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Detection of URB Series Synthetic Cannabinoids

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Abstract

The URB series of synthetic cannabinoids is emerging to replace first generation synthetic cannabinoids that have become illegal, such as JWH cannabinoids. 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. 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 drug methods of the Kentucky State Police Eastern Regional Laboratory, however those methods were insufficient for comprehensive URB detection. 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 was needed for complete elution of some standards, but also exacerbated degradation. An adequate method was developed and used to test mock evidence samples. Using these method parameters and experimental conditions, successful detection of all five drugs was inconsistent in the mock evidence samples.

Introduction

The URB series of synthetic cannabinoids is one of many that are designed to circumvent legal prohibition of marijuana, specifically the psychoactive ingredient delta-9-tetrahydrocannabinol (Δ^9-THC). Cannabinoids in general are developed to mimic the effects of

natural neurotransmitters, such as the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG). These neurotransmitters are responsible for stimuli processing, appetite regulation, and some memory regulation [1,2]. It has also been shown that endocannabinoids, as well as synthetic cannabinoids, can limit bone-reducing cells in humans. This may be of benefit to osteoporosis patients but may be detrimental to otherwise healthy users [3]. Multiple case studies have shown that cannabinoid intoxication can produce marked central nervous system depression [2]. The prescriptions Cesamet®, Marinol®, and Sativex® are all clinical uses of cannabinoids for antiemetic, appetite stimulation, and analgesic effects, however their side effects can include euphoria, dysphoria, and memory disturbances [4]. Many of the adverse psychological effects arise when the CB1 and CB2 receptors in the brain are activated.

The URB series includes URB-447, -597, -602, -754, and - 937. Each of these has slightly different pharmacological effects within the body but produce many of the outward symptoms of Δ^9-THC intoxication. URB-447 is a CB1 antagonist and CB2 agonist, mimicking the effects of endocannabinoids [5]. It cannot pass through the blood-brain barrier, therefore stimulating peripheral receptors, primarily in the gastrointestinal tract, eliminating many (if not all) of the psychological effects. URB-447 has been shown to reduce food intake and weight gain, making it of interest as an anti-obesity drug [6]. URB-597 is a fatty acid amide hydrolase (FAAH) enzyme inhibitor. FAAH is responsible for metabolism of anandamide and 2-AG. URB-597 has demonstrated a half maximal inhibitory concentration (IC50) of 4.6 nM in the brain and 0.5 nM in intact neurons, the lowest concentrations needed for enzyme inhibition among the URB series. Depressed FAAH activity has been shown to be responsible for reduced sensation of pain and URB- 597 exhibits anti-nociceptive and anxiolytic effects while lacking many of the

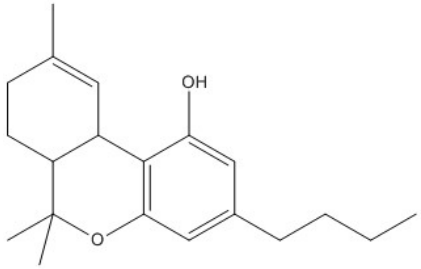
adverse cannabinoid symptoms, making it an ideal candidate for analgesic and anti-anxiety drugs [7].

URB- 602 is a monoglycerol lipase (MGL) inhibitor. MGL is responsible for the hydrolysis of 2-AG, and endogenous agonist for the CB1 receptor. Inhibition of MGL demonstrates enhanced stress-induced analgesia. URB-602 demonstrates an IC50 of 28 μM while having no impact on FAAH levels at concentrations up to 100 μM [8], making URB- 602 a possible precursor to new pain and stress management therapies. URB-754 is a non-competitive inhibitor of the monoacylglycerol lipase (MAGL) enzyme, but this has only been demonstrated in rats. No human MAGL inhibition has been show at concentrations up to 100 μM URB-754[9]. URB-937 is a peripheral FAAH inhibitor, acting on enzymes outside the central nervous system. This lack of psychological effects gives URB-937 possible use as a pain therapy drug [10].

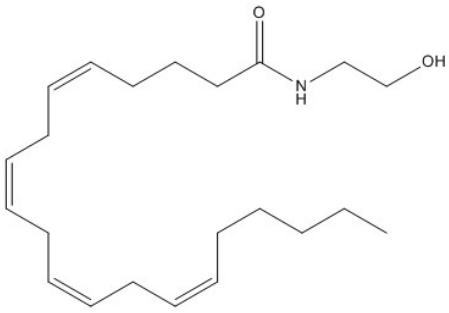
The Controlled Substance Analog Enforcement Act of 1986 requires three criteria be met in order to schedule a drug. The first requires that the substance is “substantially structurally similar” to a Schedule I or II drug. The second criterion is that it has similar stimulant, depressant, or hallucinogenic effects as the Schedule I or II drug. The final criterion is that the substance is marketed to users as having the intended effect of the Schedule I or II drug. For any substance to be scheduled as an analog, it must meet the first criterion and the second or third. The law fails to define the extent of similarity of structure, effect, or marketing. While THC is a schedule I drug, none of the URB series have structures that can be reasonably considered similar to THC (Figure 1). The pharmacology of the URB series is noticeably different from the action of THC while the end effects are similar. The applicability of the second criterion could be argued without any considerable conclusion. However, the URB series, as with all other

synthetic cannabinoids, are marketed for their mimicry to THC without the legal regulation. Because of these conditions, regulation of the URB series as THC analogs would be difficult and debated. Currently, the URB series has not been federally scheduled [11] but at least one of the series is scheduled or regulated in seven states [12] with at least 2 more having synthetic cannabinoid bans that implicitly cover the URB series. Kentucky Revised Statutes section 218A.010(44) schedules the URB series by defining synthetic cannabinoids as "any chemical compound which is not approved by the United States Food and Drug Administration or, if approved, which is not dispensed or possessed in accordance with state and federal law."[13] Despite interest and research into use of URBs in pain, stress, and appetite therapies there is no current FDA approval for their use outside of a laboratory.

