Detection of URB Series Synthetic Cannabinoids

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Abstract

The URB series of synthetic cannabinoids has emerged as a "legal" alternative to marijuana. Their structural and pharmacological identities are such that they cannot be controlled under current federal legislation and only a few states have explicitly banned the use of URBs[1]. They have become a component of "spice" products along with other controlled substances, and therefore a drug laboratory must have the ability to detect them. Because they are relatively new and not yet commonly used, research into URB detection has not adequately fulfilled the needs of a forensic drug lab. Initial efforts to detect the URB series used validated GC/MS methods of the Kentucky State Police Eastern Laboratory Branch, however those methods proved insufficient. Custom methods were developed with the hope of optimizing chromatogram quality and establishing matches to external references. It was found that a higher column temperature[3] was needed for complete elution of some standards, but also exacerbated degradation. An adequate method was developed and used to test mock evidence samples.

Introduction

- URB synthetic cannabinoids are becoming increasingly popular as part of "legal high" products sold at smoke shops.
- These compounds inhibit the enzymes that metabolize the endocannabinoids anandamide and 2-arachidonoylglycerol
- Structurally and pharmacologically different from Δ^9-THC, making them difficult to schedule under current legislation.
- Currently banned in eight states.

Materials & Methods

URB standards obtained from Cayman Chemical and were dissolved individually, as well as a mixture, in methanol in tri-spring inserts in GC vials. An Agilent 6890N gas chromatograph equipped with a Zebron ZB-DRUG-1 column coupled to an Agilent 5973N mass selection detector were used for analysis. Validated lab methods were used, however none worked appropriately, so a new method was developed as shown in Table 1. The final method derived from these parameters reduced the final hold time from 20 to 10 minutes.

Table 1. Initial developed method parameters

Injector		Oven Program		Column	
Sample washes	1	Initial temperature	100°C	Initial pressure	5.00 psi
Sample pumps	2	Initial time	0.50 min	Initial time	0.50 min
Syringe size	10.0 µL	Ramp	40°C/min	Ramp 1	75.00 psi/min
Injection volume	2.0 µL	Final temperature	300° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	20.00 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	MS Parameters		Ramp 2	150.00 psi/min
Split ratio	25:1	Solvent delay	0.42 min	Final pressure	40.00 psi
		EM voltage	905.9	Final hold time	0.50 min
		m/z range	40.0 – 550.0	Initial flow	0.5 mL/min
		Threshold	150	Avg velocity	45 cm/sec

To emulate case samples, mock evidence was created by applying the individual and mixture samples to dried leaves. Whole and crushed leaves were both used to determine if greater absorbent surface area increased the ability to detect the drugs (Figure 2). Each mock evidence sample was dried, macerated, and extracted in ethanol. All samples, as well as leaf blanks were analyzed using the final developed method.

Results

Method Development

- None of the validated laboratory methods were adequate for analysis of all five standards (URB-447, -597, -602, -754, and -937) in a mixture
- Problems included poor separation, excessive thermal breakdown, long run time, and poor mass spectral library matching
- Figure 1 shows typical TICs showing combined thermal breakdown effects of prolonged room temperature storage and higher oven temperature
- The final developed method was successful in identification of compounds in a mixture
  - Shorter run time
  - Better mass spectral library matching
    - Four of five standards identified with at least a quality match score of 78 to SWGDRUG or NIST08 libraries
- Continued issues with URB-602 identification
  - 166 m/z peak more abundant in standards
  - 169 m/z peak more abundant in SWGDRUG library spectrum
  - Peak ratio of the 213 to 195 m/z peaks was different between the standards and the libraries
    - ~26% in SWGDRUG spectrum vs. ~16% in samples
- Standard solutions stored at room temperature for extended periods of time (>two days) were found to contain an increased amount of breakdown products

Mock Evidence

- No interference from leaf material was observed in leaf blanks
- Out of 16 samples, only four successfully matched to the SWGDRUG or NIST08 libraries
- All successful matches were for URB-602
- No other drugs were identified in individual standards or mixed samples
- The amount of compound absorbed onto the leaf did not appear to increase due to crushing
- Results of mock evidence analysis shown in Table 2.

