

Increased Assay Robustness and Throughput Using Automated 96-well Solid Phase Extraction

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ABSTRACT

Sample throughput and reproducibility for Olanzapine was increased using automated 96-well solid phase extraction. A manual solid phase extraction was transferred to the 96-well format using the Tomtec Quadra 96[®]. Sample volume was reduced from 1mL to 0.75mL. Prior to loading samples, the SPE plate is conditioned with methanol and phosphate buffer. After sample loading, the plate is rinsed with phosphate buffer and a wash solution before eluting with a basic elution solution. A successfully validated method transfer was obtained. The resulting assay was more sensitive, selective, accurate and precise than the manual method. Inter-assay precision and accuracy by the automated method ranged from 3.2% to 5.6%, and 1.4% to 1.9% respectively. Inter-assay precision and accuracy for the manual method ranged from 1.7% to 10.0%, and 1.6% to 3.7% respectively. The automated assay drastically reduces sample preparation time (at least a 3-4-fold decrease) decreases labor costs and increases throughput in clinical studies. Automation reduces the tedious nature of manual extraction and also decreases the incidence of lower IS recovery. This in turn leads to better batch to batch reproducibility.

PURPOSE OF AUTOMATION

- The primary purpose of automation was to reduce the time involved with sample preparation
- Automation will aid in reducing the cumbersome nature of solid phase extraction
- In addition it will reduce labor costs and increase throughput in clinical studies

METHOD

Validation scheme for transferring the manual method to the automated method

- Fresh calibration line
- QCs at the limits of detection (n = 6)
- 3 day inter-assay precision and accuracy

Comparison of General Assay Procedure

Manual Assay

- Sample volume: 1mL
- Uses 130mg Bond Elut Certify
- Erratic recovery for both drug and ISTD causing variability in the lower points of the calibration line
- Line in singlet. Low, Mid and High QCs

Automated Assay

- Sample volume: 0.75mL
- Uses 10mg Oasis MCX
- Recovery tracks well throughout the batch. Overall there was a better recovery for both drug and ISTD
- Line in duplicate. QCs at the limits of detection

General Assay Procedure - Automation

- Load Samples (calibrators, QCs, unknowns) onto 96-well plate
- Add ISTD before loading it onto the automated platform
- Methanol and buffer are used to condition the SPE plate. Samples diluted with buffer and subsequently loaded onto the SPE plate
- Plate is rinsed with buffer and a wash solution before eluting with basic elution solution
- Evaporate the organic and reconstitute before injecting onto HPLC system using a 96-well autosampler

Calibration Standard Statistics (automated method)

Nominal Concentration	100	50.0	25.0	10.0	5.00	2.50	1.00	0.500	0.250
Average Concentration	99.3	50.3	25.3	10.0	5.00	2.51	1.002	0.487	0.250
Standard Deviation	1.44	0.372	0.488	0.093	0.079	0.0369	0.0230	0.0250	0.0179
Precision (%RSD)	1.5%	0.7%	1.9%	0.9%	1.6%	1.5%	2.3%	5.1%	7.2%
Accuracy (%RE)	-0.7%	0.6%	1.4%	0.4%	0.1%	0.5%	0.2%	-2.6%	-0.2%
N	6	6	6	5	6	6	6	5	5

Batch																		
O08T	101	98.2	50.6	50.3	25.0	24.9	10.0	9.90	4.89	4.95	2.49	2.45	0.980	0.999	0.495	0.503	0.265	0.256
O09T	98.3	98.9	50.4	50.7	26.0	25.4	C	10.1	5.09	5.08	2.54	2.53	1.02	1.03	0.501	0.443	0.233	C
O10T	101	98.3	49.8	49.9	24.9	25.8	10.0	10.1	5.03	4.99	2.54	2.53	1.01	0.974	B	0.493	0.228	0.266

A = Quality control or calibration standard dropped due to interference

B = Quality control or calibration standard dropped due to prep error

C = Quality control or calibration standard dropped due to instrument error

Inter-Assay Quality Control Sample Statistics (automated method)

Nominal Concentration	100	40.0	0.250
Average Concentration	102	40.6	0.255
Standard Deviation	3.29	1.704	0.0144
Precision (%RSD)	3.2%	4.2%	5.6%
Accuracy (%RE)	1.7%	1.4%	1.9%
N	18	18	17

Batch			
O08T	102	38.6	0.249
	101	37.6	0.252
	95.0	38.3	0.262
	97.6	38.0	0.251
	97.7	39.3	0.246
	97.4	38.3	0.248
O09T	103	41.5	0.245
	102	41.8	0.241
	102	40.4	0.258
	101	41.0	0.232
	102	41.9	0.257
	101	41.1	0.240
O10T	106	41.8	0.288
	108	41.8	0.275
	104	42.0	0.275
	105	42.7	A
	105	42.2	0.263
	102	41.6	0.251

Calibration Standard Statistics (manual method)

