Automated SPE/LC/MS method for the analysis of THC and its metabolites in biological fluids

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Introduction

Tetrahydrocannabinol (THC) is the main psychoactive constituent of Cannabis sativa products such as hash or marijuana, and it is the most abused drug in the world. The major metabolites of THC are 11-hydroxy-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) which are present in the urine and blood. However, THC-COOH is present in the urine as THC-COOH-glucuronide. The current testing methods involves initial screening using immunoassay followed by confirmatory offline (liquid-liquid extraction or SPE) liquid chromatography-mass spectrometry procedures. Because of the widespread use of these drugs, there is a demand for fast, selective and sensitive methods. As a result, this new automated method was developed for the analysis of THC metabolites in urine.

Aim of the Study

The goal in this study is to develop a complete automated SPE/LC/MS method for the analysis of THC and its metabolites in biological fluids.

Experimental

-The automated extraction unit used is the Spark Holland SymbiosisTM system that consists of a refrigerated storage compartment, autosampler, two pumps and an extraction unit.

-The mass spectrometer is the Applied Biosystems 4000 QTrap with a Turbo spray source run in the positive mode. Both ESI and APCI ionization techniques were investigated for the best sensitivity. The ESI was found to be the most sensitive for our instrument under the condition used for this method.

- In developing the method, eight SPE cartridges were evaluated to determine the optimal SPE material for the extraction of all the analytes from urine and whole blood. The cartridges screened were CN, C2, C8, C8 EC (Encapped), C18, C18 HD, Hysphere resin SH (Strong hydrophobic), and Hysphere resin GP. The results demonstrated that C8 EC performed the best, in retaining all the analytes.

- Several C8 and C18 analytical columns were evaluated. In the end, Xterra® MS C18,(5µm, 3.0 x 50mm) was found to be the best suited for this study.

- Chromatographic separation was achieved within 5.1 minutes using a gradient consisting of a gradient from 70% of 0.1% formic acid in water to 98% of 0.1% formic acid in acetonitrile. The total run time was 7 minutes, including solid phase extraction and chromatographic separation.

- In performing the SPE procedure, 50 µL of diluted urine was used and 50 uL for the pre-treated whole blood extract.

- The dueterated analogues of THC, THC-OH and THC-COOH (d_3-THC, d_3-THC-OH and d_3-THC-COOH) of THC-OH and THC-COOH were used as the internal standards.

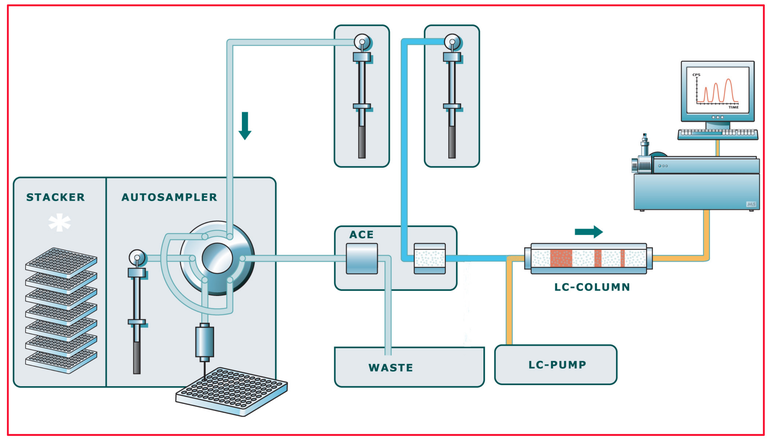

Figure 1: Schematic of Spark Holland Symbiosis™ interfaced to the Applied BioSystems 4000 QTrap

Results

This method is linearity from 6 - 900 ng/mL for urine samples and 6 - 500 ng/mL for whole blood. The within-day and between-day precision (< 15%) and accuracy (< 15%) for QC samples at 200 and 400 ng/mL in urine and whole blood.

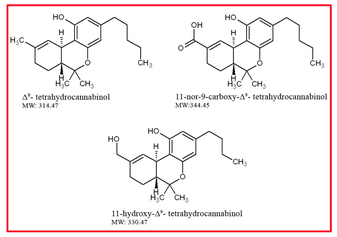

Figure 2: Structures of THC and its metabolites

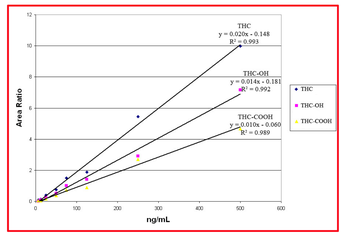

Figure 3: Standard curve of THC and its metabolites

Selectivity of the method against endogenous interferances was verified by analyzing blank urine and whole blood from several sources.

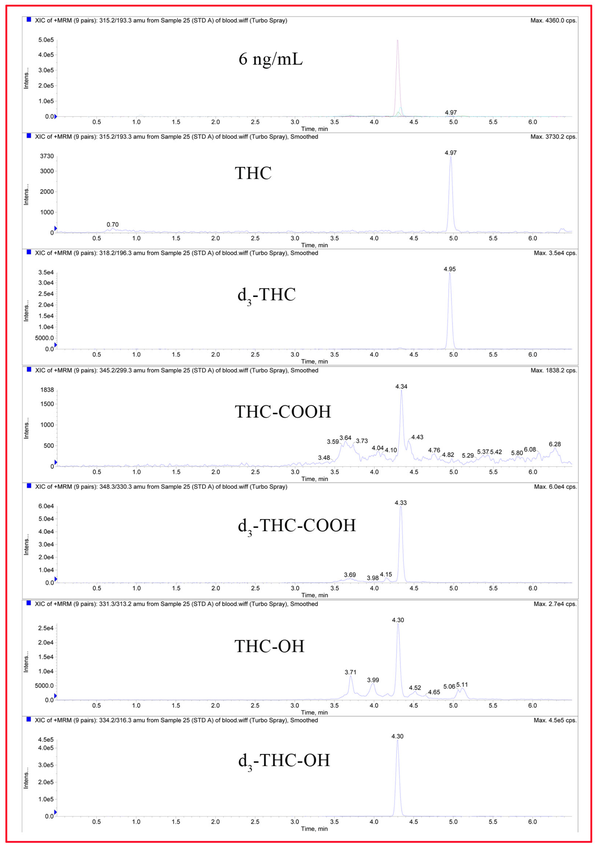

Figure 4: The MRM of THC and its metabolites extracted from a 6 ng/mL whole blood sample.

Conclusions

- Combining the Spark Holland Symbiosis™ to the Applied BioSystems 4000 QTrap so that these two instruments function as a single unit has provided an excellent automated SPE/LC/MS system.

- The results has demonstrated that this technique can be used for high throughput without compromising sensitivity and specificity.