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

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Previously, a reversed-phase liquid chromatography method with electrochemical detection was developed to quantitate olanzapine in human plasma. In order to improve the extraction reproducibility and increase the sample throughput, the method was automated using the 96-well solid phase extraction format on a TomTec Quadra 96. The resulting assay drastically reduced the sample preparation time, while maintaining excellent accuracy and precision.

Olanzapine (2-methyl-4(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*][1,5]benzodiazepine) (***F1***) is an atypical antipsychotic drug that is used in the treatment of schizophrenia. It belongs to a newer class of agents that are effective against both positive symptoms, such as delusions and hallucinations, and negative symptoms, such as social withdrawal and poverty of speech (1). Olanzapine displays high affinity for certain neurotransmitter receptors, such as those for dopamine and serotonin (2).

To monitor therapeutic dosages and assess pharmacological profiles, olanzapine is analyzed in human plasma. For this purpose, a sensitive analytical method is needed which can detect sub-ng/mL concentrations. In the mid-90s, we validated such a method using reversed phase chromatography with electrochemical detection (3). The sample preparation was based on a manual solid phase extraction (SPE) procedure. This approach yielded a fairly rugged assay, but there were a few drawbacks. First, the manual procedure was quite labor intensive, taking many hours to prepare a large batch of samples. Also, some within-batch variation of the extraction was observed, with occasional SPE tubes giving somewhat lower recovery. Although the internal standard (ISTD) (see ***F1***) generally compensated for this effect, more consistent extraction recovery was preferred.

With the advent of 96-well SPE and robotic extraction equipment, it became attractive to automate the existing assay. This report highlights the resulting automated procedure and the improvements obtained.

Experimental

Assay Summary

Matrix: heparinized human plasma
Sample Preparation: solid phase extraction
Validated Range: 0.250 - 100 ng/mL
HPLC Column: YMC Basic (4.6 x 150 mm)
Mobile Phase: 75 mM phosphate buffer/MeOH/ACN 48/26/26 v/v/v
Quantitation: peak height ratio using linear least squares regression with 1/concentration weighting
Detection: BAS LC-4C electrochemical detector

Manual Sample Preparation

- Load 1.00 mL plasma (calibrator, QC, unknown) into individual test tubes
- Add ISTD and buffer using a repeater pipet
- Precondition 130 mg Bond Elut, Certify SPE columns with MeOH and buffer
- Load sample, then rinse SPE tube with buffer followed by SPE rinse solution
- Elute compounds of interest using basic elution solution
- Evaporate to dryness, reconstitute, then inject onto HPLC system

Automated Sample Preparation

- Load 750 µL plasma (calibrator, QC, unknown) into each well of a 96-well plate
- Add ISTD using a repeater pipet and put plate onto the TomTec Quadra 96
- TomTec preconditions 10 mg Oasis MCX SPE plate by loading MeOH then buffer
- TomTec dilutes plasma samples with buffer, then loads them onto SPE plate
- Plate is rinsed with buffer and wash solution before eluting with basic elution solution
- Evaporate the organic and reconstitute before injecting onto HPLC system using a 96-well autosampler

Method Validation

Blank heparinized human plasma samples were spiked with olanzapine to obtain a standard curve. Calibration standard concentrations ranged from 0.250 ng/mL to 100 ng/mL, as shown in ***T1***. Quality control (QC) samples were prepared at three concentrations and run in each batch. Three separate batches of QC samples were analyzed to evaluate the inter-assay precision and accuracy (***T2***). Specificity and recovery were also determined (data not shown). Stability data was taken from the original manual validation (3).

Results and Discussion

Olanzapine can be extracted from human plasma using mixed-mode SPE cartridges, then eluted from the support with a basic solution. The initial manual method used in our labs employed a typical "syringe barrel" style SPE tube containing 130 mg of Bond Elut Certify. This extraction scheme included typical steps, such as preconditioning with methanol and buffer, loading the diluted plasma sample, rinsing with phosphate buffer and a wash solution, and eluting with a basic elution solvent. This procedure was performed 24 samples at a time on a manual vacuum manifold.