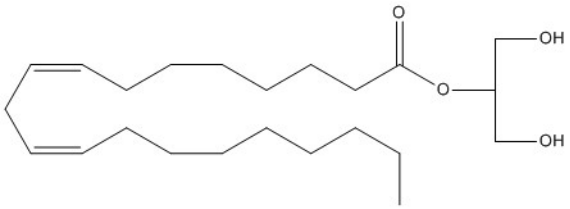
As the URB series is able to evade legal consequences, it is beginning to appear in conjunction with other drugs as a substitute for THC or other controlled synthetic cannabinoids, such as the JWH series. It is included in "potpourri" sold in head shops and by street dealers in combination with other synthetic cannabinoids and some cathinones [14]. NMS Labs has developed a blood screening test for 20 synthetic cannabinoids, but the most recent test does not include any of the URB series [15]. There has not been much focus into developing an analytical detection method for the URB series because of their scarcity compared to other synthetic cannabinoids. Using work done by researchers in Japan with street samples [16], research conducted at the Kentucky State Police Eastern Regional Laboratory aimed to develop a standard, widely applicable gas chromatography mass spectrometry (GC/MS) method capable of detecting URB compounds within seized drug samples.



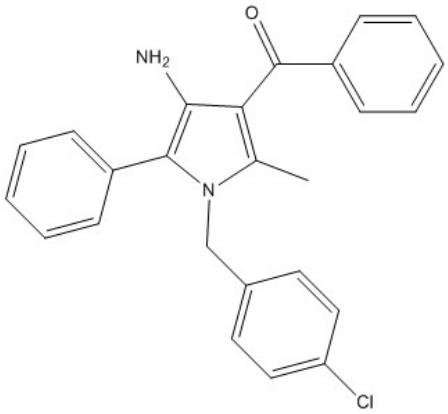
THC



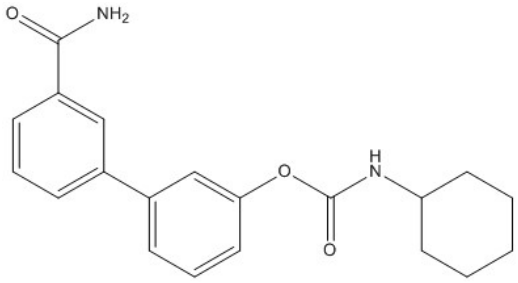
Anandamide



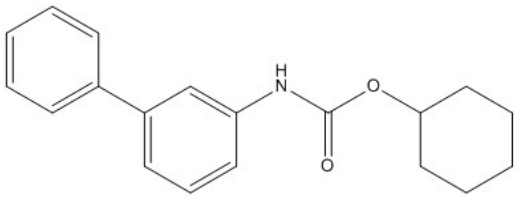
2-AG



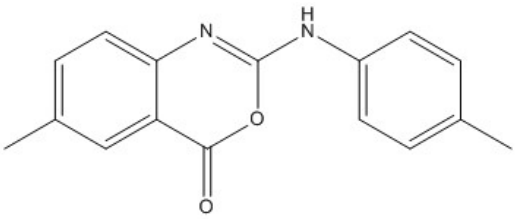
URB-447



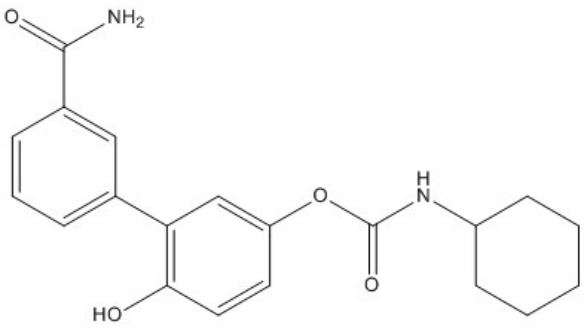
URB-597



URB-602



URB-754



URB-937

Figure 1. Chemical structures of THC, endocannabinoids, and URB series

Materials and Instrumentation

Standards of URB-447, -597, -602, -754, and -937 were obtained from Cayman Chemical (Ann Arbor, MI). Approximately one milligram of each standard was dissolved in methanol (Fisher Scientific; Fair Lawn, NJ) in a tri-spring vial insert in Agilent (Santa Clara, CA) 2-mL vials. Each standard was loaded with an Agilent 7683 Series autoinjector run on an Agilent 6890N gas chromatograph with a Zebron (Phenomenex; Torrance, CA) ZB-DRUG-1 GC column (10 m x 0.18 mm; 0.18 µm film thickness). All "DRUGS" methods used an Agilent 5973N mass selective detector in tandem with the GC and Zebron column. All "GCFID" methods used the GC in tandem with a flame ionization detector.

To test the viability of the developed method under conditions similar to seized evidence, a mixture of the URB standards was created by transferring 75 µL of each standard solution into a new vial so that it contained all five standards in final concentrations of 0.74 (URB-447), 0.46 (URB-597), 0.97 (URB-602), 0.69 (URB-754), and 0.74 (URB-937) mg/mL. Leaves were cut from a dogwood tree, rinsed in methanol, pressed and air dried for one week. Three samples of mock evidence, M1-M3, were created by applying a small amount of the mixture onto a portion of individual dried leaves. One drop of each individual standard solution was also applied to isolated areas of a single leaf. The samples for URB-602 and -754 bled with the Sharpie® used to outline the sample area therefore an additional leaf was used for clean URB-602 and -754 samples. All samples were allowed to dry for four hours. In total, fifteen samples including five leaf blanks were taken for mock evidence analysis. Each sample was cut from the leaf and covered with methanol in an open extraction vial, macerated, and extracted for two hours. The liquid volume of each sample was transferred to an insert within a GC vial like all previous standards. Extracted samples were stored in the freezer until runtime.

An additional mixture was made by directly combining the five solid standards in one GC vial insert and dissolving in methanol with URB-477, -597, -602, -754, and -937 concentrations of 2.7, 2.1, 4.8, 3.2, and 7.2 mg/mL respectively. This mixture was applied to both a whole leaf and separate crushed leaf material to create samples Ma-Mf. These samples were dried, extracted, and run as before.

