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Introduction

Cannabis testing labs face increasing demand of regulatory requirements. Robust testing methods are required for variety of products, including potency tests for CBD oil and isolate and CBD drinks, pesticide residue testing in plant materials and final products. A series of sample preparation and (U)HPLC methods were developed to target these analyses.

Instruments and Materials

Instruments: LC-MS/MS method used a AB Sciex Exion LC system coupled to an API 4500 mass spectrometer with an ESI source operated in positive ion mode. HPLC-UV method used an Agilent LC system with DAD detector. UHPLC-UV method used a Waters Acquity H-Class UHPLC system.

Materials: Cannabidiolic acid, cannabigerol, cannabidiol, cannabinol, tetrahydrocannabinol, cannabichromene, and tetrahydrocannabinolic acid standards were purchased from Cayman Chemical. CBD oil was provided by Kazmira, LLC. Mass spec grade methanol, acetonitrile, water and formic acid (FA) were purchased from Pharmco-Aaper. Orochem specialty QuEChERS tubes for hemp (Orochem Technologies Inc.) were used for all pesticide extractions. Five pesticides (Bifenazate, Spiromesifen, Pyrethrin I, Pyrethrin II and Bifenthrin), phosphoric and formic acid and NaCl were purchased from Sigma-Aldrich. Gazelle C18 and Biphenyl UHPLC columns, Monitor C18 and Orosil C18 HPLC column were obtained from Orochem Technologies Inc

