

# Analysis of Synthetic Cannabinoids from Whole Blood for Forensic Toxicology Using Ostro Sample Preparation Plates and CORTECS UPLC Solid-Core Columns

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## APPLICATION BENEFITS

- Single, universal method for analysis of synthetic cannabinoids and metabolites
- Linear response for all analytes and metabolites
- Greater than 90% recovery for most analytes
- Matrix effects less than 15% for most compounds
- Baseline resolution of structural isomers with identical mass spectra

## WATERS SOLUTIONS

Ostro™ Sample Preparation Plates

CORTECS™ UPLC® Column

ACQUITY UPLC® System

Xevo® TQD Mass Spectrometer

## KEY WORDS

Synthetic cannabinoids, designer drugs, spice compounds, UPLC, forensic toxicology, whole blood, sample preparation, CORTECS

## INTRODUCTION

Synthetic cannabinoids, often referred to or marketed as “Spice” compounds, constitute a growing challenge for law enforcement agencies and forensic laboratories. These designer drugs mimic the psychoactive effects of natural cannabinoids. Often labeled as “not for human consumption” and marketed as a legal alternative to natural cannabis, their popularity and use have risen substantially in the last several years.<sup>1,2</sup> 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 application note details a strategy for the successful extraction and analysis of representatives of several different classes of synthetic cannabinoids from whole blood samples for forensic toxicology. Twenty-two 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 compounds with different chemical properties. Extraction recoveries ranged from 73% to 105% with an average of 92%, and matrix effects were less than 15% for most compounds. Calibration curves were linear ranging from 2 to 500 ng/mL, with accurate and precise results from quality control samples. Analytical separation was achieved using the Waters® solid-core particle CORTECS Column packed with sub-2- $\mu$ m particles, resulting in optimal performance and separation efficiency. The analysis of several different classes of these drugs should render this method applicable to newly developed related compounds with minimal, if any, modification necessary.

## EXPERIMENTAL

## UPLC conditions

|                   |   |
|-------------------|---|
| System:           | ACQUITY UPLC  |
| Column:           | CORTECS C <sub>18</sub> 1.6 μm,<br>2.1 x 100 mm<br>(p/n 186007095)  |
| Column temp.:     | 30 °C   |
| Injection volume: | 10 μL   |
| Flow rate:        | 0.6 mL/min  |
| Mobile phase A:   | 0.1% formic acid<br>in MilliQ water   |
| Mobile phase B:   | 0.1% formic acid in ACN   |
| Gradient:         | Initial conditions at 30% B.<br>The %B was increased to 50%<br>over two minutes, and held<br>at 50% B for one minute,<br>increased to 90% B over four<br>minutes, then returned to 30%<br>over 0.2 minute. The system<br>was allowed to re-equilibrate<br>for 0.8 minute. The entire cycle<br>time was 8.0 minutes. |
| Vials/plates:     | Ostro 96-well sample<br>collection plates, 2.0 mL<br>(p/n 186005518)  |

## MS conditions

|                        |   |
|------------------------|---|
| Mass spectrometer:     | ACQUITY® TQD                                    |
| Ionization mode:       | ESI Positive                                    |
| Acquisition mode:      | MRM (Table 1 for transitions)                   |
| Capillary voltage:     | 1 kV  |
| Collision energy (eV): | Optimized for individual<br>compounds (Table 1) |
| Cone voltage (V):      | Optimized for individual<br>compounds (Table 1) |

## Data management

MassLynx® Software

## Sample description

AM2233, JWH-015, RCS-4, JWH-203, RCS-8, JWH-210, JWH-073, and JWH-018 were purchased from Cerilliant (Round Rock, TX). All other compounds and metabolites were purchased from Cayman Chemical (Ann Arbor, MI).

Individual stocks (1 mg/mL) were prepared in methanol, DMSO, or 50:50 DMSO/methanol. A combined stock solution of all compounds (10 μg/mL) was prepared in methanol. Working solutions were prepared daily by preparing standards in matrix (whole blood), and performing serial dilutions to achieve the desired concentrations. Calibrator concentrations ranged from 2 to 500 ng/mL for all analytes. Quality control samples were prepared at 7.5 ng/mL, 75 ng/mL, and 300 ng/mL in whole blood.