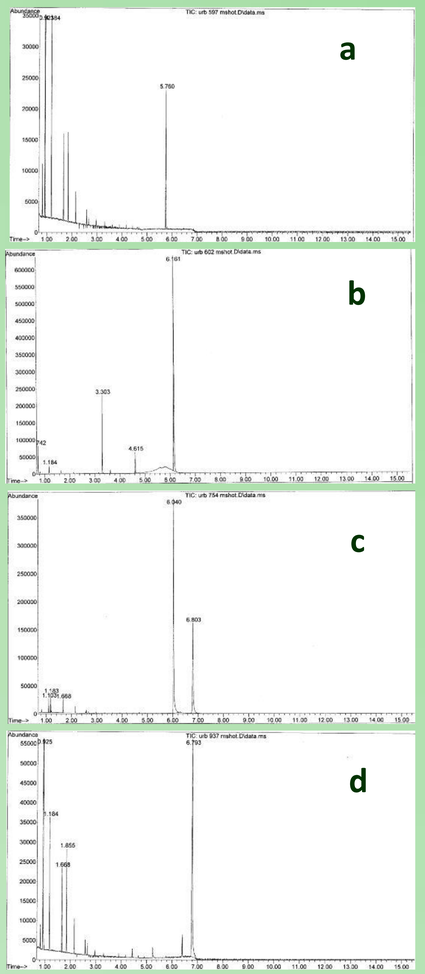

Figure 1. Typical TIC of a)URB-597 b)URB-602 c)URB-754 d)URB-937

Figure 2. Photographs of mock evidence samples

Table 2. Analysis summary of mock evidence with library quality match score

Sample	Replicate	Compounds detected‡
Mixture on whole leaves	1	None
	2	None
	3	URB-602 (QMS=87)
Individual standards applied to whole leaves	URB-447	None
	URB-597	None
	URB-602	None
	URB-754	None
	URB-937	None
	Add'l URB-602	URB-602 (QMS=96)
	Add'l URB-754	None
Concentrated mixture† applied to whole leaves	a	None
	b	None
	c	URB-602 (QMS=89)
Concentrated mixture† applied to crushed leaves	d	None
	e	None
	f	URB-602 (QMS=99)

†mixture ~10 times more concentrated than original
‡sample determined a match when listed in top ten library results

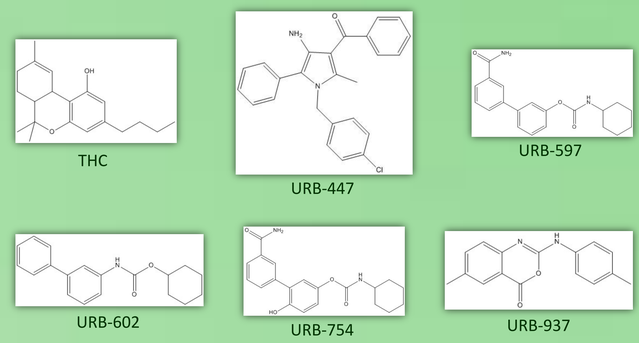

Figure 3. Structures of Δ^9-THC and the URB series

Discussion & Conclusion

- The higher oven temperature created significant degradation for methanolic standards

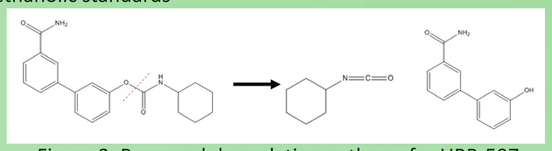

Figure 3. Proposed degradation pathway for URB-597

- Derivatization methods may protect cleavage point
- Derivatization may also improve the MS matching of URB-602
- The developed method adequately identified the URB series as standard solutions
- Identification remains problematic in mock evidence samples

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