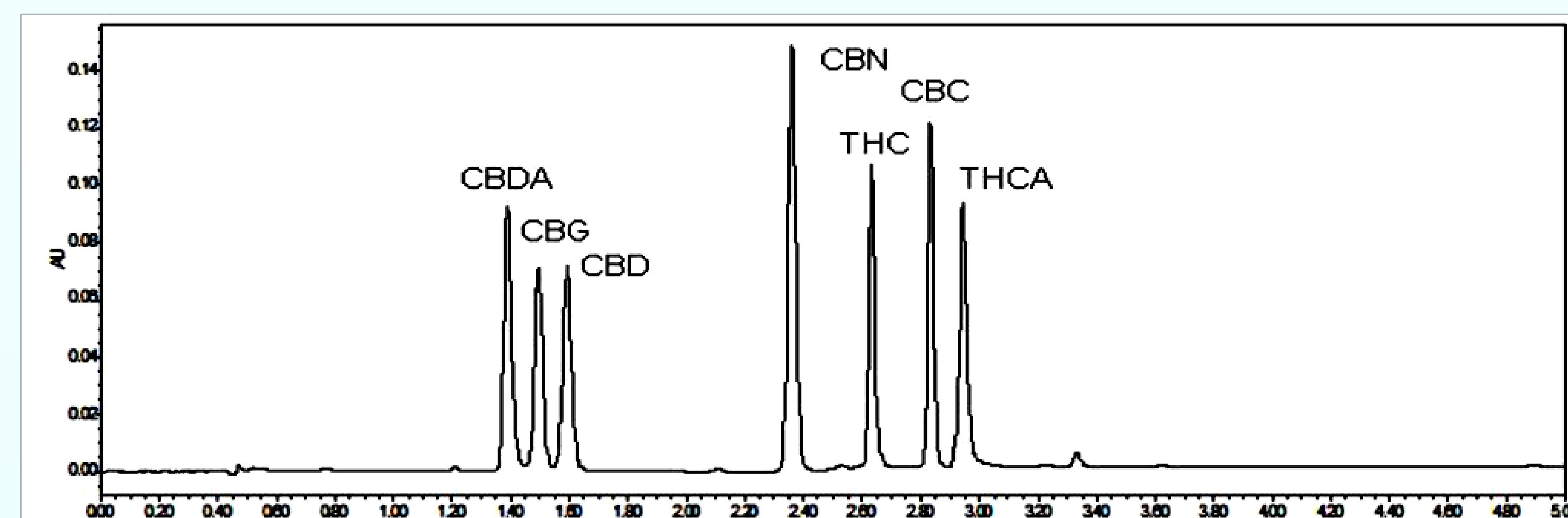
Potency Test

Sample preparation : Dilute CBD oil with organic solvent

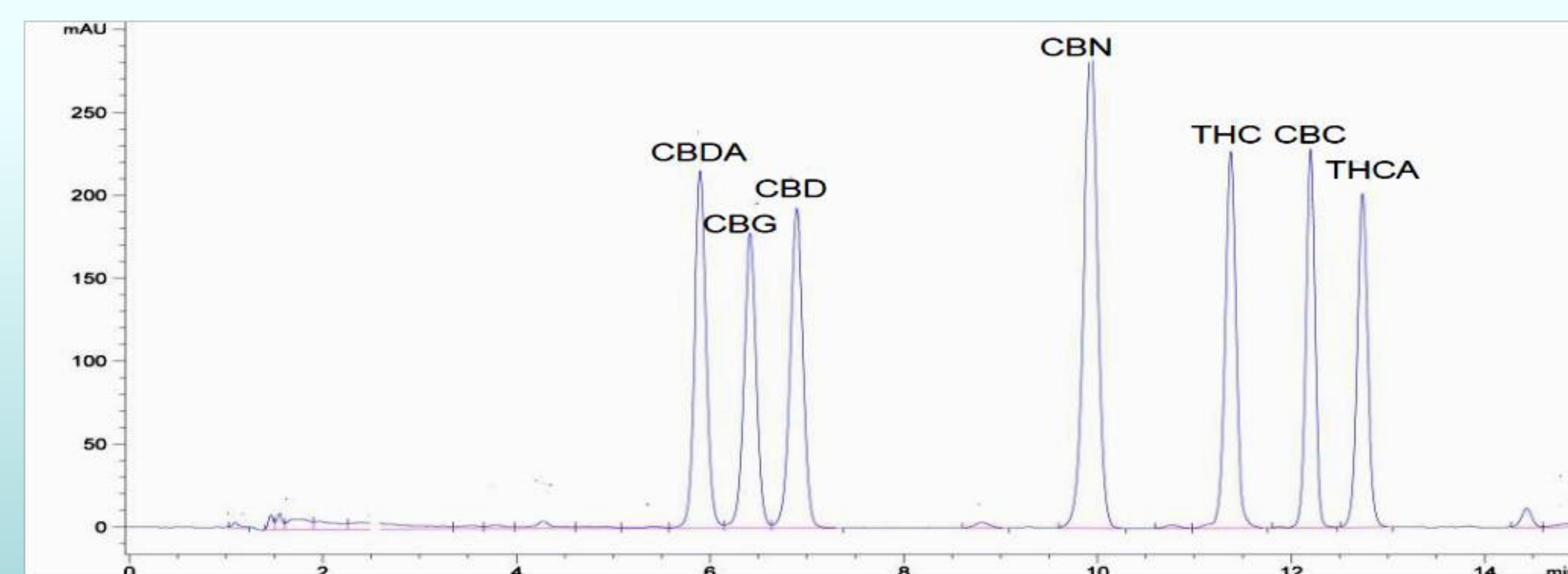
HPLC-UV Conditions:

Column: Orochem Gazelle C18 UHPLC column 2.1x100 mm, 1.7 μm; or Monitor C18 HPLC column, 4.6x150 mm, 3 μm

Mobile phases: acetonitrile-phosphoric acid gradient



HPLC-UV Chromatogram of cannabinoids using Gazelle C18 UHPLC column, total run time 4 min



HPLC-UV Chromatogram of cannabinoids using Monitor C18 HPLC column, total run time 15 min

Pesticide Residue Test

Pesticides recovery in QuEChERS method from Cannabis oil

Samples comprised 10.0 mg/mL dilution of Cannabis oil in acetonitrile with 0.1% formic acid. 2 mL of solution was then added to two varieties of QuEChERS tube filled with a specialty dSPE mixture (Orochem Technologies Inc.) and vortexed for 30 sec. After centrifugation at 2500 rpm for 5 min, supernatant was filtered through 200 nm filter and analyzed by LC-MS to determine pesticides recovery.

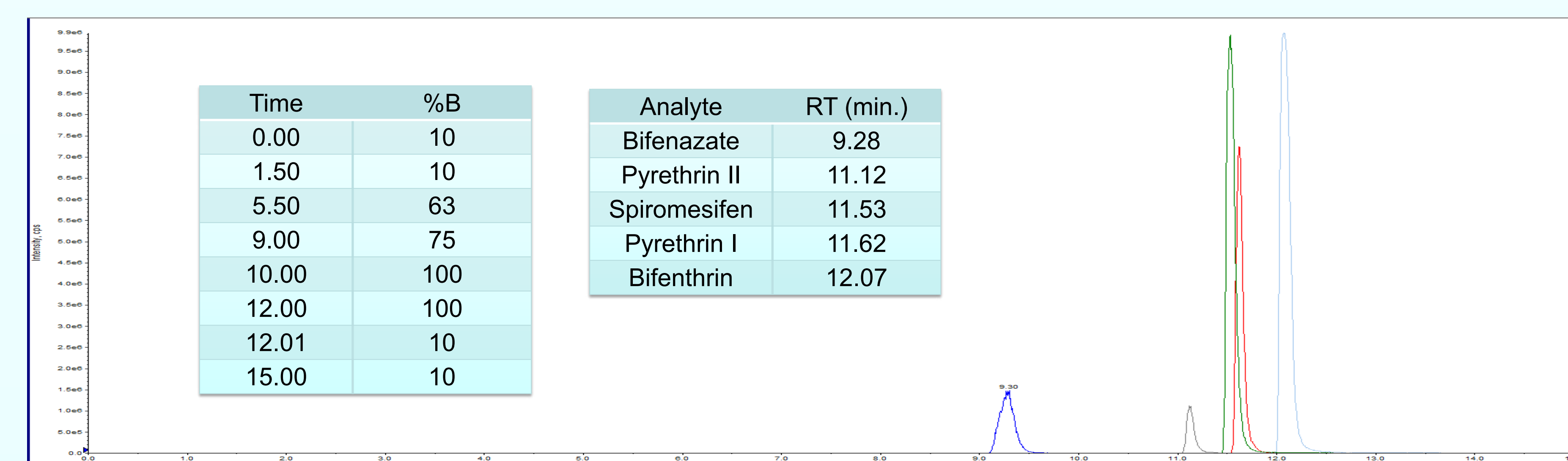
| | Bifenazate | Spiromesifen | Pyrethrin I | Pyrethrin II | Bifenthrin |
|------------------------|------------|--------------|-------------|--------------|------------|
| Que A Recovery; Neat | 93.4% | 93.5% | 96.2% | 105.7% | 115.9% |
| Que B Recovery; Neat | 85.6% | 79.1% | 88.9% | 93.8% | 89.0% |
| Que A Recovery; Matrix | 85.4% | 102.9% | 91.0% | 87.5% | 90.0% |
| Que B Recovery; Matrix | 71.6% | 89.2% | 83.9% | 74.8% | 86.6% |