The 22 compounds analyzed, listed in Table 1, constitute a panel which includes various classes of forensically relevant synthetic cannabinoids. These include adamantoylindoles such as AM 1248 and AKB48, naphthoylindoles such as JWH 022, phenylacetyl indoles such as RCS-4 and RCS-8, and tetramethylcyclopropylindoles (UR-144, XLR11). Major metabolites of JWH-073 and JWH-018 were also included, as some of these compounds are structural isomers with identical mass spectral fragments that require adequate chromatographic separation for accurate quantification.

## Sample preparation

Samples were extracted using Ostro 96-well Sample Preparation Plates. 50 μL of whole blood was added to wells containing 150 μL of an aqueous solution of 0.1 M ZnSO<sub>4</sub>/NH<sub>4</sub>CH<sub>3</sub>COOH, and vortexed for five seconds to lyse the blood cells. 600 μL of ACN was then added to each well, and all samples were vortexed for three minutes to fully precipitate the samples. All samples were eluted into a 2-mL 96-well collection plate. 10 μL was injected onto the LC/MS/MS system.

Analyte recovery was calculated according to the following equation:

$$\%Recovery = \left( \frac{Area A}{Area B} \right) \times 100\%$$

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:

$$Matrix Effects = \left( \left( \frac{Peak area in the presence of matrix}{Peak area in the absence of matrix} \right) - 1 \right) \times 100\%$$

The peak area in the presence of matrix refers to the peak area of an extracted matrix sample in which the compounds were added post-extraction. The peak area in the absence of matrix refers to analytes in a neat solvent solution.

## RESULTS AND DISCUSSION

## Chromatography

A representative chromatogram of all compounds from a 20-ng/mL calibration standard is shown in Figure 1. Peak assignments are listed in Table 1. Using a CORTECS UPLC C<sub>18</sub> 1.6 μm, 2.1 x 100 mm Column, all analytes were analyzed within 7.5 minutes with a total cycle time of 8.0 minutes. Peak shape was excellent for all compounds, with no significant tailing or asymmetries, and all peak widths were less than three seconds. Peaks 9 and 10, an isobaric pair of metabolites with identical precursor and product ions, were nearly baseline resolved, with a calculated resolution of 1.04, enabling unambiguous identification that would not be possible if the two compounds co-eluted. When the same mix of compounds was analyzed on a BEH UPLC C<sub>18</sub> column, adequate separation was not achieved for these two compounds (data not shown). Co-elution of compound pairs 5 and 6, and 7 and 8 were also seen on the hybrid column. A more thorough comparison of the CORTECS UPLC Column and two analogous fully-porous UPLC columns (BEH and HSS T3) revealed that peak widths on the fully-porous columns ranged from the equivalent of those seen on the CORTECS UPLC Column to more than 2X as wide. On average, peak widths on the BEH and HSS T3 columns were 15% and 30% greater, respectively, than those seen using the CORTECS UPLC Column.

