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Down in the Weeds: Automated Fast Screening Workflow for Cannabinoids in Whole Blood Using High Resolution Mass Spectrometry

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Introduction

Distinguishing psychoactive tetrahydrocannabinol (THC) isomers and their metabolites from nonpsychoactive isobars and novel semi-synthetic cannabinoids like hexahydrocannabinol (HHC) is increasingly important to forensic toxicologists, as is the ability to remain up to date with novel compounds that are introduced into the population. This study describes an automated two-step chromatographic workflow that utilizes an initial fast screen method followed by reinjection with specific chromatography to resolve isobars if a suspect is detected in the fast screen on a Revident LC/Q-TOF (Figure 1). This allows for a larger number of samples to be screened more quickly while ensuring chromatographic resolution of isomers for confirmation. The data acquisition mode enables laboratories to interrogate data retrospectively to determine if novel compounds were present in any given sample.

Figure 1. Revident LC/Q-TOF with 1290 Infinity II LC.

Experimental

Whole blood was spiked with cannabinoid standards made from working stock solutions. Samples were crashed with cold ACN:MeOH, vortexed, and centrifuged to precipitate out proteins. The supernatant was loaded onto Captiva EMR-Lipid filtration cartridges, then rinsed with an ACN:H_2O mixture to enhance analyte recovery. The eluate was dried under nitrogen at 45°C and reconstituted in 50:50 MeOH:H_2O, as shown in Figure 2. Samples were injected onto a C18 column for an MS-only fast screening method.

Experimental

Aliquot
- Add 500 µL whole blood to Eppendorf tube

Protein Crash
- Add 1.2 mL of cold ACN:MeOH (85:15 v:v) to Eppendorf tube
- Vortex
- Centrifuge

Elution
- Transfer supernatant to 3 mL Captiva EMR-Lipid cartridge
- Apply positive pressure (1 drop/3-5 sec)

Final Prep
- Evaporate to dryness under nitrogen
- Reconstitute in 100 µL MeOH:water (50:50 v:v)
- Vortex
- Transfer samples to autosampler vials

Figure 2. Extraction procedure for Captiva EMR-Lipid[1]

Any analytes identified above a defined cutoff level were reflexed to a longer All Ions method to chromatographically separate isobars for the target analyte masses and observe fragments. Samples were analyzed in positive mode, and run times were 4 minutes for the MS-only screen and 15 minutes for the All Ions method (conditions detailed in Tables 1 and 2).

Table 1. LC Conditions with 1290 Infinity II LC.

LC Conditions				
Column	Agilent Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 µm (pn 699675-902) and Agilent Poroshell 120 PFP, 2.1 x 100 mm, 2.7 µm (pn 695775-408)			
Column temp	55 °C			
Injection	5 µL		Autosampler	5 °C
Mobile phase	A = H_2O + 0.1% formic acid B = MeOH			
Flow rate	0.800 mL/min		0.500 mL/min	
	Short		Long	
Gradient program	Time	%B	Time	%B
	0.0	70	0.0	55
	0.30	70	5.85	59
	1.30	75	6.5	59
	2.00	98	6.6	64
	2.50	98	10.5	64
	2.60	70	11.5	100
	3.00	70	13.5	100

Results and Discussion

Table 2. Source parameters with Revident LC/Q-TOF.

Parameters	Value
Ion mode	AJS, positive
Gas temperature	325 ºC
Drying gas flow	11 L/min
Nebulizer gas	35 psi
Sheath gas temperature	350 ºC
Sheath gas flow	11 L/min
Capillary voltage	4500 V
Nozzle voltage	500 V

The Revident LC/Q-TOF combined with intelligent reflex automated injection logic allows for a larger number of samples to be analyzed faster in toxicology laboratories while only utilizing a second reflexive reinjection analysis if a suspect is detected above a user-defined cutoff concentration. If a suspect is detected in the first stage, then it will be reinjected on a more specific chromatographic method to confidently distinguish between psychoactive isomers and other isobaric interferences, as shown in Figure 3.

