INTRODUCTION

Synthetic cannabinoids, often referred to or marketed as “Spice” or “K2”, represent a growing challenge for law enforcement agencies and forensic laboratories. These drugs mimic the psychoactive effects of natural cannabinoids, and their popularity and use have risen substantially in the last several years (1, 2). While recent legislation has banned some of these compounds, minor modifications to existing structures have resulted in a proliferation of substances designed to circumvent existing laws. This current work details a strategy for the successful extraction and analysis of representatives of some different classes of synthetic cannabinoids from whole blood samples for forensic toxicology. A total of 22 synthetic cannabinoids and metabolites were extracted from whole blood samples using a rapid and universal sample preparation strategy that provides effective sample cleanup and is generic enough to use on a variety of chromatographic columns with different chemical properties. Analytical separation was achieved using Waters® newly developed Ostri Sample Preparation Plates on an ACQUITY UPLC® solid-core column with optimally packed 1.6 μm particles, resulting in exceptional performance and separation efficiency. Extraction recoveries ranged from 73 to 105% with an average of 98.4% matrix effects were less than 10% for all compounds with only 3 greater than 15%. Calibration curves were linear from 2.5 to 500 ng/mL, with accurate and precise results from quality control samples. The analysis of several different classes of these drugs should render this method applicable to newly developed related compounds with little, if any, additional modification necessary.

METHODS

Chemicals and Materials

All target compounds and metabolites were obtained from Cerplant (Round Rock, TX) and Cayman Chemical (Ann Arbor, MI). Sample Preparation

50 μL whole blood was added to 150 μL 0.1% ZnSO4/NaH2PO4/Dixon in Octane sample preparation plate wells. Samples were vortexed for 5 sec. 600 μL ACN was then added, and the sample was vortexed for 30 sec. Samples were then eluted under vacuum into 2 mL 96-well collection plates. 10 μL was injected onto the UPLC/MS system.

Equipment

Sample Prep: Ostri Sample Preparation Plates
UPLC System: ACQUITY UPLC
MS: ACQUITY QD
Column: CORTECS™ UPLC C18, 2.1 x 100 mm 1.6 μm Water + 0.1% formic acid (FA)
Gradient Table

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Recovery and Matrix Effects

Analyte recovery was calculated according to the following equation:

\[ \text{Recovery} = \frac{\text{Area}_{\text{sample}}}{{\text{Area}}_{\text{standard}}} \times 100\% \]

Where A = the peak area of an extracted sample and B = the peak area of an extracted matrix sample in which the compounds were added post-extraction.

Matrix effects were calculated according to the following equation:

\[ \text{Matrix Effect} = \frac{\text{Peak area in the presence of matrix}}{\text{Peak area in the absence of matrix}} \times 100\% \]

RESULTS

A representative chromatogram of all compounds from a 25 ng/mL calibration standard is shown in Figure 1. Peak assignments are listed in Table 1. Peak shape was resolved to the compound of significant meaning, and all peak widths were under 3 seconds. Peaks 9 and 10, an isobaric pair of metabolites with identical parent ion and product ions, were nearly baseline resolved, with a calculated resolution of 1.39, enabling unambiguous identification that would not be possible if the two compounds co-eluted. When the same mixture of compounds was analyzed on an ACQUITY UPLC® BEH C18 column, adequate separation was not achieved for these two compounds (Figure 1B). Co-elution of peaks 5 and 6, 7 and 8, 11 and 13, and 14 and 16, as well as the lower end of the spectrum, was achieved on the ACQUITY UPLC® column. A total of 22 synthetic cannabinoids and metabolites were successfully extracted from whole blood and analyzed on an ACQUITY UPLC® C18 column with optimally packed 1.6 μm particles, resulting in exceptional performance and separation efficiency. Extraction recoveries ranged from 73 to 105% with an average of 98.4% across all QC levels and the bottom row shows accuracy averages for all compounds at each QC level. Linearity, accuracy and precision

Calibration curves were extracted at concentrations ranging from 2.5 to 500 ng/mL for all compounds. Quality control samples (4) were prepared at 7.5, 75, and 300 ng/mL. Table 3 summarizes R2 values from the calibration curves and QC summary data for all compounds. All compounds displayed excellent linearity over the entire calibration range with R2 values of >0.99 for 21 of the 22 compounds. Signal-to-noise ratios were excellent with all compounds demonstrating linear responses down to 2 ng/mL. Quality control (QC) results were accurate and precise across all medium and high concentrations. Accuracies for low level QC samples (7.5 ng/mL) ranged from 78.0–108.4% with an average of 89.6%. The results for the medium and high QC samples were excellent, with accuracies within 10% of expected values. Analytical precision was excellent with most RSDs less than 15% and none greater than 13%. When QC accuracy was assessed over all levels (low, medium, and high), the mean ranged from 85.5% to 101.7%

CONCLUSIONS

Improved resolution on CORTECS UPLC solid-core particle column vs. fully porous particle columns

Successful extraction of 22 synthetic cannabinoids and metabolites from whole blood using Ostri sample preparation plates

Excellent recovery and minimal matrix effects

Linear, accurate, and precise performance for all compounds

References
