K46 Determination of Synthetic Cannabinoids in Whole Blood From Recreational Users

Robert Kronstrand, PhD, and Markus Roman, BSc, Nat'l Board of Forensic Med, Dept of Toxicology, Artillerigatan 12, Linkoping, SWEDEN*

After attending this presentation, attendees will understand how synthetic cannabinoids may be analyzed and will have an insight in the common findings in recreational users as well as the challenges these compounds present to the forensic toxicologist. This paper presents a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method for synthetic cannabinoids in whole blood and toxicological findings from recreational users.

This presentation will impact the forensic science community by adding findings for substances that have paucity in data.

Analysis was performed on a Waters ACQUITY ultra performance LC connected to an API 4000 triple-quadrupole instrument equipped with an electrospray interface. The following compounds were included in the method: JWH-007, JWH-015, JWH-018, JWH-019, JWH-020, JWH-073, JWH-073-methyl, JWH-081, JWH-098, JWH-122, JWH-147, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM-2201, AM-1241, RCS-4, RCS-8, and WIN 55,212-2. JWH-018-d_{11} was used as internal standard. The analytes were chosen because they either were scheduled by the Swedish government or because they were reported as possible drugs of abuse.

To 1g whole blood was added 0.5mL TRIS-buffer (0.5M, pH=8.5) and 3mL of tert-butylmethylether:chlorobutane (50:50). The organic phase was transferred to new tubes, evaporated to dryness, and reconstituted in 100µL of 10mM ammonium formate and acetonitrile (50:50). 5µL was injected into the LC/MS/MS. Chromatography was performed using an ACQUITY high-strength silica T3 column (1.8µm, 50 x 2.1mm, Waters) and operated at 0.6mL/min with a total run time of 6 minutes. Mobile phase A consisted of 0.05% formic acid in 10mM ammonium formate and phase B was 0.05% formic acid in acetonitrile. The chromatographic system was run in a linear gradient from 35% to 70% phase B.

Method validation included selectivity and matrix effect studies, investigations of calibration models, accuracy, within and between day precision, and dilution integrity. The method was applied to 1,609 authentic cases where the police had requested synthetic cannabinoids.

The method validation experiments showed overall good results for the 23 analytes. Matrix effects were seen especially for the late eluting compounds and an interference for the transitions of RCS-8 appeared in most chromatograms close to the retention time of RCS-8. All analytes were best fitted to quadratic calibration curves between 0.05ng/g to 5.0ng/g.

The overall positive rate was approximately 30% with AM-2201 as the most common finding. The positive findings changed over time, sometimes so that substances that were scheduled decreased to be replaced by new unscheduled analogs. Eleven of the analytes were found in one or more cases.

Analyte	*N*	**Median (ng/g)**	**Mean (ng/g)**	**Range (ng/g)**
JWH-018	80	0.10	0.62	0.05 – 9.7
JWH-019	7	0.65	0.44	0.07 – 0.78
JWH-081	8	0.20	0.24	0.06 – 0.65
JWH-122	51	0.42	3.6	0.05 – 88
JWH-203	3	13	27	0.06 – 68
JWH-210	31	0.75	1.6	0.07 – 11
JWH-250	3	0.48	0.45	0.14 – 0.73
AM-694	10	0.12	0.43	0.05 – 1.5
AM-2201	290	0.34	1.0	0.05 – 29
RCS-4	5	0.23	0.80	0.06 – 3.4

Concentrations were typically in the subnanogram range, but some cases had very high concentrations (see table). It has been reported that synthetic cannabinoids, in comparison to cannabis, seem to be more dangerous and potent, causing several unwanted symptoms in the users. In the material, case histories were not received in more than a few cases where the subjects had suffered from severe side effects and been brought to hospital. These subjects presented with unconsciousness, vomiting, incontinence, and hallucinations and relatively high concentrations of JWH-018, JWH-203, or JWH-210, sometimes in combination with another synthetic cannabinoid.

 ** Presenting Author*

This study concludes synthetic cannabinoids appear in very low concentrations and the changing panorama of substances requires a flexible approach to the analytical methodology.

Cannabinoids, LC/MS/MS, Recreational Users

 ** Presenting Author*