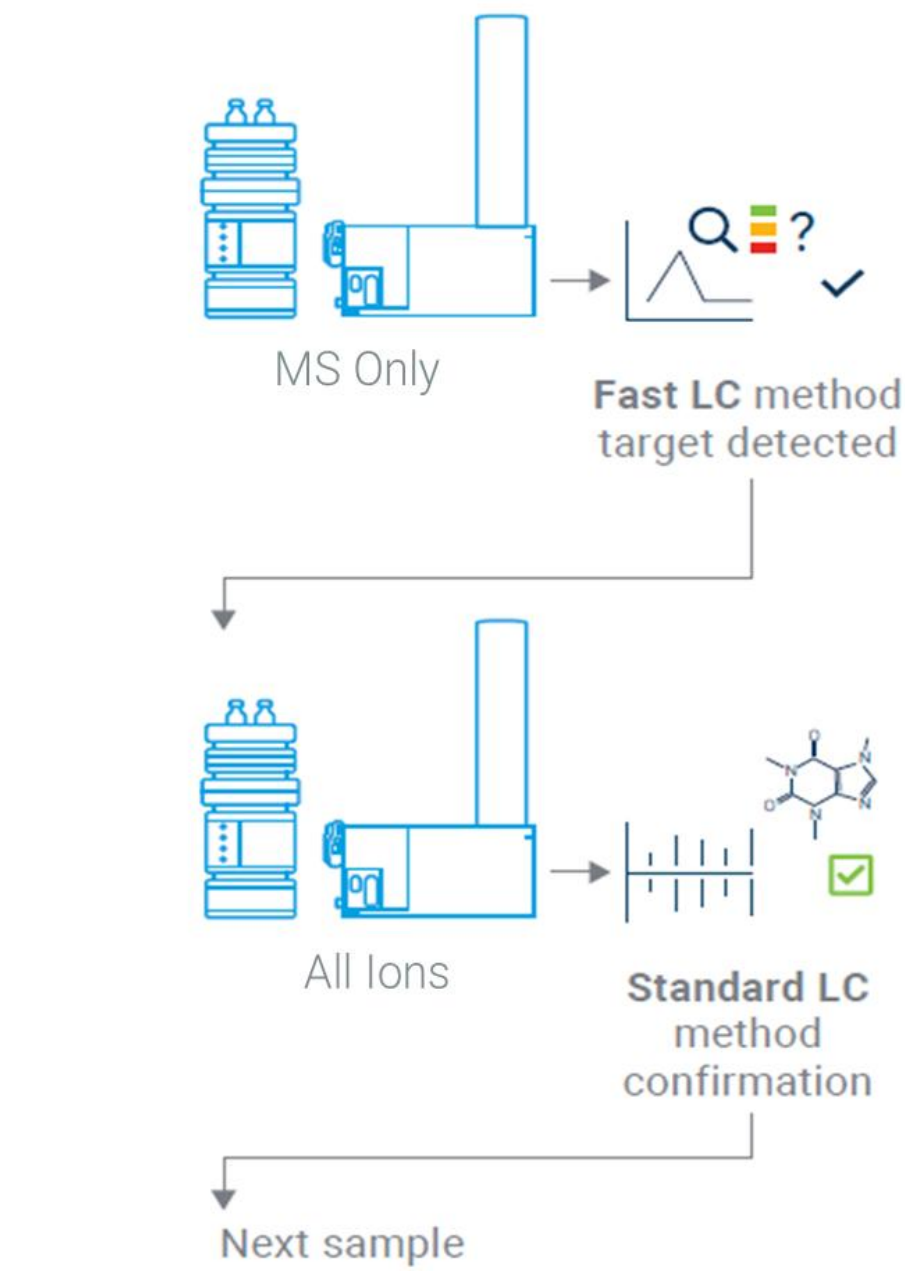

Figure 3. Workflow summary utilized on the Revident LC/Q-TOF for fast screening with reflexive reinjection of putative positives.