LC-MS parameters

Column: Gazelle C18, 1.7 μm, 50 x 2.1 mm - with 10x2.1 mm Gazelle C18 Guard Column

Mobile Phase A: 5 mM Ammonium Acetate in Water + 0.1% Formic Acid

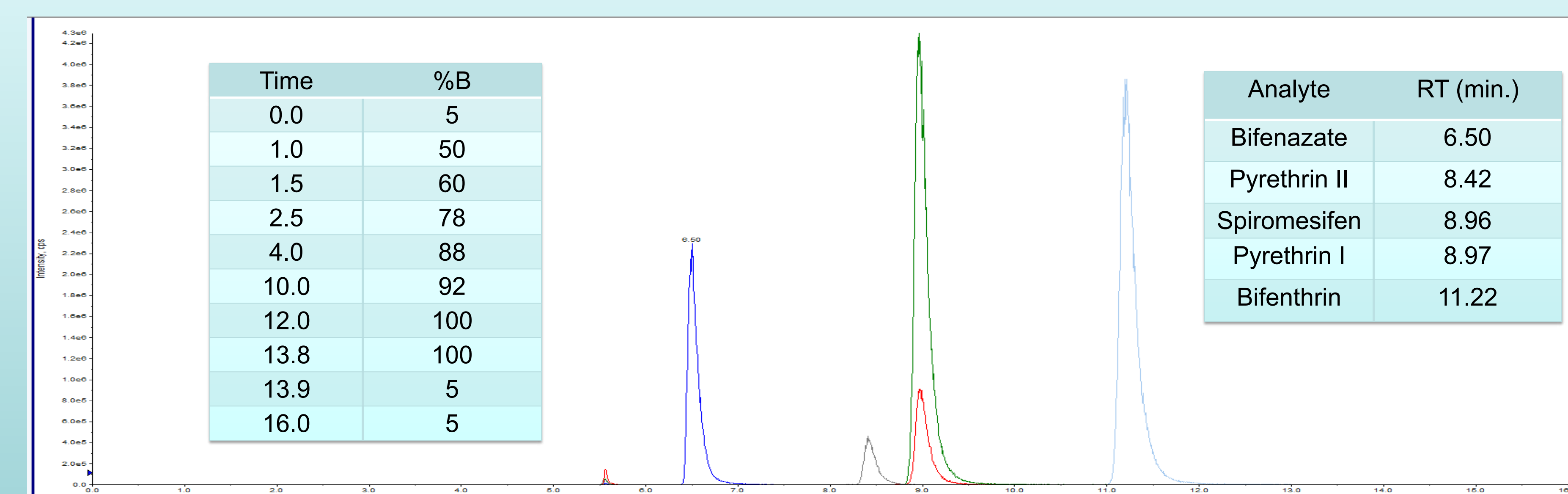
Mobile Phase B: MeOH



Column: Gazelle Biphenyl, 1.7 μm, 50 x 3.0 mm- with 10x2.1 Gazelle Biphenyl Guard Column

Mobile Phase A: 5 mM Ammonium Acetate in Water + 0.1% Formic Acid

Mobile Phase B: 5 mM Ammonium Acetate in 98:2 MeOH:Water



Conclusions

For potency test of CBD related products, an ultra-fast UHPLC-UV method was developed to detect seven cannabinoids in 4 minutes; For an HPLC method, a LC-UV method was validated to quantify cannabinoids with 15 minutes analysis run time. QuEChERS sample preparation allowed for excellent recovery of five tested pesticides from cannabis oil solutions with minimal or no effect from present matrix.