Results and Discussion

Method Development

A standard must be validated in order for the laboratory to use the standard in future casework. This includes analysis by both GCFID and GC/MS. Two reference matches, a computer match and a literature reference match, must be made for each mass spectrum and the corresponding GCFID peak must also be found. All peaks and library matches must be labeled. Each standard was analyzed with various Eastern Laboratory validated methods (GCFID10, GCFID50, GCFID50LG, DRUGS10, DRUGS25LG, DRUGS50; Appendix 1), however multiple problems arose.

URB-447 was analyzed using the GCFID50LG and DRUGS25LG methods. The chromatogram of the GCFID showed a second peak that was just beginning to elute but was cut off when the run ended at 15 minutes (Fig 2). However, the compound eluted slightly faster on the GC/MS, allowing the second peak to completely elute. This peak was a match to URB-447 with a quality match score of 99 in the SWGDRUG library. The initial run of URB-597 under the DRUGS10 method immediately after the previous URB-447 run showed the presence of 447 in the last peak of the GC/MS run as matched in the SWGDRUG library (Fig 3). URB-937 was run under the DRUGS50 method and was matched to the SWGDRUG library with a relatively

low quality match score of 78 (Fig 4). URB-602 and -754 both made successful SWGDRUG matches with quality match scores of 95 and 91, respectively. When a library match could not be made by the software, a visual match of ion peaks and ratios was made to reference spectra from the Southern Association of Forensic Scientists Forendex database, however these visual matches could not be the sole consideration for validation.