Using this workflow, samples were screened with the fast 4-minute method, and any putative positives were automatically *reflexed* to the secondary method with specific THC chromatography. These reflexed samples can be inserted immediately after the fast-screening method, or they can be appended to the end of the worklist (Figure 4) and run following a first-pass screen of every sample in the batch. For increased efficiency, the append workflow option was employed in this experiment.

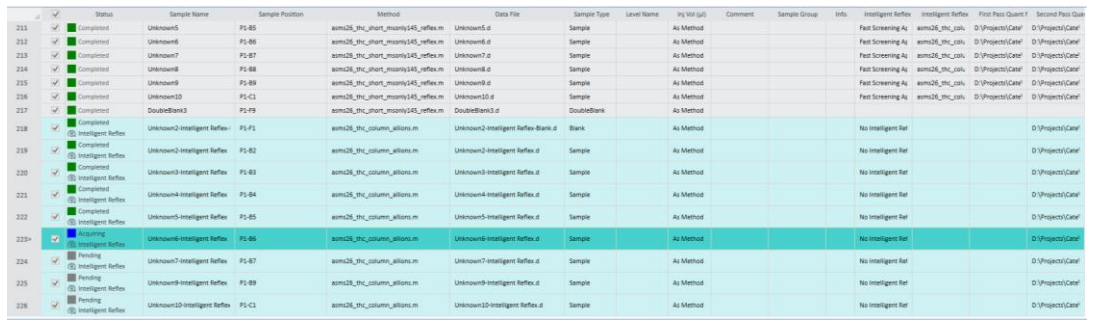

Figure 4. Fast Screening worklist showing automatic addition of positive samples to the end of the worklist (append workflow). These samples have a blank inserted before the first reflexed injection to allow the method to switch over and equilibrate the column. QCs can also be included after a set number of injections on the secondary method.

The first-pass fast screening method required simple method development to ensure the method was relatively quick but also did not push endogenous interferences into the peaks of interest and cause false positives that would reflex every sample to the long method. Representative chromatography for the shorter, fast method is shown in Figure 5, with a matrix example shown in Figure 6. Development of this fast method focused almost exclusively on maintaining separation of matrix interference peaks to minimize the number of false positives.

