

Increased Sample Throughput For Ketoconazole Analysis By Automated 96-Well Sample Preparation And Multiplexed HPLC

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ABSTRACT

Purpose. In order to increase the sample throughput of a manual LC-FL assay for ketoconazole, the method was automated and a multiplexed HPLC system was employed.

Methods. The original ketoconazole method involved manual protein precipitation, followed by HPLC-FL analysis with a run time of approximately 15 minutes. The sample preparation was converted to the 96 well format and automated using the Tomtec Quadra96. In addition, a multiplexed HPLC system was configured to cut the HPLC run times in half. The LC configuration enabled alternate sample injections to travel down one of two HPLC columns. The injections were staggered such that the eluting peaks could be diverted to a single FL detector.

Results. The resulting assay was fully validated and readily met $\pm 15/15/15\%$ acceptance criteria. Based on the assay's performance statistics, the automated method was found to be robust, even though two separate analytical columns were used for the same batch. Additional data using high-flow HPLC on a monolithic rod column also looks very promising.

EXPERIMENTAL

Matrix: 250 μL heparinized human plasma

HPLC Column: Waters Symmetry C18 (150 x 3.9 mm) with Javelin BDS C18 precolumn (20 x 3 mm) or Chromolith Performance RP-18e column (100 x 4.6 mm)

Mobile Phase: 40% ACN : 60% 50 mM Phosphate Buffer, pH 7

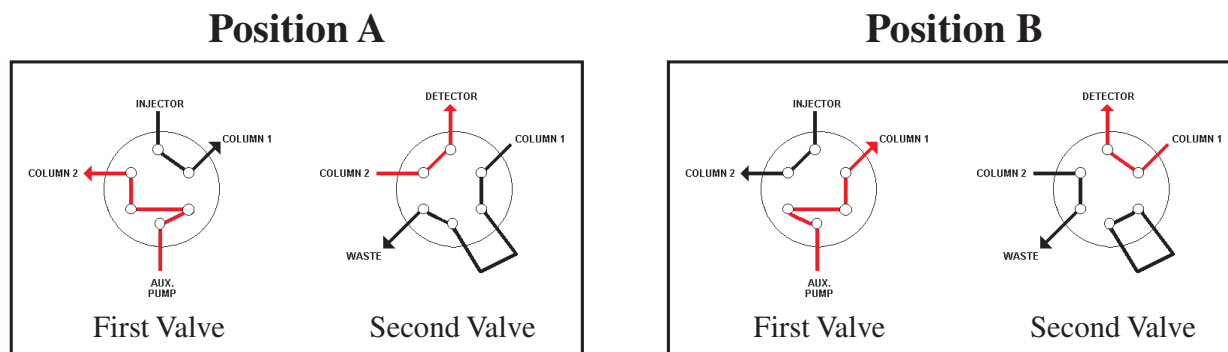
Detector: Hitachi L-7480 fluorescence detector. $\lambda_{\text{ex}} = 260$ $\lambda_{\text{em}} = 375$

Validated Range: 0.2 to 50 $\mu\text{g}/\text{mL}$

Manual Sample Prep: Protein precipitation by combining 250 μL heparinized human plasma and 500 μL acetonitrile. Vortex mix, then centrifuge for 10 min. Transfer 500 μL of the supernatant to an autosampler vial, dilute with an equal volume of water, vortex mix, then inject 100 μL .

96-Well Sample Prep: Aliquot 250 μL of each sample into a 1.2 mL 96-well plate. The Tomtec Quadra96 adds 500 μL acetonitrile. Cap plate, vortex, and centrifuge for 5 min. The Tomtec then transfers 250 μL of the supernatant to a fresh plate, dilutes with an equal volume of water, and mixes. Centrifuge plate for 5 minutes, apply foil seal, then inject 100 μL .

Multiplexing Configuration: An autosampler alternately injects onto one of 2 columns via a switching valve. Another synchronized switching valve selects one column to the detector and one column to waste. (see schematic) Since the peak of interest elutes near the end of the chromatogram, the first half of each run is diverted to waste.