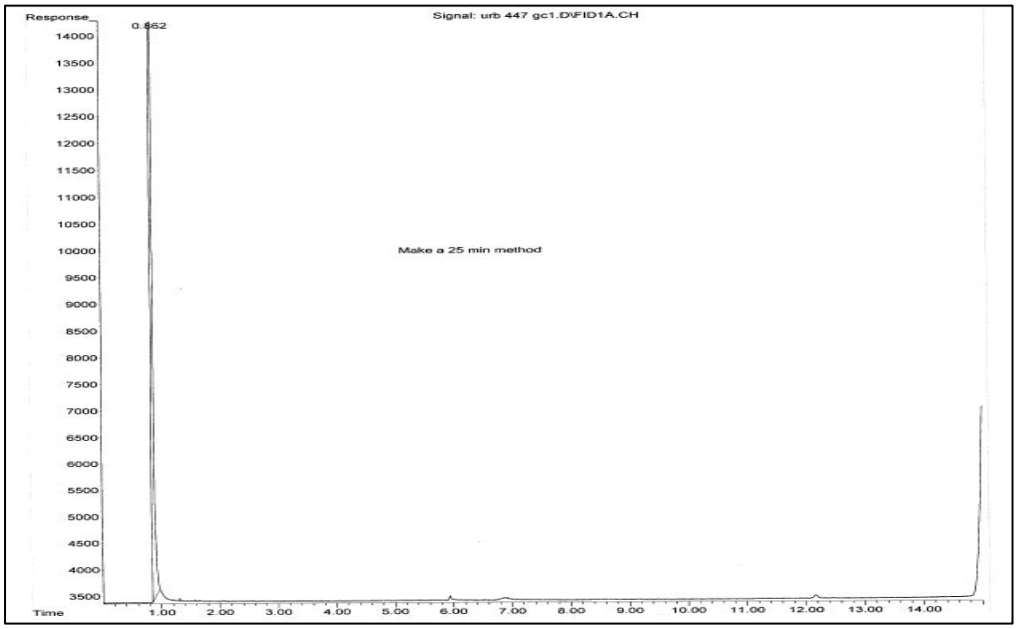


Figure 2. Incomplete GCFID50LG elution of URB-447

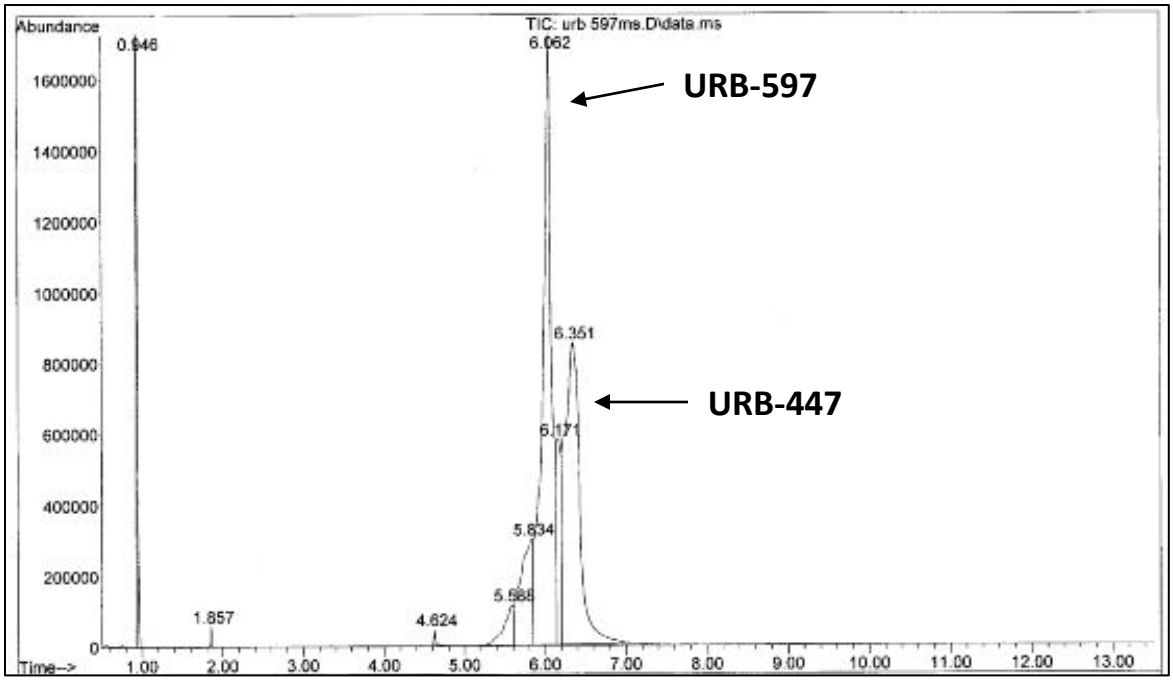


Figure 3. URB-447 bleed in DRUGS10 URB-597 spectrum

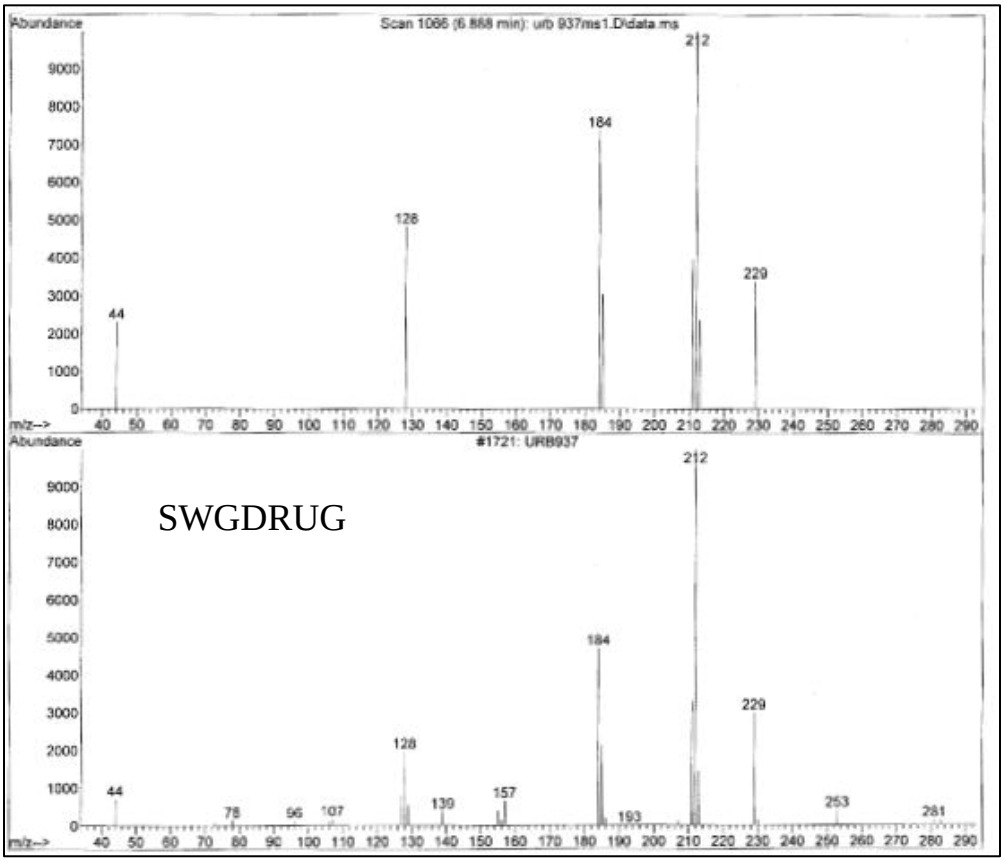


Figure 4. Low-quality library match of URB-937

Based on the results using previously validated methods, custom methods "GCFID50xLG" and "DRUGS25xLG" were developed that increased both the final temperature from 280°C to 300°C and extended the hold time from 15 minutes to 20 minutes. To simplify analysis and provide a more equal comparison, each standard was rerun under these methods. The longer runtime allowed URB-447 to fully elute unlike previously (Fig 5), while the URB-597 spectrum was much cleaner and sharper (Fig 6). With the exception of URB-602, a library match to SWGDRUG could be made in all cases with a quality match score of at least 91. URB-602 required a visual match to confirm its presence.