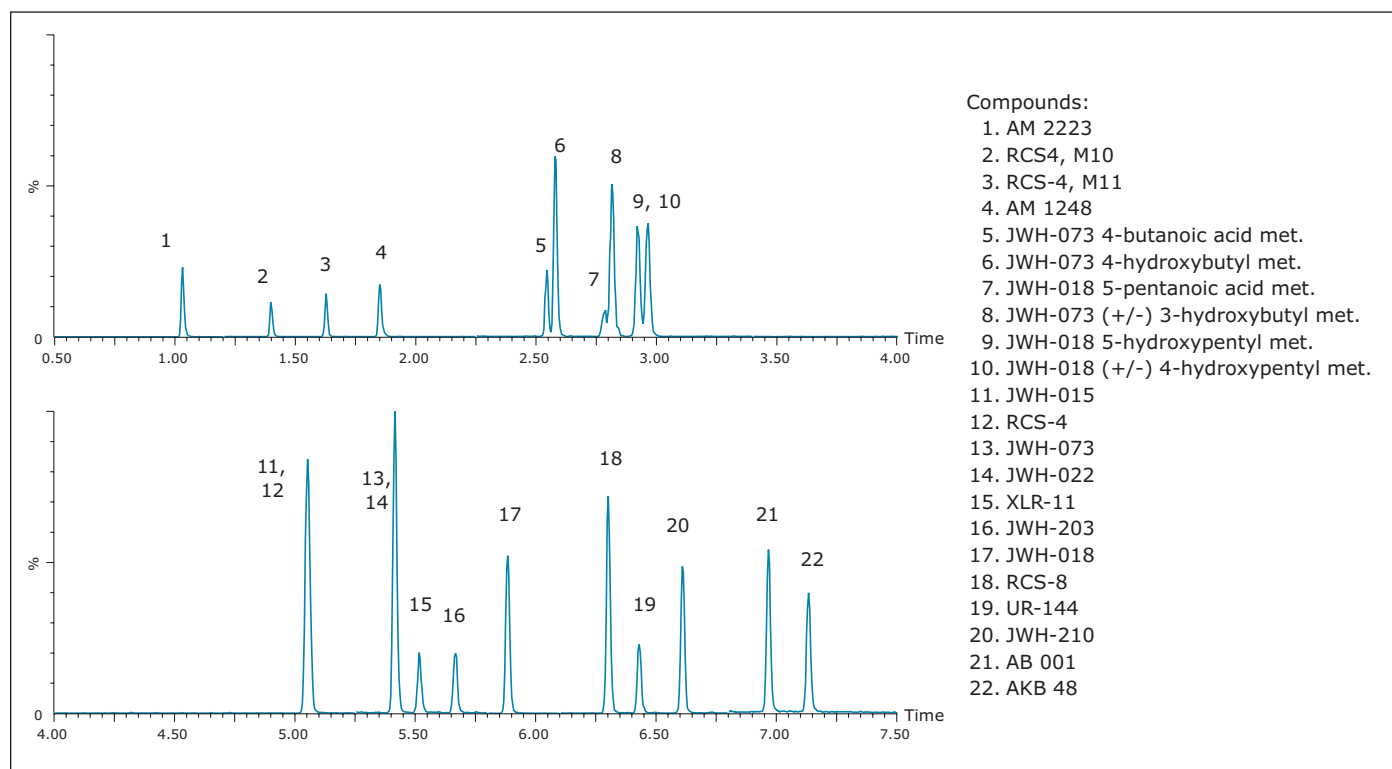


Figure 1. UPLC/MS/MS chromatogram for 22 synthetic cannabinoids and metabolites. Peak assignments are listed in Table 1.