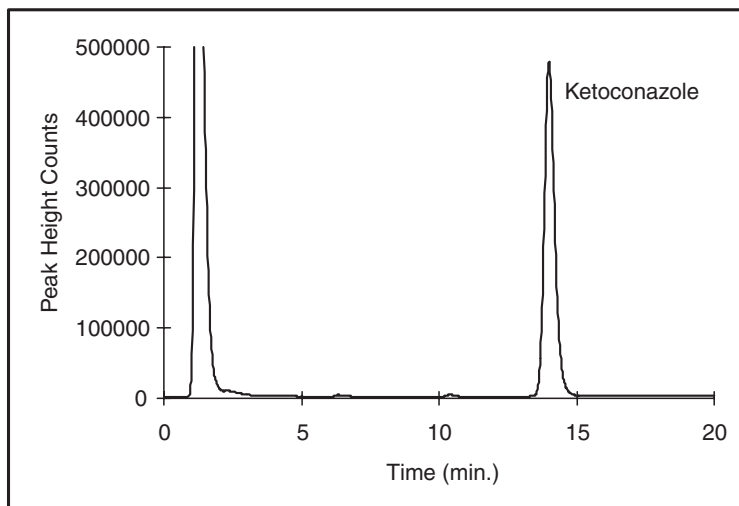


RESULTS

Throughput Comparison

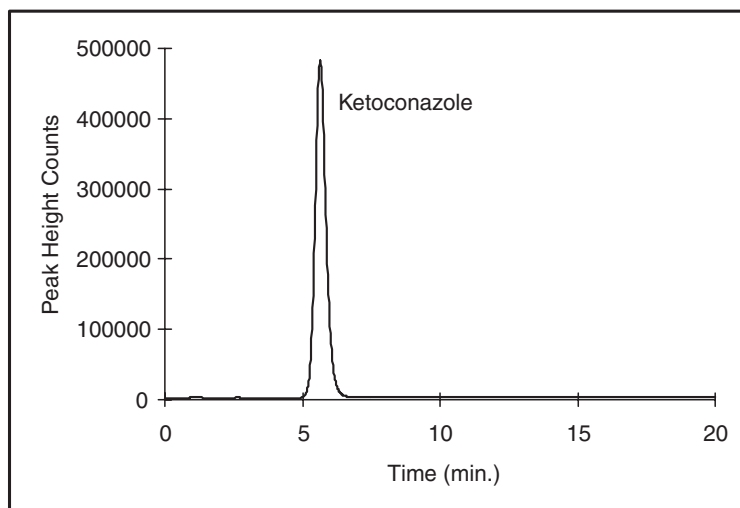
Configuration	Sample Prep	LC Run	Total time
Manual – Sequential	1.5 hr	26.0 hr	27.5 hr
TomTec – Multiplexed	0.5 hr	13.0 hr	13.5 hr
TomTec – Chromolith	0.5 hr	6.0 hr	6.5 hr

Manual - Sequential



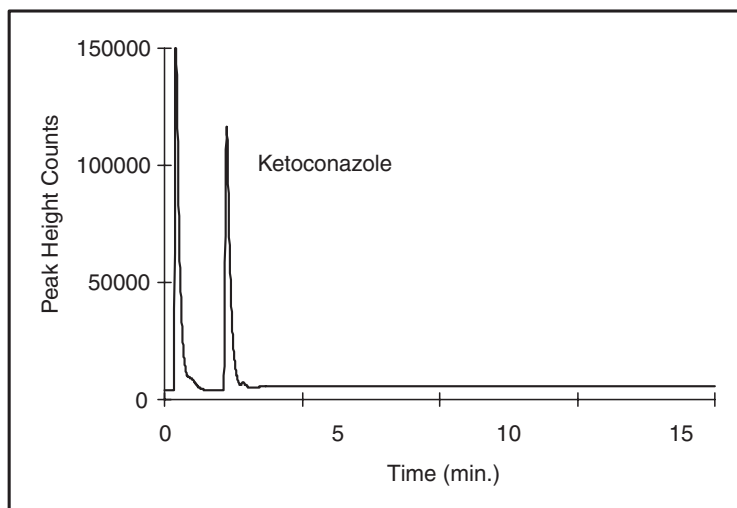
Nominal Concentration (ng/mL)	40000	20000	600
Average Concentration (ng/mL)	40279	20356	623
Standard Deviation	763	561	36.0
Precision (%)	1.9%	2.8%	5.8%
Accuracy (%)	100.7%	101.8%	103.9%
N	18	18	18

TomTec - Multiplexed



Nominal Concentration (ng/mL)	40000	20000	600
Average Concentration (ng/mL)	40424	20134	598
Standard Deviation	961	334	17.5
Precision (%)	2.4%	1.7%	2.9%
Accuracy (%)	101.1%	100.7%	99.6%
N	18	18	18

TomTec - Chromolith



Nominal Concentration (ng/mL)	40000	20000	600
Average Concentration (ng/mL)	39319	18997	627
Standard Deviation	1151	341	27.5
Precision (%)	2.9%	1.8%	4.4%
Accuracy (%)	98.3%	95.0%	104.6%
N	18	18	18

CONCLUSIONS

- Use of the Tomtec Quadra96 and multiplexed HPLC allows significant time savings without a loss of quality.
- Although typically associated with LC/MS/MS assays, multiplexed HPLC is useful with other detectors as well.
- The Chromolith HPLC column appears to provide definite speed advantages without sacrificing chromatographic performance.
- The data demonstrate the ability to injection a single batch onto two HPLC columns without compromising the assay.