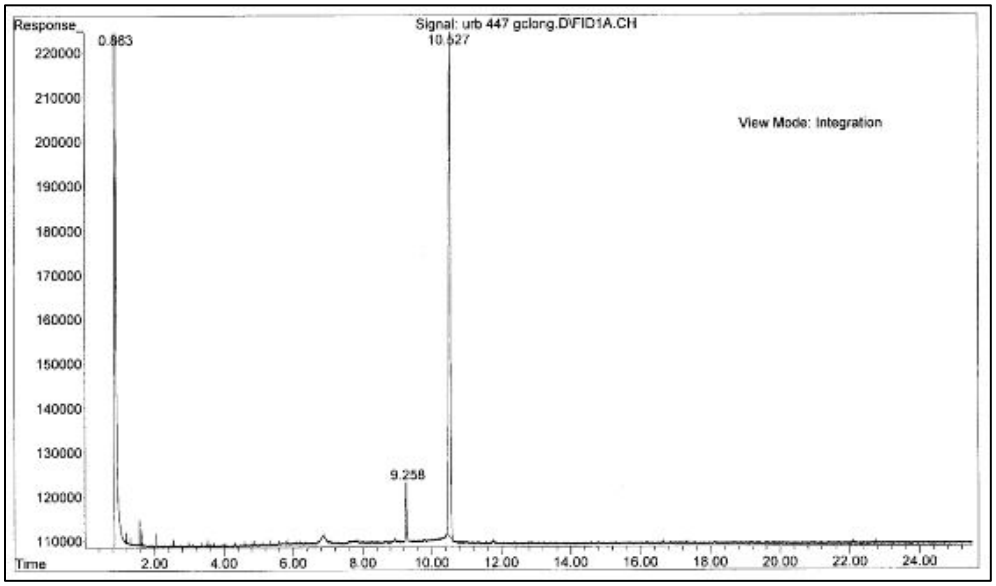


Figure 5. Complete GCFID50xLG elution of URB- 447

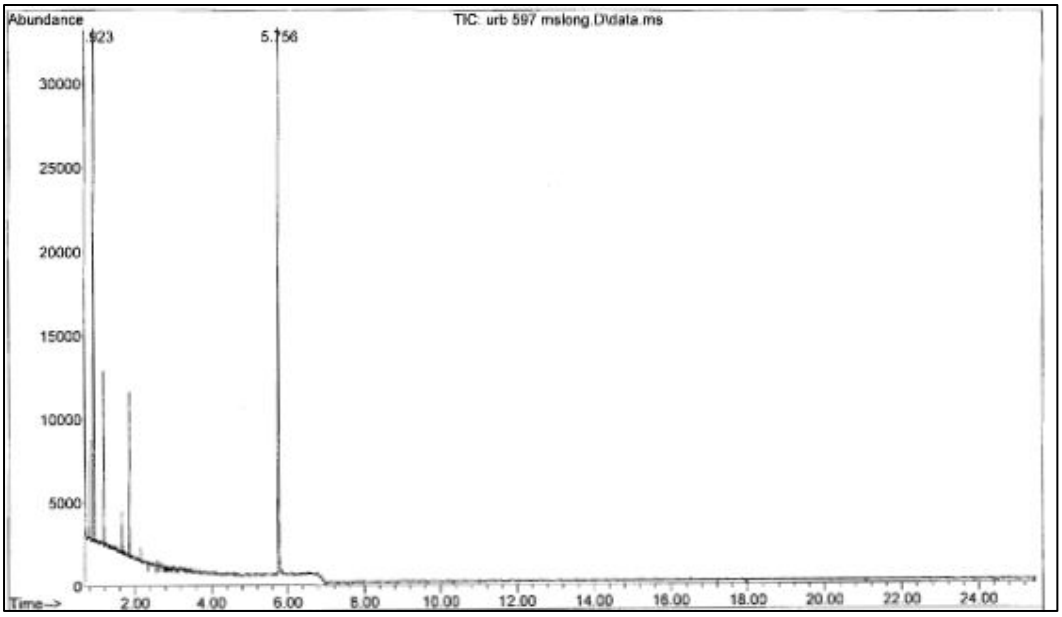


Fig 6. Improved DRUGS25xLG URB-597 elution

These methods corrected many of the problems encountered by the validated methods, however shorter methods were also tested for more practical use in casework, “GCFID50HOT” and “DRUGS25HOT”. These methods retained the increased temperature, but decreased the hold time to 10 minutes for a total runtime of 15.50 minutes. The same benefits of the “xLG” methods were observed using the “HOT” methods and each standard matched to its reference spectrum with a quality match score of at least 90, with the exception of URB- 602, which required a visual comparison to reference spectra provided by the Southern Association of Forensic Scientists Forendex database. However, a general increase in peaks with retention times under 2.00 minutes and an increase in possible degradation products was observed in most standards as well as a decrease in analyte signal (Fig 7, 8). Each standard was re-run on the “HOT” method the day following the initial run to help determine if the increase in extraneous signal was most likely the result of degradation due to extended incubation at room temperature, or whether the increased column temperature caused more extensive breakdown of the standard upon injection. It was hypothesized that further breakdown would be seen if it was a result of

room temperature conditions, but breakdown would remain constant if it were strictly from the temperatures of the column. The rerun standards were not considerably different, but continued degradation of URB-754 was observed (Fig 9).