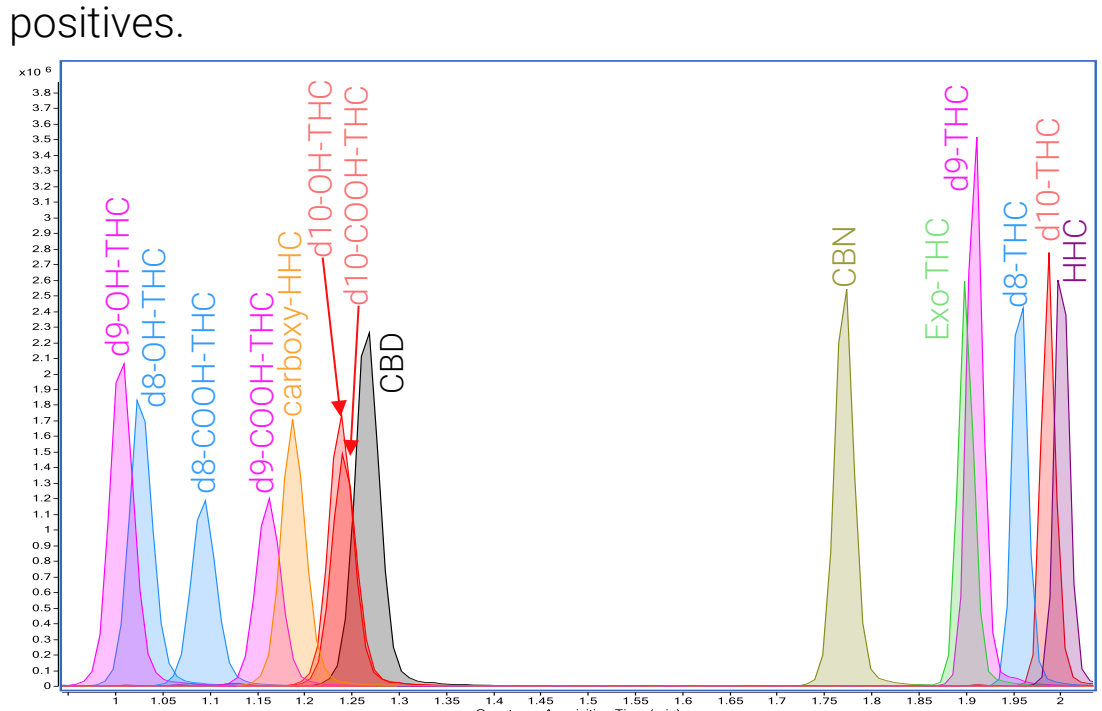

Figure 5. Representative chromatography of each individual standard overlaid, acquired using the shorter first-pass method.