| No. | Compound                           | RT   | Mol. formula                                     | Cone voltage | MRM transitions | Coll. energy |
|-----|------------------------------------|------|--|--------------|-----------------|--------------|
| 1   | AM2233                             | 1.04 | C <sub>22</sub> H <sub>23</sub> N <sub>2</sub> O | 40           | 459.2→98.05     | 34           |
|     |                                    |      |  | 40           | 459.2→112.1     | 22           |
| 2   | RCS-4, M10                         | 1.40 | C <sub>20</sub> H <sub>21</sub> NO <sub>3</sub>  | 40           | 324.2→121.0     | 22           |
|     |                                    |      |  | 40           | 324.2→93.0      | 46           |
| 3   | RCS-4, M11                         | 1.62 | C <sub>20</sub> H <sub>19</sub> NO <sub>3</sub>  | 36           | 322.2→121.0     | 22           |
|     |                                    |      |  | 36           | 322.2→93.0      | 46           |
| 4   | AM 1248                            | 1.87 | C <sub>26</sub> H <sub>34</sub> N <sub>2</sub> O | 56           | 391.4→135.1     | 28           |
|     |                                    |      |  | 56           | 391.4→112.1     | 30           |
| 5   | JWH-073 4-butanoic acid met.       | 2.54 | C <sub>23</sub> H <sub>19</sub> NO <sub>3</sub>  | 50           | 358.2→155.1     | 26           |
|     |                                    |      |  | 50           | 358.2→127.1     | 48           |
| 6   | JWH-073 4-hydroxybutyl met.        | 2.57 | C <sub>23</sub> H <sub>21</sub> NO <sub>2</sub>  | 50           | 344.2→155.1     | 22           |
|     |                                    |      |  | 50           | 344.2→127.1     | 40           |
| 7   | JWH-018 5-pentanoic acid met.      | 2.77 | C <sub>24</sub> H <sub>21</sub> NO <sub>3</sub>  | 46           | 372.2→155.1     | 24           |
|     |                                    |      |  | 46           | 372.2→127.1     | 50           |
| 8   | JWH-073 (+/-) 3-hydroxybutyl met.  | 2.81 | C <sub>23</sub> H <sub>21</sub> NO <sub>2</sub>  | 44           | 344.2→155.1     | 26           |
|     |                                    |      |  | 44           | 344.2→127.1     | 46           |
| 9   | JWH-018 5-hydroxypentyl met.       | 2.91 | C <sub>24</sub> H <sub>23</sub> NO <sub>2</sub>  | 40           | 358.2→155.1     | 24           |
|     |                                    |      |  | 44           | 358.2→127.1     | 48           |
| 10  | JWH-018 (+/-) 4-hydroxypentyl met. | 2.96 | C <sub>24</sub> H <sub>23</sub> NO <sub>2</sub>  | 40           | 358.2→155.1     | 24           |
|     |                                    |      |  | 44           | 358.2→127.1     | 48           |
| 11  | JWH-015                            | 5.04 | C <sub>23</sub> H <sub>21</sub> NO               | 42           | 328.2→155.1     | 24           |
|     |                                    |      |  | 42           | 328.2→127.1     | 42           |
| 12  | RCS-4                              | 5.05 | C <sub>21</sub> H <sub>23</sub> NO <sub>2</sub>  | 44           | 322.2→135.1     | 26           |
|     |                                    |      |  | 44           | 322.2→92.0      | 64           |
| 14  | JWH-022                            | 5.41 | C <sub>24</sub> H <sub>21</sub> NO               | 50           | 340.2→155.1     | 26           |
|     |                                    |      |  | 50           | 340.2→127.1     | 54           |
| 13  | JWH-073                            | 5.41 | C <sub>23</sub> H <sub>21</sub> NO               | 48           | 328.2→155.1     | 26           |
|     |                                    |      |  | 48           | 328.2→127.1     | 48           |
| 15  | XLR-11                             | 5.52 | C <sub>21</sub> H <sub>28</sub> FNO              | 48           | 330.3→125.1     | 26           |
|     |                                    |      |  | 48           | 330.3→97.1      | 32           |
| 16  | JWH-203                            | 5.66 | C <sub>21</sub> H <sub>22</sub> ClNO             | 46           | 340.2→125.0     | 26           |
|     |                                    |      |  | 46           | 340.2→188.1     | 20           |
| 17  | JWH-018                            | 5.88 | C <sub>24</sub> H <sub>23</sub> NO               | 44           | 342.2→155.1     | 26           |
|     |                                    |      |  | 44           | 342.2→127.1     | 42           |
| 18  | RCS-8                              | 6.30 | C <sub>25</sub> H <sub>29</sub> NO <sub>2</sub>  | 42           | 376.3→121.1     | 26           |
|     |                                    |      |  | 42           | 376.3→91.0      | 50           |
| 19  | UR-144                             | 6.43 | C <sub>21</sub> H <sub>29</sub> NO               | 46           | 312.3→125.1     | 24           |
|     |                                    |      |  | 46           | 312.3→214.2     | 25           |
| 20  | JWH-210                            | 6.61 | C <sub>26</sub> H <sub>27</sub> NO               | 48           | 370.2→183.1     | 26           |
|     |                                    |      |  | 48           | 370.2→155.1     | 38           |
| 21  | AB 001                             | 6.97 | C <sub>24</sub> H <sub>31</sub> NO               | 52           | 350.3→135.1     | 30           |
|     |                                    |      |  | 52           | 350.3→93.0      | 46           |
| 22  | AKB 48                             | 7.13 | C <sub>23</sub> H <sub>31</sub> N <sub>3</sub> O | 38           | 366.3→135.1     | 22           |
|     |                                    |      |  | 38           | 366.3→93.1      | 50           |

Table 1. Molecular formulae, retention times, and MS/MS conditions for the synthetic cannabinoid compounds and metabolites in this application.

### Recovery and matrix effects

Recoveries and matrix effects were calculated according to the equations described in the experimental section with the results shown in Figure 2. All compounds demonstrated excellent recoveries, with all but one compound at 80% or greater and an average recovery of 92% for all compounds. Matrix effects were minimal. Three compounds had matrix effects of 16%, and all remaining compounds had matrix effects less than 15%. This extraction protocol results in nearly complete recovery and minimizes matrix effects for all the compounds tested. The high recoveries and minimal matrix effects for this variety of synthetic cannabinoids indicate that this method should give similar results for other related compounds.