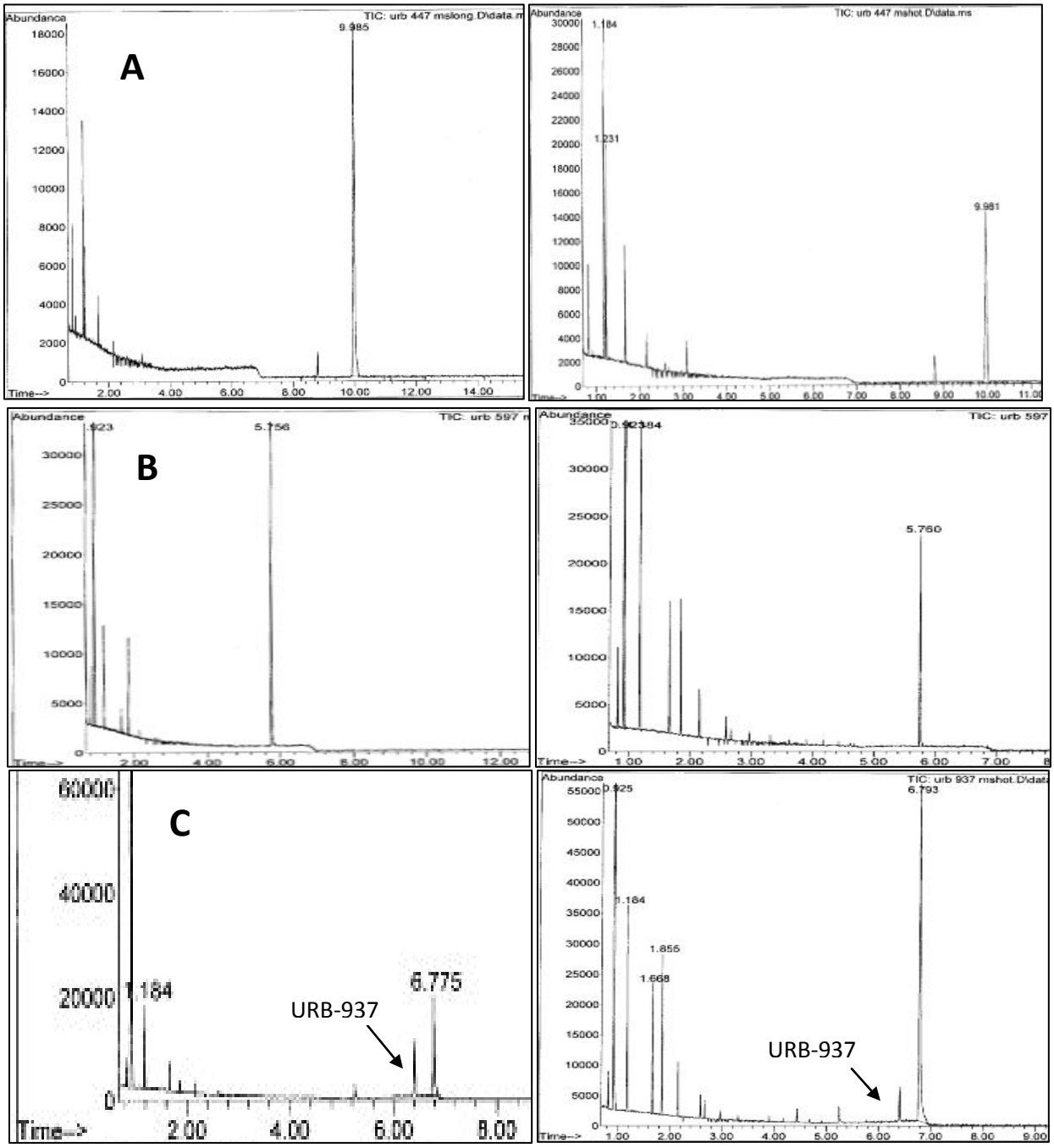


Figure 8. Standard degradation from DRUGS25xLG to DRUGS25HOT a) URB-447 b) UBR-597 and c)URB-937

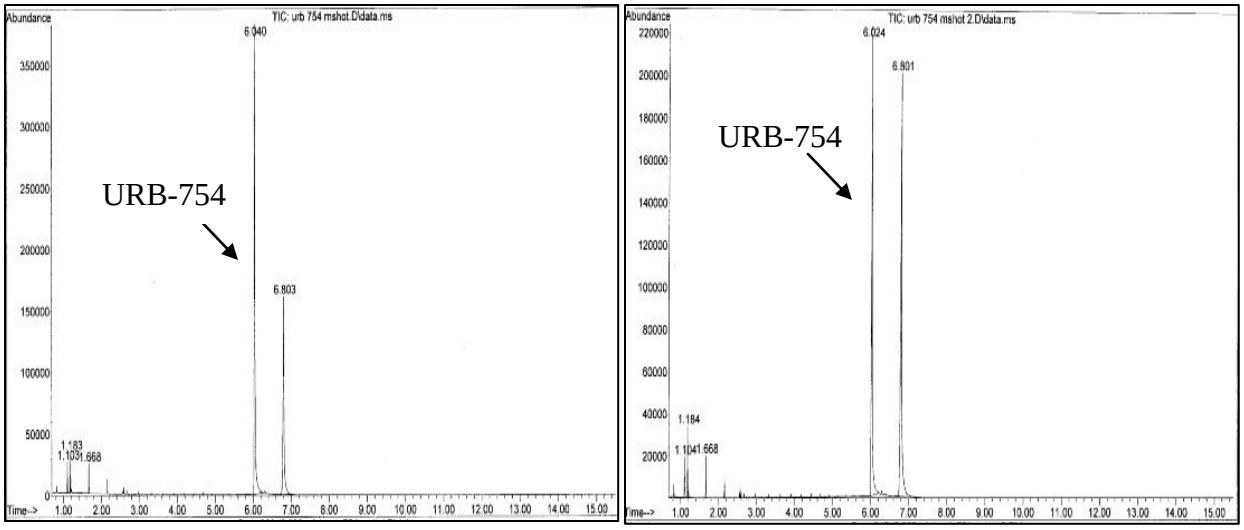


Figure 9. Continued degradation of URB-754 between first (left) and second "HOT" runs

To determine if the degradation was predominantly a result of room temperature conditions, new standards were prepared and stored at -20°C. The first "HOT" run showed improvement in spectral quality and peak intensity, however URB-602 continued to fail to match to the library and URB-937 did not appear to have eluted. This lack of elution could have been caused by column alteration when basic samples had been applied, hindering URB-937's interactions with and elution from the column. When analyzed on a different GC/MS instrument, URB-602 again failed to match the library but URB-937 demonstrated an improved spectrum relative to the first, degraded standard[*]. The final spectra and library match results for URB-447, -597, -754, and -937 are shown in Figures 10 and 11. The final spectra for URB-602 and its most abundant breakdown product are included in Figure 12 with the Forendex spectra used for visual comparisons.

[*] The column was also showing problems with casework. The column was changed and the URB-937 standard was shown to elute on the new column.

