Results

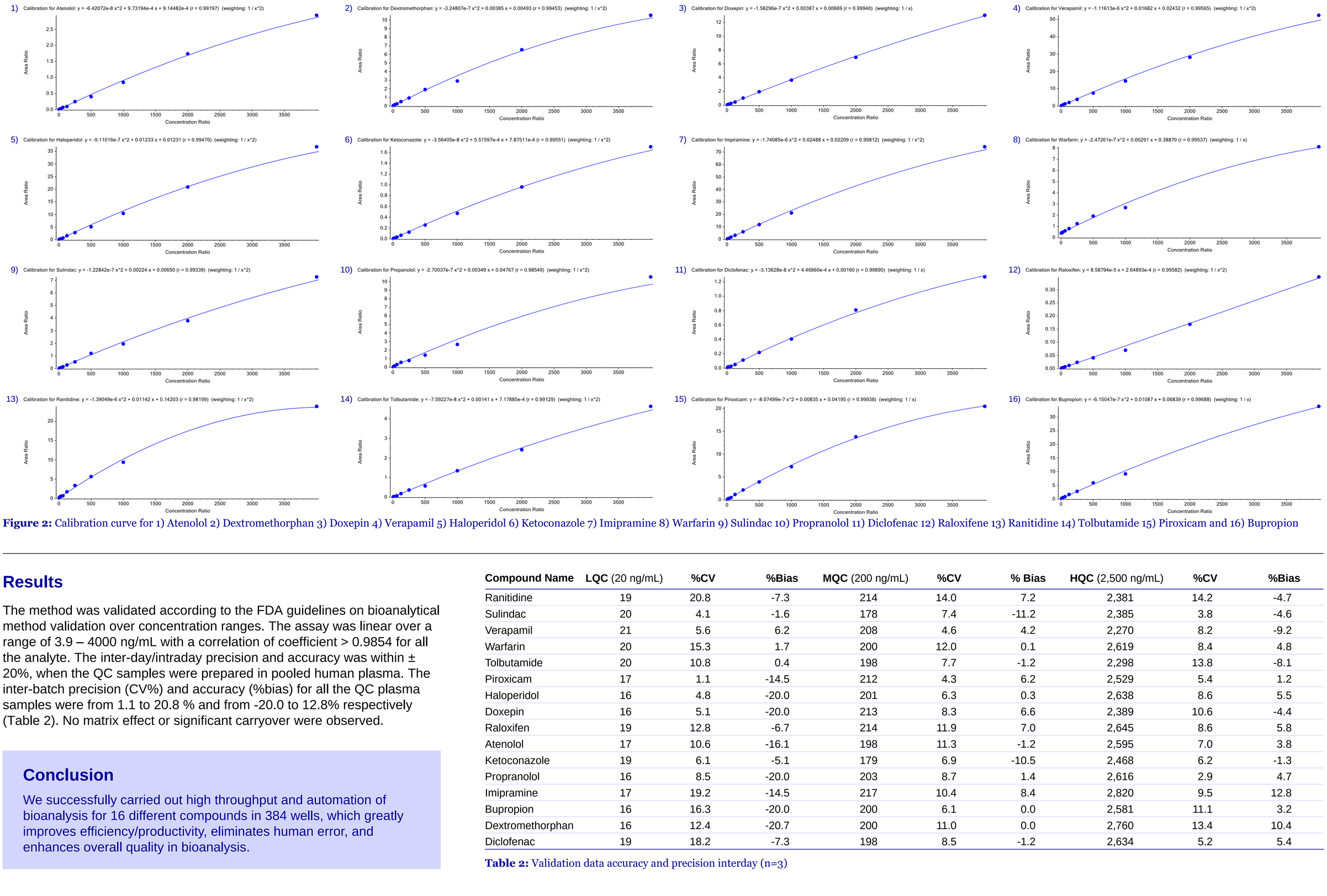

Figure 2: Calibration curve for 1) Atenolol 2) Dextromethorphan 3) Doxepin 4) Verapamil 5) Haloperidol 6) Ketoconazole 7) Imipramine 8) Warfarin 9) Sulindac 10) Propranolol 11) Diclofenac 12) Raloxifene 13) Ranitidine 14) Tolbutamide 15) Piroxicam and 16) Bupropion

Results

The method was validated according to the FDA guidelines on bioanalytical method validation over concentration ranges. The assay was linear over a range of 3.9 – 4000 ng/mL with a correlation of coefficient > 0.9854 for all the analyte. The inter-day/intraday precision and accuracy was within ± 20%, when the QC samples were prepared in pooled human plasma. The inter-batch precision (CV%) and accuracy (%bias) for all the QC plasma samples were from 1.1 to 20.8 % and from -20.0 to 12.8% respectively (Table 2). No matrix effect or significant carryover were observed.

Conclusion

We successfully carried out high throughput and automation of bioanalysis for 16 different compounds in 384 wells, which greatly improves efficiency/productivity, eliminates human error, and enhances overall quality in bioanalysis.

Compound Name	LQC (20 ng/mL)	%CV	%Bias	MQC (200 ng/mL)	%CV	% Bias	HQC (2,500 ng/mL)	%CV	%Bias
Ranitidine	19	20.8	-7.3	214	14.0	7.2	2,381	14.2	-4.7
Sulindac	20	4.1	-1.6	178	7.4	-11.2	2,385	3.8	-4.6
Verapamil	21	5.6	6.2	208	4.6	4.2	2,270	8.2	-9.2
Warfarin	20	15.3	1.7	200	12.0	0.1	2,619	8.4	4.8
Tolbutamide	20	10.8	0.4	198	7.7	-1.2	2,298	13.8	-8.1
Piroxicam	17	1.1	-14.5	212	4.3	6.2	2,529	5.4	1.2
Haloperidol	16	4.8	-20.0	201	6.3	0.3	2,638	8.6	5.5
Doxepin	16	5.1	-20.0	213	8.3	6.6	2,389	10.6	-4.4
Raloxifen	19	12.8	-6.7	214	11.9	7.0	2,645	8.6	5.8
Atenolol	17	10.6	-16.1	198	11.3	-1.2	2,595	7.0	3.8
Ketoconazole	19	6.1	-5.1	179	6.9	-10.5	2,468	6.2	-1.3
Propranolol	16	8.5	-20.0	203	8.7	1.4	2,616	2.9	4.7
Imipramine	17	19.2	-14.5	217	10.4	8.4	2,820	9.5	12.8
Bupropion	16	16.3	-20.0	200	6.1	0.0	2,581	11.1	3.2
Dextromethorphan	16	12.4	-20.7	200	11.0	0.0	2,760	13.4	10.4
Diclofenac	19	18.2	-7.3	198	8.5	-1.2	2,634	5.2	5.4

Table 2: Validation data accuracy and precision interday (n=3)