Nominal Concentration	100	50.0	25.0	10.0	5.00	2.50	1.00	0.500	0.250
Average Concentration	101	49.6	24.8	9.97	4.91	2.50	1.00	0.489	0.263
Standard Deviation	1.00	0.917	0.306	0.113	0.0436	0.0351	0.0252	0.0292	0.0126
Precision (%)	1.0%	1.8%	1.2%	1.1%	0.9%	1.4%	2.5%	6.0%	4.8%
Accuracy (%)	1.0%	-0.8%	-0.7%	-0.3%	-1.8%	0.1%	0.1%	-2.2%	5.1%
N	3	3	3	3	3	3	3	3	3

Batch									
L01O	101	49.4	25.1	10.1	4.86	2.50	0.989	0.516	0.251
L02O	100	50.6	24.9	9.91	4.93	2.54	0.984	0.458	0.276
L03O	102	48.8	24.5	9.90	4.94	2.47	1.03	0.493	0.261

Inter-Assay Quality Control Sample Statistics (manual method)

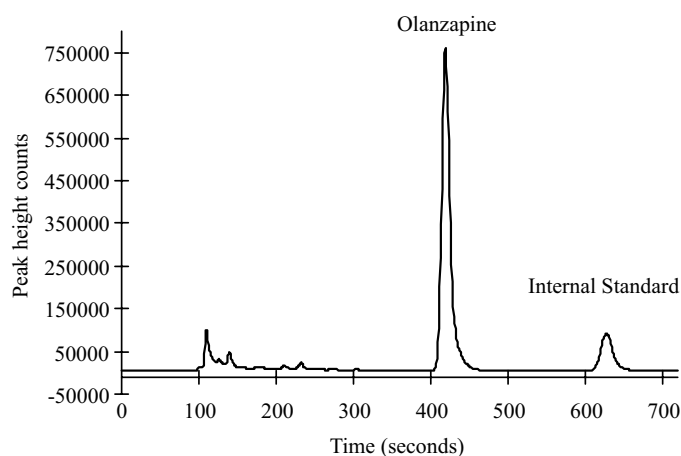
Nominal Concentration	80.0	40.0	0.640
Average Concentration	81.2	41.4	0.653
Standard Deviation	4.25	1.52	0.0421
Precision (%)	5.2%	3.7%	6.4%
Accuracy (%)	101.4%	103.4%	102.0%
N	16	16	16

Batch			
L01O	85.1	42.2	0.720
	80.7	42.8	0.712
	78.2	41.0	0.678
	69.0	41.7	0.711
	82.2	42.6	0.684
L02O	79.0	39.7	0.600
	81.0	39.3	0.615
	79.9	39.3	0.625
	78.8	40.6	0.598
	80.1	39.6	0.596
	79.2	39.2	0.628
L03O	83.7	43.6	0.673
	84.7	42.7	0.677
	86.1	42.6	0.657
	84.8	42.6	0.627
	86.0	42.1	0.647

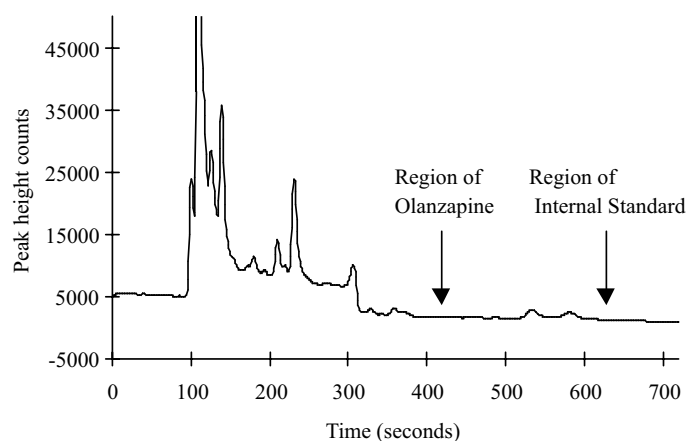
Assay Specifics

- Sample Volume: 750 μ L
- Sample Preparation: Solid Phase Extraction
- Validated Range: 0.250 - 100 ng/mL
- Column: YMC Basic column
- Mobile Phase: 75 mM PO_4 buffer/MeOH/ACN 48/26/26 v/v/v
- Quantitation: Linear regression with 1/concentration weighting.
Quantitation by peak height ratio
- Detection: BAS LC - 4C Electrochemical Detector

Representative 100ng/mL Calibrator Chromatogram



Representative Blank Chromatogram



Assay throughput increased using automation

100 samples take about 6 -7 hours to extract manually

400 samples take about 6 -7 hours to extract using automation

Results

- Automation improves precision and accuracy of the method
- Changing to a polymer based cation exchanger SPE column eliminates the secondary silanol interactions and improves recovery for both drug and ISTD

Conclusion

- Automation drastically reduces sample preparation time (3 - 4 fold decrease) and reduces the tedious nature of manual extractions
- Changing from silica to polymer based SPE gives better batch to batch reproducibility
- Better recoveries are obtained for both drug and ISTD improving low end robustness
- Automating the assay helps with increasing throughput in clinical studies