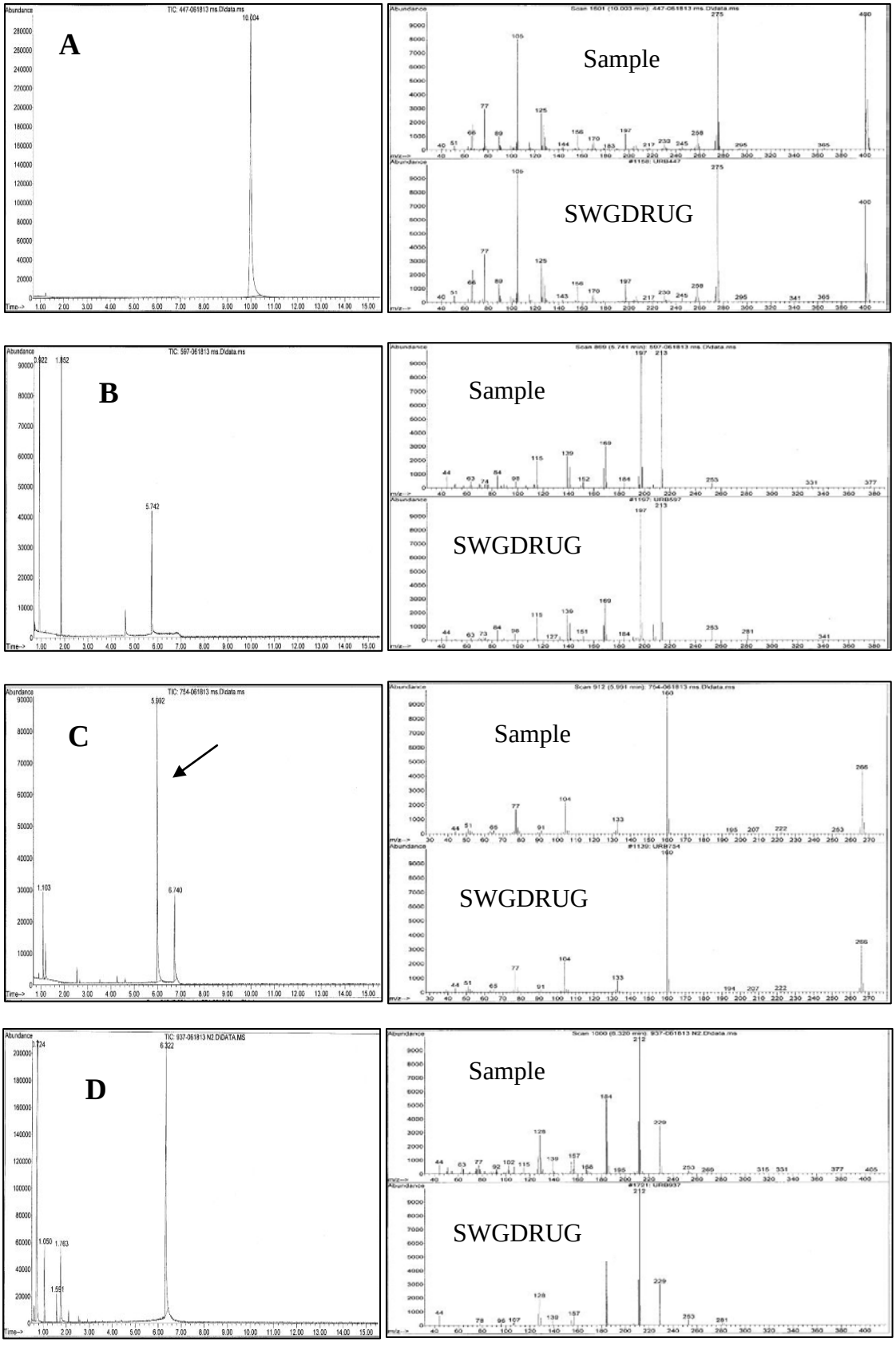


Figure 11. Final spectra and library match for A) URB-447, B) URB-597, C) URB-754, and D) URB-937

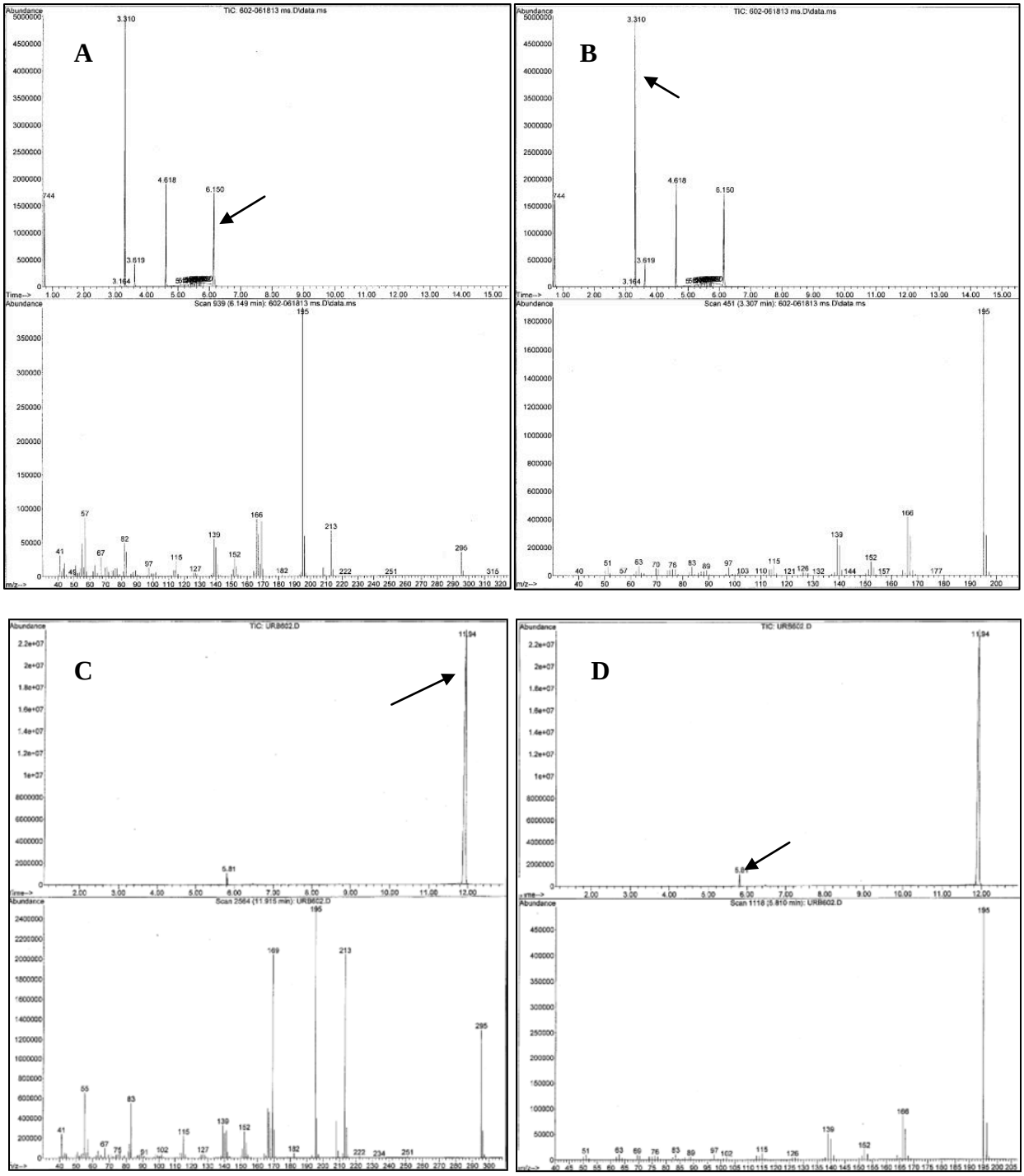


Figure 12. Final spectra for A) URB-602 and B) its potential breakdown product; Forendex reference spectra for C) URB-602 and D) its potential breakdown product