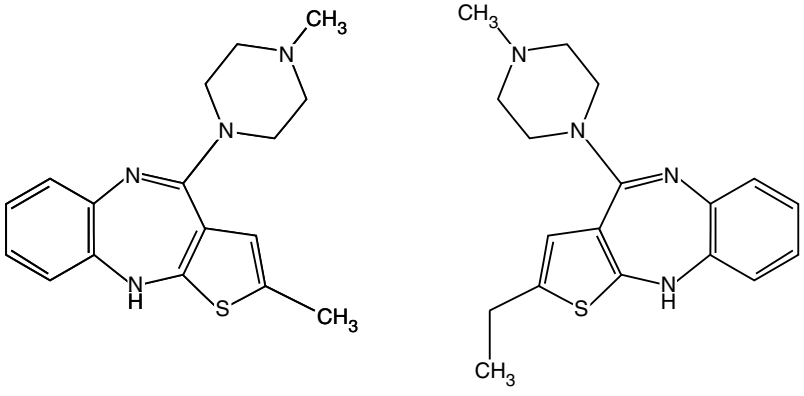
Only slight changes were needed to convert this method to the 96-well format. The ionic strength of the buffer was increased, but the other solutions remained the same. Smaller liquid volumes had to be used due to the capacity of the sample wells in the plate. The bed mass in the SPE plate was reduced to 10 mg for the automated method, and the sorbent was changed to Oasis MCX. Prior to loading samples, the extraction plate was conditioned with methanol and phosphate buffer. After sample loading, the plate was rinsed with phosphate buffer and a wash solution before eluting with a basic elution solution.

The liquid handling expertise of the TomTec Quadra 96 was then exploited to automate the 96-well method. Once the TomTec was programmed, all the normal SPE functions could be carried out automatically. Initial pipetting of the plasma and internal standard into the 96-well plate were still performed manually. The plate also had to be covered and heat-sealed, centrifuged and placed into the autosampler by the analyst. The autosampler could inject directly from the plate onto the chromatography column.

A successfully validated method transfer was obtained. Automation reduced the tedious nature of manual extraction and minimized the chance of errors. The automated assay also reduced the sample preparation time by a factor of four. For example, a batch of approximately one hundred samples takes about six to seven hours to extract manually. However, four batches of this size can be prepared in the same amount of time when the automated method is used. This is significant because reduced sample preparation time decreases labor costs and greatly increases sample throughput for large clinical studies.

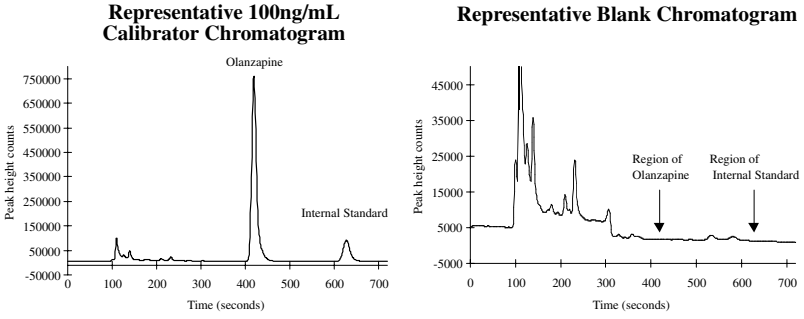
F1

Structures of olanzapine and ISTD.



F2

Chromatograms



T1

Automated calibration standards table.

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

T2

Automated QC stats.

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

In addition to increased speed, the automated assay was more sensitive. In fact, only 0.75 mL of plasma was needed to achieve a 0.25 ng/mL limit of quantitation using the automated method, versus 1 mL of plasma with the manual procedure. The automated method was just as accurate and precise as its predecessor. The inter-assay precision and accuracy for the automated method ranged from 3.2% to 5.6% and 1.4% to 1.9%, respectively (***T2***). Furthermore, the automated assay had more consistent recovery (data not shown) and showed good selectivity. A typical chromatogram of a plasma extract containing olanzapine and the internal is shown in ***F2***, along with a chromatogram of a blank plasma extract. The scale for the blank extract chromatogram has been expanded to emphasize the absence of plasma components coeluting with the analytes. Thus, overall the automated method proved to be rapid, sensitive, precise and accurate.

Conclusion

A manual solid phase extraction method was transferred successfully to the 96-well format and automated on the TomTec Quadra 96 with minimal change in solutions or procedures. Automation drastically reduced the sample preparation time (four-fold decrease) and eliminated the tedious nature of manual extraction. Less plasma volume was needed in the automated method, yet it achieved the same lower limit of quantitation. Thus, the automated assay helped increase the sample throughput for clinical studies, while simultaneously improving the method's sensitivity. All of this was achieved while maintaining good accuracy and precision.

References

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2. *R.W. Fuller and H.D. Snoody, Res. Comm. Chem. Path. Pharmacol. 77 (1992) 545.*
3. *BAS Analytics Standard Analytical Procedure #820-0192, entitled "Olanzapine (LY170053) in Heparinized Human Plasma."*