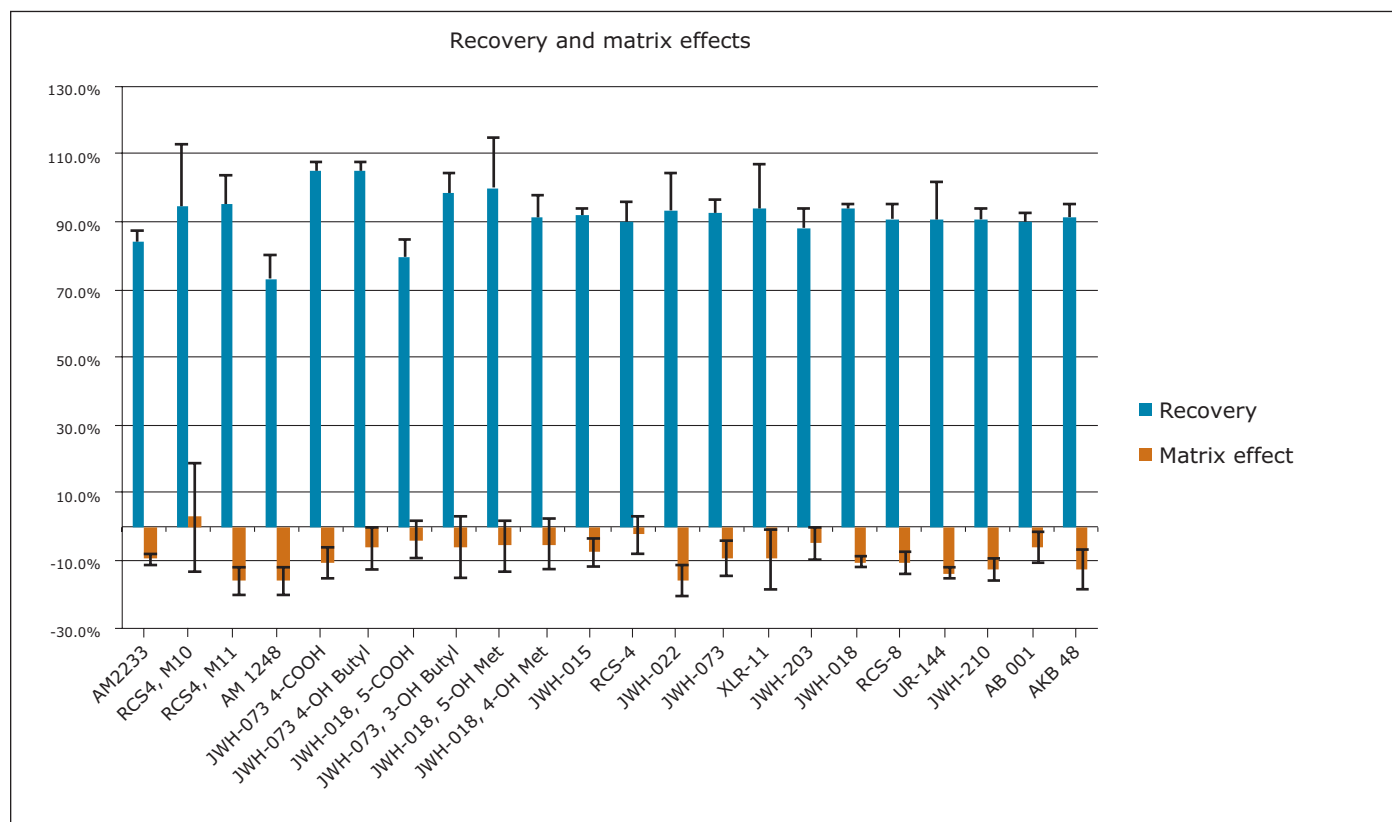


Figure 2. Recovery and matrix effects of synthetic cannabinoid compounds from whole blood following extraction with Ostro sample preparation plates. Bars and error bars represent mean and standard deviations (N=4), respectively.

### Linearity and analytical sensitivity

In order to assess linearity and analytical sensitivity, calibration curves were extracted at concentrations ranging from 2 to 500 ng/mL for all components. Quality control samples (N=4) at 7.5, 75.0, and 300.0 ng/mL were also extracted and analyzed. Table 2 summarizes  $R^2$  values from the calibration curves and QC summary data for all compounds. All compounds showed excellent linearity over the entire calibration range with  $R^2$  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 at low, medium, and high concentrations. Accuracies for low-level QC samples (7.5 ng/mL) ranged from 79.0% to 104.4% with an average of 89.4%. The results for the medium and high QC levels were excellent for all analytes, with all accuracies within 10% of expected values. Analytical precision was excellent with most %RSDs less than 10%, and none greater than 13%. When recovery was assessed over all levels (low, medium, and high), the means ranged from 89.5% to 101.7%.