Results and Discussion

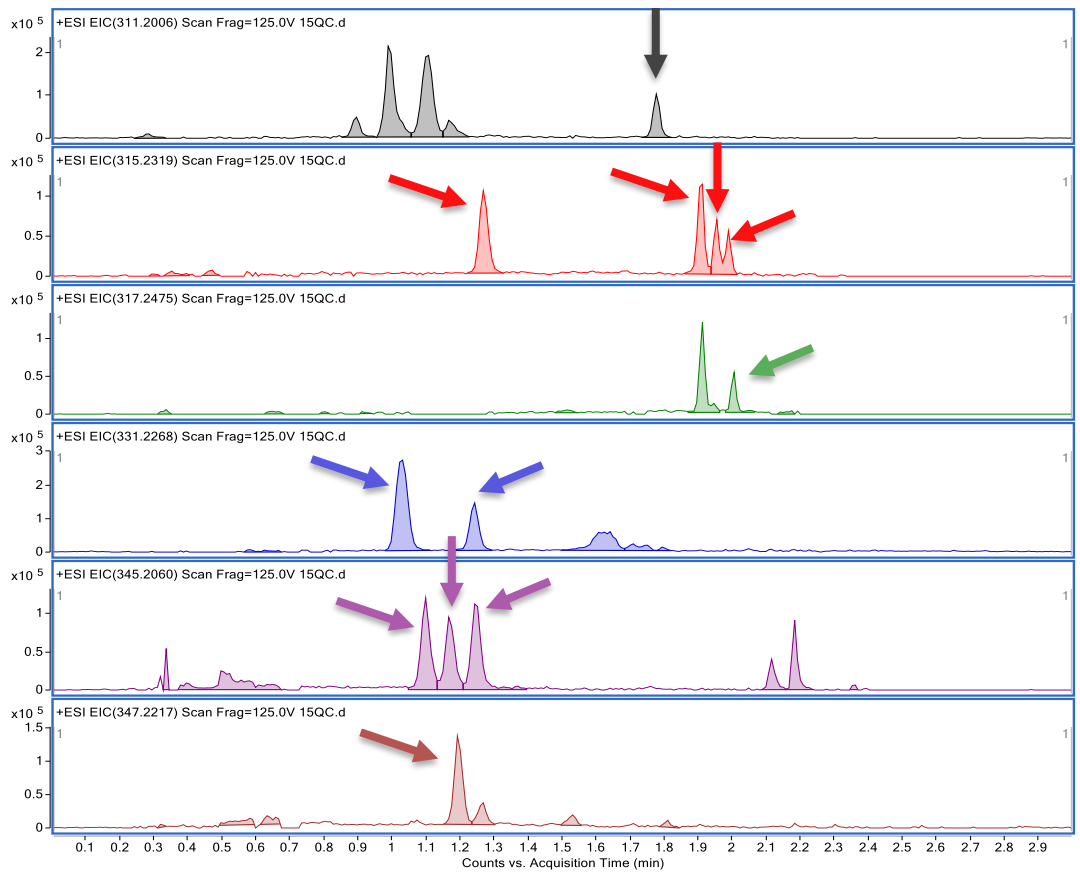

Figure 6. Representative chromatography of all compounds spiked at 15 ng/mL into whole blood. Peaks of interest are marked with arrows; all other peaks are matrix interferences that were monitored for adequate separation from targets using the fast chromatographic method.

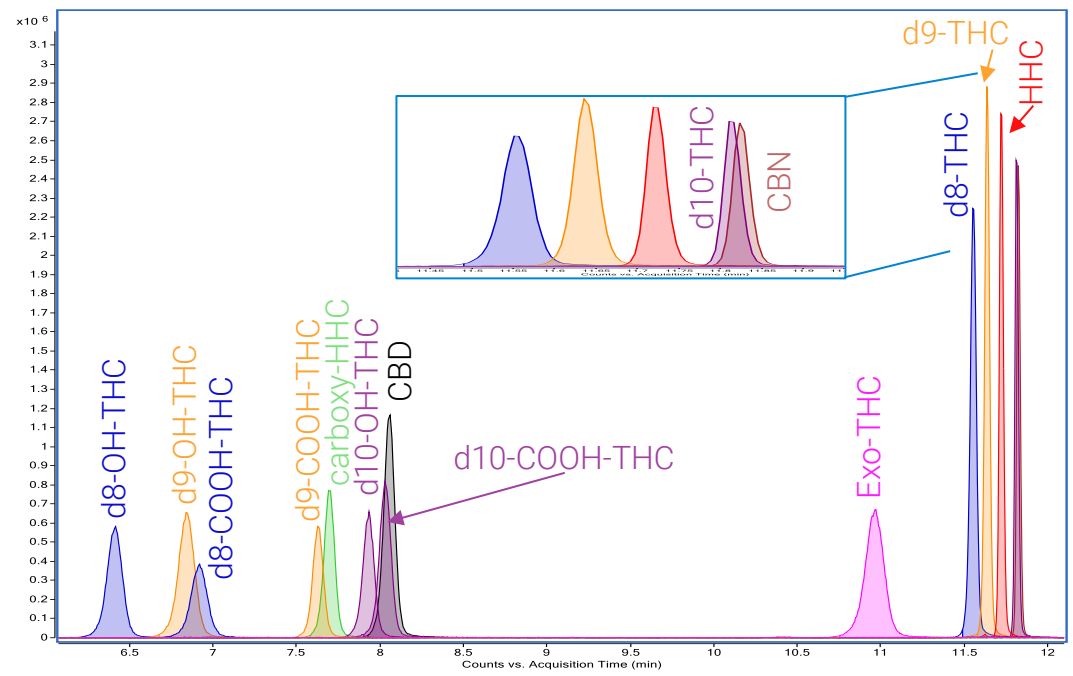

Figure 7. Representative chromatography of all compounds acquired with the longer second-pass method, showing baseline resolution of the THC isobars in the inset.

The second-pass longer chromatographic method had been optimized and established previously[2], and the chromatographic separation is shown in Figure 7. Each THC isomer, along with their hydroxy and carboxy metabolites, is well resolved by the Poroshell 120 PFP column for confident identification, and any overlapping peaks are easily mass resolved with the Revident LC/Q-TOF system.

