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Detection of 11-Nor-9-carboxy- Δ^9 -Tetrahydrocannabinol in Urine using Thomson eXtreme|FV®

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BACKGROUND / INTRODUCTION

- To determine and validate a more time effective extraction method for quantifying 11-Nor-9-carboxy- Δ^9 -Tetrahydrocannabinol (THC-COOH) in urine
- Tetrahydrocannabinol or THC is one of the most commonly used illicit drugs, but has some medical acceptance
- The main metabolite of THC in urine is THC-COOH in a glucuronide form
- Solid Phase Extraction (SPE) using the Zymark Rapid Trace extracts THC-COOH in urine from other compounds by its chemical and physical properties.
- The Thomson eXtreme|Filter Vial® (TV) filters out impurities from the sample and can run instantly on the LC/MS/MS.
- Thomson eXtreme|FV® 0.2 μ m PVDF was used to lower sample preparation time.

METHODS

Quality Control

- The controls and patient samples were run on a 5 point calibration curve for validation. The curves were spiked at 15, 30, 75, 750 and 3000 ng/mL. The standards were obtained from Cerilliant, Round Rock, Texas, at concentrations of 100 μ g/mL or 1.0 mg/mL.

Sample Preparation

- Urine samples were hydrolyzed with 90 μ l of β -glucuronidase and incubated at 55°C for 30 minutes. 200 μ l of sample and 200 μ l of 2% methanol in HPLC grade water were placed into Thomson eXtreme|FV® 0.2 μ m PVDF. Samples were pressed to remove impurities before LC/MS/MS analysis.

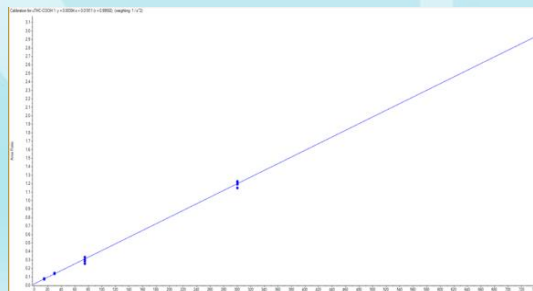
Instrumentation

- All hydrolyzed samples were run on a Shimadzu Prominence Sciex 5500 Qtrap LC/MS/MS.



Figure 1: Thomson eXtreme|FV® during filtering process.

OUTCOMES

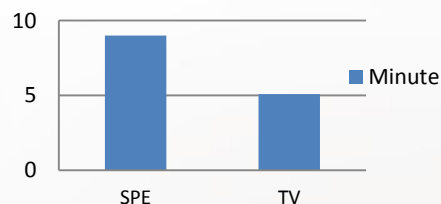


Graph 1: A calibration curve for the analyte, THC-COOH, over 5 days had an R² value of 0.99592.

Between Run		
Sample	TV % Accuracy	SPE % Accuracy
LOQ	103.3	103.3
LOW CONTROL	92.2	95.4
MIDDLE CONTROL	91.1	-
HIGH CONTROL	84.6	84
LOW STABILITY	89.6	-
HIGH STABILITY	88.5	-

Figure 2: Accuracy of quality control samples between all 5 days.

Time Evaluation Per Sample



Graph 2: Comparison of time between two extraction methods.

RESULTS

- Figure 1 depicts the process of the Thomson eXtreme|FV® removing macroparticles from the sample that are not necessary for sample injection.
- Based off of Graph 1, the method reached accurate and precise results with an R² value of 0.99592 over 5 days.
- Figure 2 shows the limit of quantification (LOQ) for both methods have the same accuracy.
- The low and high control are very close in precision between both methods.
- Graph 2 shows that the new Thomson vial method takes 5 minutes per samples and the old Rapid Trace method took 9 minutes per sample.

CONCLUSIONS

- The accuracy and precision did not change the LOQ when using the new Thomson eXtreme|FV® 0.2 μ m PVDF method compared to the old SPE Rapid Trace method. The high and low controls accuracy was only a minor difference, but still having correctness.
- By using the Thomson eXtreme|FV® method, the time decreases by 4 minutes. Increasing the extraction efficiency increases the turn around time for reporting of patient samples.

Acknowledgments

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