

Quantitation of Fentanyl and Norfentanyl from Urine Using On-line High Throughput System

Francois A. Espourteille, Ph. D., Thermo Fisher Scientific, Franklin, MA

Introduction

The use of the Thermo Scientific Aria TLX-4 system with TurboFlow™ methods for automated on-line sample cleanup of a biological sample is well documented in the literature¹. The Aria™ TLX-4 system enhances the sensitivity, specificity, and precision for mass spectrometric detection of fentanyl and norfentanyl. Increasing demand in clinical research laboratories for higher sample throughput has put the emphasis on automated methods and platforms that have the ability to quickly ramp up throughput to meet demand.

The Aria TLX-4 system extracts both fentanyl and its metabolite, norfentanyl, from the many interferences found in urine and chromatographically separates them from each other, before sending them to the mass spectrometer. TurboFlow extraction methods exclude both high molecular weight species and salts while the stationary phase coating retains the analyte(s) through reverse phase column chemistry. This results in fast, efficient, on-line separation of fentanyl and its metabolite prior to introduction into the mass spectrometer.

Goal

- Eliminate the need for SPE extraction of urine samples for fentanyl/norfentanyl assay
- Significantly increase sample throughput by running multiple samples simultaneously in front of one mass spectrometer
- Confirm the stability of the on-line assay

Key Words

- TurboFlow Technology
- TSQ Quantum Access
- Clinical Research
- Aria TLX-4

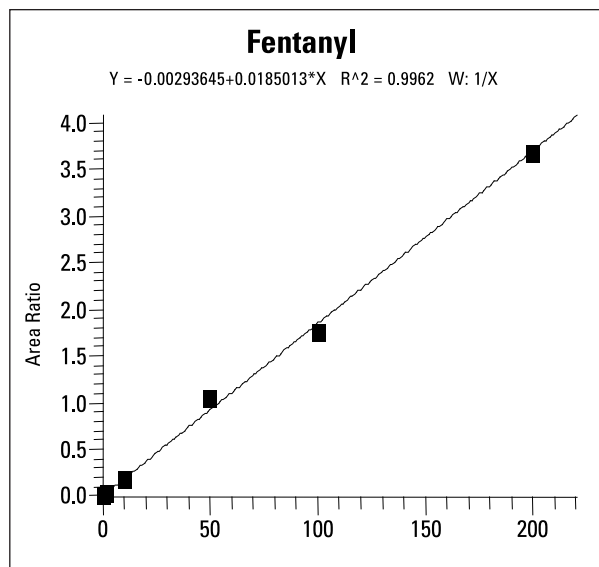


Figure 1: Calibration curves of Fentanyl from 4 channels of Aria TLX-4 System. Data courtesy of Dennis Crouch, Ameritox, LTD.

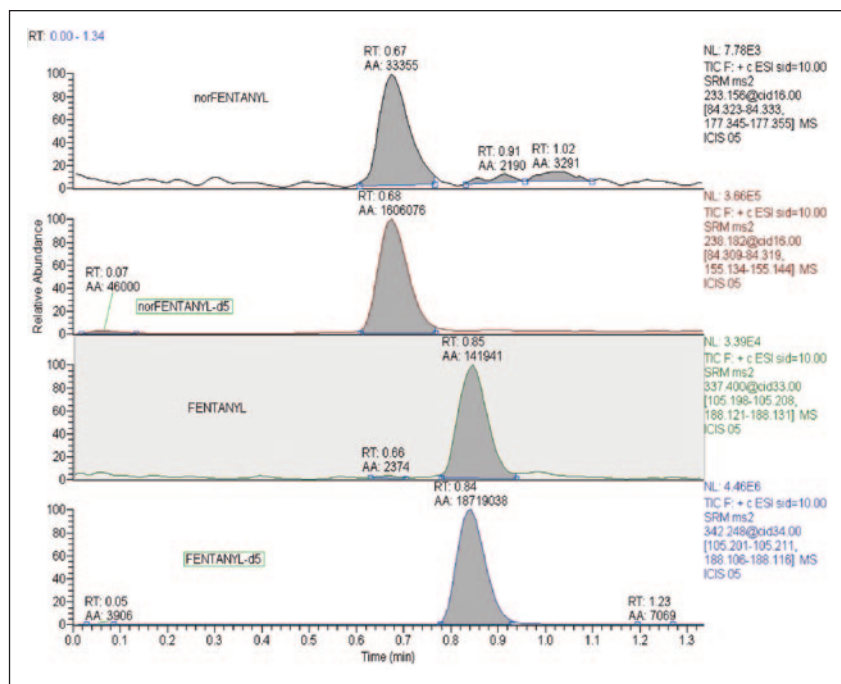


Figure 2: Excellent Signal/Noise at LOQ for (A) Norfentanyl and (B) Fentanyl at 0.5 ng/mL calibration. Data courtesy of Dennis Crouch, Ameritox, LTD.

Methods

This method describes the analysis for the determination of fentanyl and its metabolite, norfentanyl, from a urine sample. Human urine was used as the test matrix. An LOQ of 0.5 ng/mL was seen in human urine, with an LOD below 0.1 ng/mL. Instrumentation used is identified in Table 1.

Table 1. Instrumentation used in this method

| | |
|--------------------|--|
| LS-MS/MS: | Aria TLX-4 with Thermo Scientific TSQ Quantum Access triple quadrupole mass spectrometer |
| Extraction column: | Thermo Scientific TurboFlow XL C18 P 0.5x50 mm |
| Analytical column: | Thermo Scientific Hypersyl GOLD aQ 3x50, 5 µm |

Experimental Conditions:

A working solution containing fentanyl and norfentanyl at 1000 ng/mL was made. Subsequent dilutions yielded a curve from 200 ng/mL to 0.5 ng/mL. An internal standard solution containing both fentanyl-D5 and norfentanyl-D5 was added to all standards. Samples were vortexed and then centrifuged at 10,000 RCF for 5 minutes and analyzed immediately.

Results:

The data in Figure 1 shows linear regression for 0.5 ng/mL to 200 ng/mL, with 1/x weighing. Figure 2 demonstrates the limit of quantitation with excellent signal to noise ratio.

Conclusion:

The Aria TLX-4 system powered by TurboFlow technology provides a fast, efficient, and automated on-line separation technology for the extraction and analysis of fentanyl and its metabolite, norfentanyl. The ability to run 5.5 minute methods on four channels further decreases analysis time and increases the efficiency of the TSQ Quantum Access™ mass spectrometer. The Aria TLX-4 coupled with the TSQ Quantum Access can run one sample every 86 seconds with a 92.9% sample completion rate with 7.1% re-injection². The method run time was 5.5 minutes. This system provides a reliable high throughput method of fentanyl and norfentanyl for clinical research labs.

References

1. Sauvage et al. 2006. Therapeutic Drug Monitoring 28(1), pp. 123-130.
2. Crouch, Dennis. *The Analysis of Fentanyl and Norfentanyl using TurboFlow Column Analyte Isolation and Multiplex-HPLC/MS/MS*. Oral presentation, AAFS, Washington DC February 17-20 2008.

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