Excellent sensitivity, linearity, and reproducibility were obtained for all analytes on both chromatographic methods. A representative calibration curve is shown in Figure 8. The concentration ranges tested during these experiments varied from 1 ng/mL to 500 ng/mL in blood for every analyte. Most compounds demonstrated an LOQ of 5 ng/mL, with HHC at 10 ng/mL.

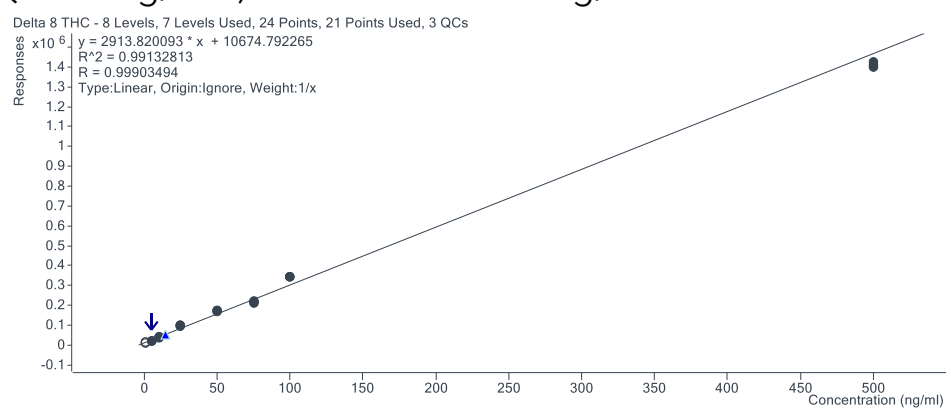

Figure 8. Example calibration curve for delta 8 THC over a range of 5-500 ng/mL (1 ng/mL excluded).

For the intelligent reflex fast screening workflow, a cutoff concentration of 10 ng/mL was set based on SWGTOX recommendations and experimental determination. Unknowns that had any compound detected above that level were reflexively reinjected using the second-pass longer method, which was acquired using All Ions (Figure 9).

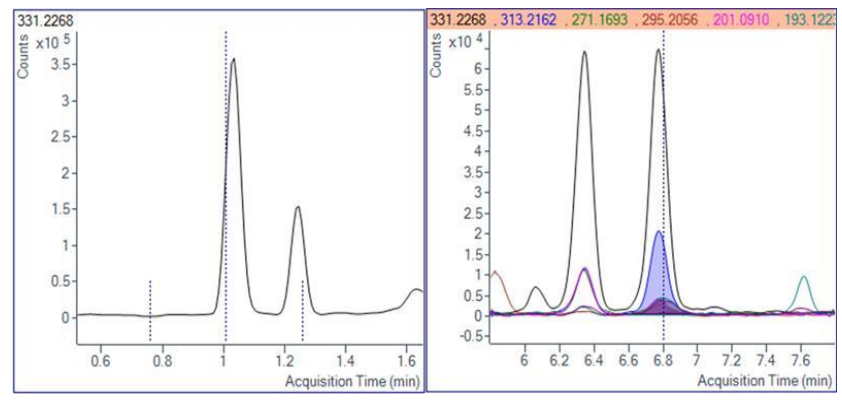

Figure 9. Peak to peak comparison of MS only data for d8/d9-OH-THC (first peak on left) to All Ions data for the same compounds on right (d8 is first peak, then d9).

Conclusions

- Clear and concise software workflow for quick screening analysis
- Baseline resolution of THC isomers and interferences in longer method to allow for confident identification
- All Ions acquisition method allows for retrospective data interrogation with fragment analysis

References

[1] Stevens, J. and Zhao, L. Efficient Quantitative Analysis of THC and its Metabolites in Whole Blood Using Agilent Captiva EMR-Lipid and LC-MS/MS. Agilent Application Note 5991-8635EN, **2020**.

[2] Stone, PJW et al. Quantitative Separation of THC Isomers and Metabolites from Whole Blood. Agilent Application Note 5994-8664EN, **2025**.

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