



Rapid Cleanup and Comprehensive Screening of Pain Management Drugs in Urine

Highlights

- Automated disposable pipette extraction LC-MS/MS allows sensitive determination of pain management drugs in urine samples
- Fully automated sample preparation
- High throughput automation for just-in-time screenings

Overview

This study focuses on the automated extraction of small volumes of urine samples (< 500 µL) using disposable pipette extraction (DPX) for the comprehensive screening of pain management drugs by LC-MS/MS.

Using a GERSTEL MPS autosampler, DPX extractions of hydrolyzed urine were performed, using a reversed phase (DPX-RP-S) sorbent. The resulting eluents were diluted and analyzed by LC-MS/MS allowing rapid, "just-in-time" sample preparation for high throughput screenings, averaging an injection to injection cycle time of 7 min/sample.

Introduction

Toxicology laboratories are trying to find ways to minimize sample preparation and enhance productivity. The adaptation of LC-MS/MS instrumentation is desired due to its high sensitivity, selectivity, omission of analyte chemical derivatizations, less sample volume use and low detection limits (e.g., 1 ng/mL).

Disposable Pipette Extraction (DPX) was developed as an alternative to traditional SPE, combining efficient and rapid extraction with significantly reduced solvent consumption.

Described within is a fast and automated DPX urine cleanup method using a GERSTEL MPS multipurpose sampler for the comprehensive screening of 49 pain management drugs with LC-MS/MS.

The DPX method allows the removal of salts and proteins present in urine, resulting in reduced matrix effects.

Since the extraction time (3 mins) is less than the analytical LC-MS/MS acquisition time the extraction of one sample can be performed during the chromatographic analysis of the previous sample, achieving high throughput while processing each sample "just-in-time" so all samples are treated identically.

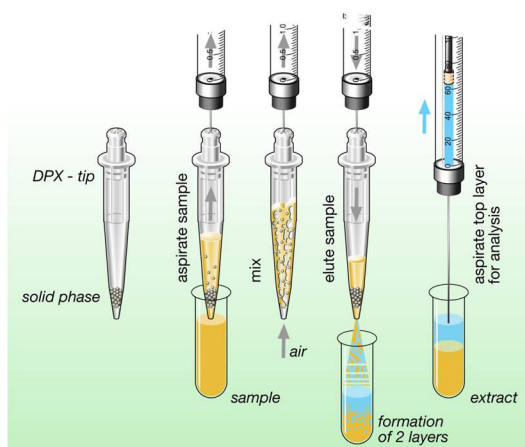


MPS robotic^{Pro} multi-purpose sampler with DPX option

Experimental

Automated DPX Prep Sequence

All automated DPX PrepSequences were performed using a MPS robotic^{Pro} multi-purpose sampler with the GERSTEL DPX option.



Automated DPX urine cleanup process

Aliquots of 260 μL of hydrolyzed urine samples were added into clean shell vials for automated cleanup and injection. The automated DPX extraction used for this method consisted of the steps shown below.

LC

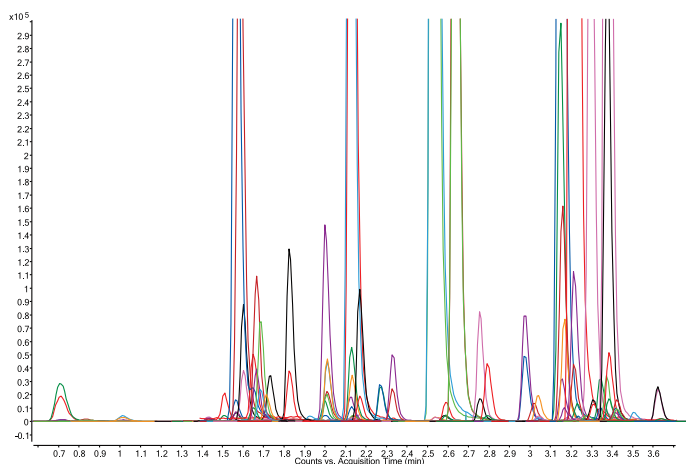
An Agilent 1290 LC system with a Zorbax Eclipse Plus C18 column (2.1 x 50 mm, 1.8 μm , 600 bar), with a gradient of eluent A 5 mM ammonium formate with 0.05% formic acid and eluent B 0.05% formic acid in methanol was used at a flow rate of 0.5 mL/min. The injection volume was set to 50 μL .

MS/MS

An Agilent 6460 triple quadrupole mass spectrometer with jet stream electrospray source (ESI) was used. A total of 124 MRM were monitored in a 4 minute analytical window followed by a column regeneration time of 2.5 minutes. A retention time window value of 30 seconds was used for each positive ion transition being monitored during the course of the dynamic MRM experiment.

Results and Discussion

The figure below shows representative dynamic MRM chromatograms for all 49 pain management drugs and internal standards, from a hydrolyzed urine sample spiked sample at the minimum reporting limit (MRL) concentrations after the automated DPX cleanup procedure.



Overlaid chromatograms for all 124 dynamic MRM transitions from an extracted urine sample

LLOQ concentrations are higher (5 fold factor increase) in comparison to those in a previous work performed with an automated concentration step using a solvent evaporation station. Note that the LLOQs of this modified cleanup method are still well below the reported MRLs.

Summary

- The automated DPX cleanup method using the GERSTEL MPS robotic^{Pro} sampler for pain management drug screenings in urine was modified to provide cycle times of approximately 7 min/sample allowing throughput of over 200 samples per day.
- 49 pain management drugs can be rapidly and reproducibly isolated from hydrolyzed urine samples using an automated DPX cleanup procedure coupled to LC/MS/MS analysis using the Agilent 6460 Triple Quadrupole Mass Spectrometer.
- The automated DPX method incorporates the GERSTEL Prep-Ahead concept enabling “just-in-time” sample handling for maximum productivity.

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