

High throughput workflow analysis: Dextrorphan in Human Plasma

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Keywords: High-throughput, extraction-analysis, LDTD, Tandem mass spectrometry, Dextrorphan, H. Plasma

Overview

- High-throughput extraction and analysis of Dextrorphan in human plasma is performed by an Agilent Bravo robot system and a LDTD-MS/MS system;
- Extraction and analysis of 384 samples within 69 minutes.

Instrumentation

- Agilent extraction system, Bravo
- Phytronix Technologies LDTD ion source.
- Agilent 6410 mass spectrometer system.

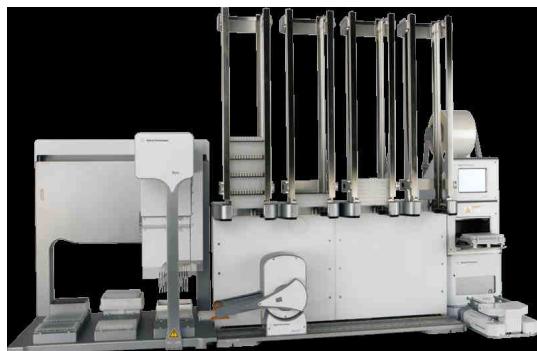


Figure 1. Extraction system



Figure 2. LDTD-MSMS system

Workflow process

The extraction procedure of 384 samples is allowed in 9 minutes while the LDTD-MS/MS analysis takes 60 minutes. Therefore operation both process in parallel (figure 3) allows 69 minutes for 384 samples.

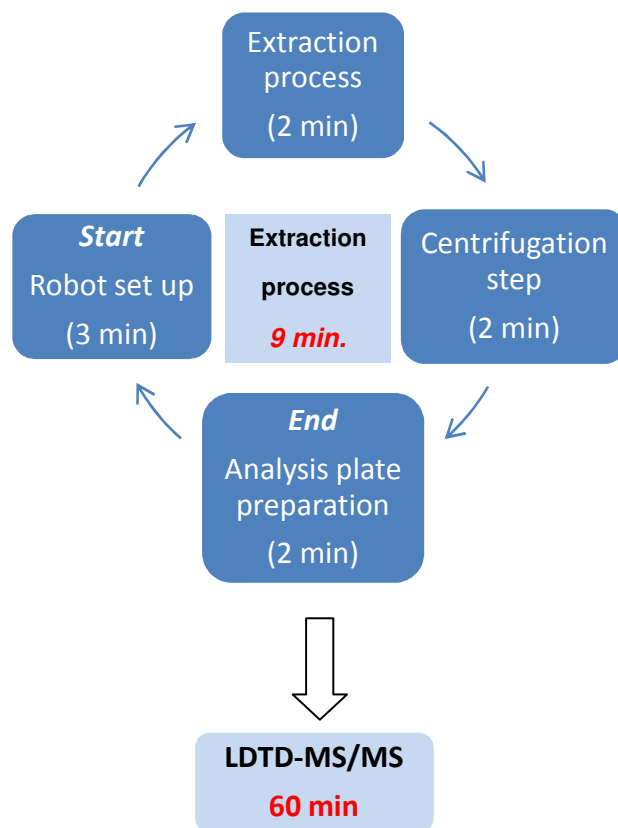


Figure 3 Workflow process of sample extraction and analysis for 384 samples

Samples Preparation

Liq-Liq extraction:

Human plasma sample was spiked with Dextrorphan and Dextrorphan-d3 was used as internal standard (ISTD). Extraction was performed as follows:
 - 50 µL of Ethyl acetate was transferred into the extraction plate.

- 2 µL of ISTD was transferred into the extraction plate.
- 10 µL of sample was transferred into the extraction plate.
- Solutions were mixed by aspirate/dispense tip system
- Add 2µL of extracted sample into the LazWell
- Samples were dried with air flow using 384 pins dryer

MS Parameters

Gas temperature	325°C
Gas flow	5 L/min
Dwell	20
Fragmentor	135
Collision energy	38
Polarity	Positive
Delta EMV (+)	900
Dextrorphan	258.3 → 157.2 amu
Dextrorphan-d3	261.3 → 157.2 amu

LDTD Parameters

Laser power pattern: 0 to 45% in 3.0 sec. Hold at 45% for 2 sec.

Carrier gas flow: 3 L/min (Air)

LDTD ionization process

The LDTD ion source uses an infrared laser diode to desorb sample that have been dried onto a well of a LazWell™ (384-well plate). The desorbed gas phase molecules are carried into a corona discharge region to undergo APCI, and then they are transferred directly into the mass spectrometer for detection.

Results and Discussion

The extraction system and LDTD-MS/MS allows high-throughput extraction and analysis of Dextrorphan in human plasma where 2304 samples/hours are extracted and 384 samples/hours are analyzed.

Dextrorphan Calibration Curve

Quantitative determination of Dextrorphan in human plasma liquid-liquid extraction can be achieved over a nominal concentration range of 1.6 to 800 ng/mL (**Figure 4**). An excellent linearity is obtained over the concentration range ($R^2 = 0.991$).

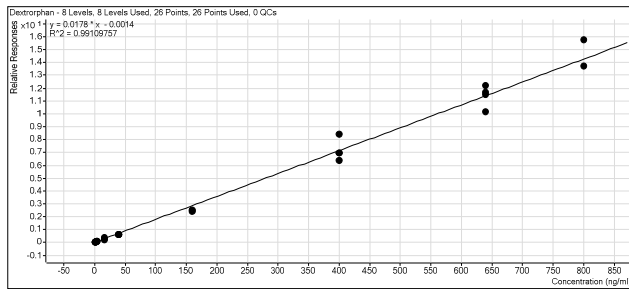


Figure 4 Standard curve of Dextrorphan

Extraction and analysis time comparison

Classic extraction and analysis procedure time is compared (**table 1**). The following parameters are used to evaluate manual extraction:

- Sample set: 192 samples per technician.
- Analyst extraction time: 8 hours
- LC/MS/MS run time: 3 minutes / samples

Process	Workflow system	Classic analysis
Quantity	Samples : 384	Samples : 384*
Extraction	Time : 9 minutes	Time : 480 minutes
Analysis	Time :60 min.	Time :1152 minutes
Total	69 min. (1.09h)	1632 min. (27h)

*For two technicians.

Table 1 Comparison process time

Conclusions

The combination of Bravo extraction system and LDTD-MS/MS (Agilent 6410 system) allows ultra-fast **extraction-analysis of 384 samples in 1.09 hours** while it takes 27 hours to do the same in LC/MS/MS. Therefore using the workflow setup during the same 27 hours period over **9000 samples** could be analyzed instead of only 384 with classic analysis.

This extraction procedure may be applied for protein precipitation.

For more information about your specific application, visit www.phytronix.com

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