**Introduction**

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) provides excellent testing specificity and accuracy with rapid analysis times. However, many forensic labs are interested in improving sample throughput to better utilize their testing instrumentation. While analysis times can be shortened through appropriate LC method choices, a user is often only interested in a portion of the total data collected by an LC/MS system. Typically, there is time during each chromatographic separation where no compounds of interest are being analyzed by the mass spectrometer, leaving the instrument under-utilized for a large period of time.

This work demonstrates the ability to increase mass spectrometer productivity for the analysis of Δ⁹-tetrahydrocannabinol (THC) through the automated use of a dual channel high performance liquid chromatography (HPLC) system. A newly developed software interface intelligently determines the timing of all HPLC components and coordinating the analytical utilization of the mass spectrometer.

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**Results and Discussion**

A previously developed LC/MS method for the analysis of Δ⁹-THC was used for testing the capabilities of the Agilent StreamSelect solution. The standard method uses an autosampler, a binary pumps, an HPLC columns, and a temperature-controlled column compartment. With a runtime of 4.5 minutes, Δ⁹-THC reaches the mass spectrometer between approximately 2 minutes to 3 minutes; more than 50% of the data collected by the mass spectrometer is of no interest. The standard method utilizes what is considered a single HPLC stream. The StreamSelect system mirrors certain components of this single stream system to provide a second stream, operating in parallel to the first. By loading the standard method and window of interest into the automation software, the software is able to determine the most efficient method of injecting and analyzing a list of samples. By staggering injections on parallel streams and switching between the two streams at the appropriate time, throughput of the integrated expanded system can double the throughput achieved with the standard method.

![Figure 1. Structure of Δ⁹-tetrahydrocannabinol](image)

**Sample Preparation**

Calibrators were prepared by making serial dilution of THC (Cerilliant) using synthetic negative saliva containing extraction buffer (Immunalysis). QC s were obtained from Immunalysis. Samples were collected using Quantisal collection devices (Immunalysis).

Combine 1 mL of sample with 1mL of 50 ng/ml internal standard solution and vortex.

1. Condition Bond Elut-Certify II, 200mg, 3mL SPE cartridges with 2 mL of methanol followed by 2mL of 95:5 0.1M Sodium Acetate: Methanol
2. Load sample onto SPE cartridge
3. Wash with 1 mL of 0.1M Sodium Acetate and 1mL of Hexane
4. Elute with 2.0 mL of 95:5 Hexane:Ethyl Acetate

Evaporate to dryness with nitrogen at 40°C, reconstitute in 250µL of 30:70 methanol:water, and transfer samples to a 96-well plate for analysis.

**Instrumentation**

The complete, integrated LC/MS/MS system is comprised of a triple quadrupole mass spectrometer coupled to a configurable HPLC system, all controlled by a single software application. For the purposes of this work, the expanded HPLC system consists of a high-capacity autosampler, two binary pumps, two HPLC columns, and two temperature-controlled column compartments. To operate the system, a standard data file collected by LC/MS/MS is loaded into the software. The data analysis method is extracted from the data file and a window of interest is specified using the data file’s chromatogram. Based on that information, the software automatically coordinates all timing related to running the HPLC system.

**HPLC Column**

Poroshell 120 EC-C18, 2.1 x 50mm, 2.7µm

**Injection Volume (µl)**

20

**Column Temp (°C)**

50

**Flow Rate (ml/min)**

0.3

**Mobile Phase A**

Water + 5mM Ammonium Formate

**Mobile Phase B**

Methanol

**Table 1. LC parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC Column</td>
<td>Poroshell 120 EC-C18, 2.1 x 50mm, 2.7µm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>20 µl</td>
</tr>
<tr>
<td>Column Temp</td>
<td>50°C</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.3 ml/min</td>
</tr>
<tr>
<td>Mobile Phase A</td>
<td>Water + 5mM Ammonium Formate</td>
</tr>
<tr>
<td>Mobile Phase B</td>
<td>Methanol</td>
</tr>
</tbody>
</table>

![Figure 2. Agilent StreamSelect LC/MS Solution with Online Sample Cleanup](image)

The two parallel LC systems displayed excellent agreement when comparing quantitative results (figure 3) with an R² > 0.998. Retention times were very reproducible, both within and between streams (figure 4). Each stream showed less than 1% CV within a stream and less than a 2% difference was observed between the two streams.

![Figure 3. Overlay a calibration curve from LC1 (black) and LC2 (red) from 0.5 ng/mL to 1000 ng/mL](image)

![Figure 4. Overlay of 50 injections from LC1 (black) and LC2 (red).](image)

Cutoff quality controls (Immunalysis) were used to verify method results. Levels were supplied at 4 (below cutoff), 8 (cutoff calibrator), and 16 (above cutoff) ng/mL according to the supplied datasheet. Accuracies for these samples were 96.32%, 93.33%, and 99.10% respectively.

**Conclusions**

Fully automated software controlling a completely integrated LC/MS system consisting of two parallel LC streams has been developed and implemented in the forensic analysis of THC. No special method development is required; the user supplies a standard method and defines a window of interest, allowing the software to determine all necessary timing and coordination of the analysis. Throughput for this method has been doubled through the use of the Agilent StreamSelect system.