|                     | $R^2$              | QC concentrations (ng/mL) |      |              |      |             |      | Mean % acc. |
|---------------------|--------------------|---------------------------|------|--------------|------|-------------|------|-------------|
|                     |                    | 7.5                       |      | 75           |      | 300         |      |             |
|                     |                    | %Acc.                     | %RSD | %Acc.        | %RSD | %Acc.       | %RSD |             |
| AM2233              | 0.997              | 100.5                     | 2.0  | 103.6        | 3.3  | 100.5       | 2.0  | 101.5       |
| RCS4, M10           | 0.986              | 97.5                      | 3.9  | 106.1        | 5.7  | 101.7       | 8.4  | 101.7       |
| RCS4, M11           | 0.991              | 91.3                      | 16.3 | 108.8        | 5.1  | 96.8        | 12.0 | 98.9        |
| AM 1248             | 0.993              | 83.1                      | 10.0 | 106.1        | 5.7  | 105.4       | 6.4  | 98.2        |
| JWH-073 4-COOH      | 0.991              | 96.1                      | 9.8  | 99.3         | 7.4  | 106.2       | 9.1  | 100.5       |
| JWH-073 4-OH Butyl  | 0.996              | 88.7                      | 21.3 | 98.1         | 3.5  | 102.2       | 3.9  | 96.3        |
| JWH-018, 5-COOH     | 0.992              | 90.7                      | 15.2 | 97.8         | 3.8  | 103.7       | 10.6 | 97.4        |
| JWH-073, 3-OH Butyl | 0.993              | 79.0                      | 8.6  | 92.9         | 8.3  | 96.6        | 2.9  | 89.5        |
| JWH-018, 5-OH Met   | 0.995              | 82.8                      | 10.3 | 100.0        | 10.4 | 100.1       | 3.4  | 94.3        |
| JWH-018, 4-OH Met   | 0.992              | 82.3                      | 17.9 | 103.1        | 6.3  | 96.0        | 1.9  | 93.8        |
| JWH-015             | 0.993              | 87.1                      | 4.3  | 101.8        | 3.9  | 101.3       | 2.1  | 96.8        |
| RCS-4               | 0.993              | 92.5                      | 8.1  | 99.6         | 5.0  | 97.3        | 3.6  | 96.4        |
| JWH-022             | 0.993              | 85.3                      | 4.9  | 100.3        | 4.8  | 97.8        | 4.2  | 94.5        |
| JWH-073             | 0.994              | 89.6                      | 6.5  | 99.4         | 6.6  | 97.6        | 4.9  | 95.5        |
| XLR-11              | 0.993              | 101.4                     | 10.4 | 99.6         | 2.8  | 99.7        | 5.0  | 100.2       |
| JWH-203             | 0.990              | 82.1                      | 12.2 | 96.1         | 12.2 | 94.6        | 9.3  | 91.0        |
| JWH-018             | 0.994              | 88.4                      | 2.9  | 97.2         | 3.9  | 98.8        | 3.6  | 94.8        |
| RCS-8               | 0.992              | 94.3                      | 2.6  | 101.9        | 4.6  | 99.4        | 4.7  | 98.5        |
| UR-144              | 0.994              | 85.1                      | 5.4  | 97.0         | 6.7  | 99.2        | 3.7  | 93.8        |
| JWH-210             | 0.994              | 92.7                      | 6.4  | 96.3         | 4.5  | 95.6        | 5.3  | 94.8        |
| AB 001              | 0.992              | 84.4                      | 8.1  | 101.0        | 4.7  | 100.2       | 10.6 | 95.2        |
| AKB 48              | 0.992              | 92.8                      | 9.9  | 98.5         | 4.8  | 97.7        | 8.4  | 96.4        |
|                     | <b>Mean % acc.</b> | <b>89.4</b>               |      | <b>100.2</b> |      | <b>99.5</b> |      |             |

Table 2.  $R^2$  values and quality control results for all compounds.

## CONCLUSIONS

A panel of 22 synthetic cannabinoid drugs and metabolites were extracted from whole blood and analyzed by UPLC/MS/MS. The use of Ostro Sample Preparation Plates resulted in excellent recoveries for all analytes with minimal matrix effects. All compounds demonstrated excellent linearity from 2 to 500 ng/mL. Separation using the CORTECS UPLC Column enabled the analysis of all compounds in a short analysis time with baseline resolution of a critical isobaric pair. Separation efficiency and peak widths were superior to fully-porous columns of matching dimensions. This method enables the rapid and reliable extraction and analysis of this critical class of compounds for forensic toxicology. The excellent performance seen on this variety of compounds and the universal nature of the extraction method should allow its use on other synthetic cannabinoids and metabolites, an important feature for the rapid development of new, related compounds.

## References

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