Mock Evidence

The extracted evidence samples were analyzed using the developed "HOT" method. Each leaf used also had its own blank sample. The leaf blanks were all consistent and only indicated a peak at approximately 2.8 minutes (beta-l-Arabinopyranoside, methyl; QMS=74, NIST08) that does not co-elute with any of the analytes (Fig 13). The M1 sample displayed a peak at approximately 5.8 minutes (Fig 14) whose mass spectrum contained six fragments, including four major fragments consistent with URB-602. However, because the peak is of such low abundance and the mass spectrum is fairly nondescript, the peak cannot be confidently identified. No other URBs were indicated in the M1 sample. The M2 sample was also lacking any significant peaks. The M3 sample was the only mixture sample to match to the library. It contained a peak at 5.8 minutes that matched to URB-602 with a quality match score of 87 in the SWGDRUG library (Fig 15).

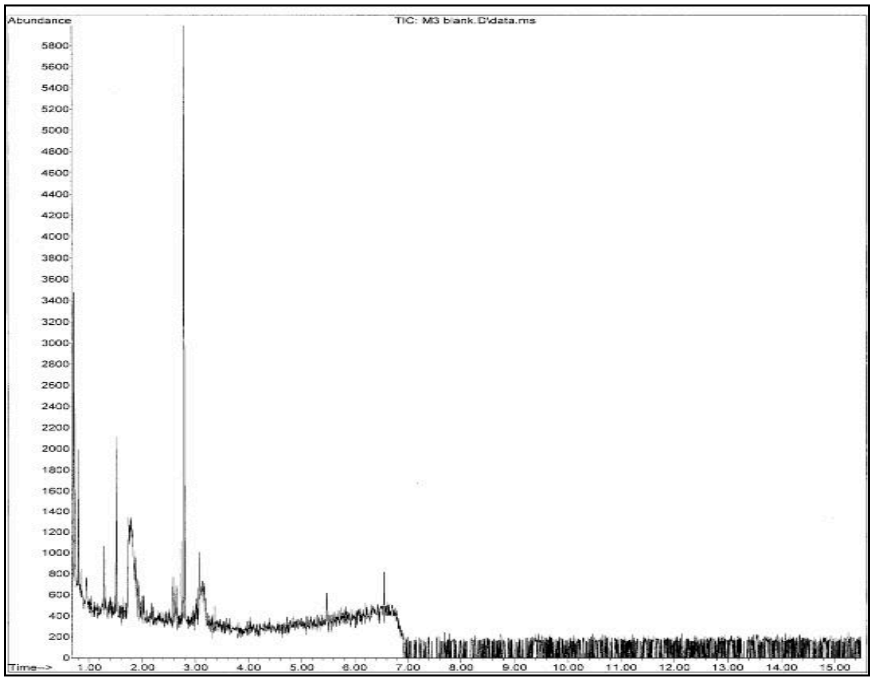


Figure 13. Leaf blank showing no co-elution with URBs

The isolated samples of URB-447, -597, -602, -754, and -937 were tested with the "HOT" method and were undetected. URB-602 was likely present based on retention time but was unmatched to the library. It is probable that either the sample concentrations were too low or that degradation had progressed too far for any analyte to be detected.

Additional mix samples Ma-Mf used the more concentrated mixture, "mix 071513". Ma-Mc were applied to a whole leaf just as M1-M3 were, and Md-Mf were applied to crushed leaf material with the hope of improving absorption of the solution onto the leaf. The results of each mock evidence sample are summarized in Table 1. Mix 071513 was also tested as a solution for the presence of all standards. URB-447 and URB-937 were matched from the SWGDRUG library and the possible presence of URB-602 was indicated by retention time without a library match.

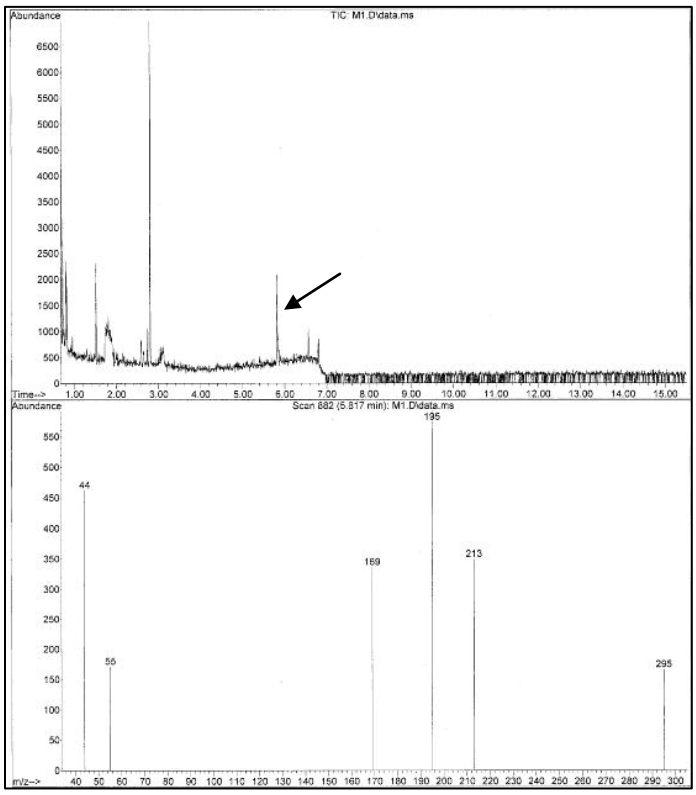


Figure 14. Mixture M1 with TIC of peak

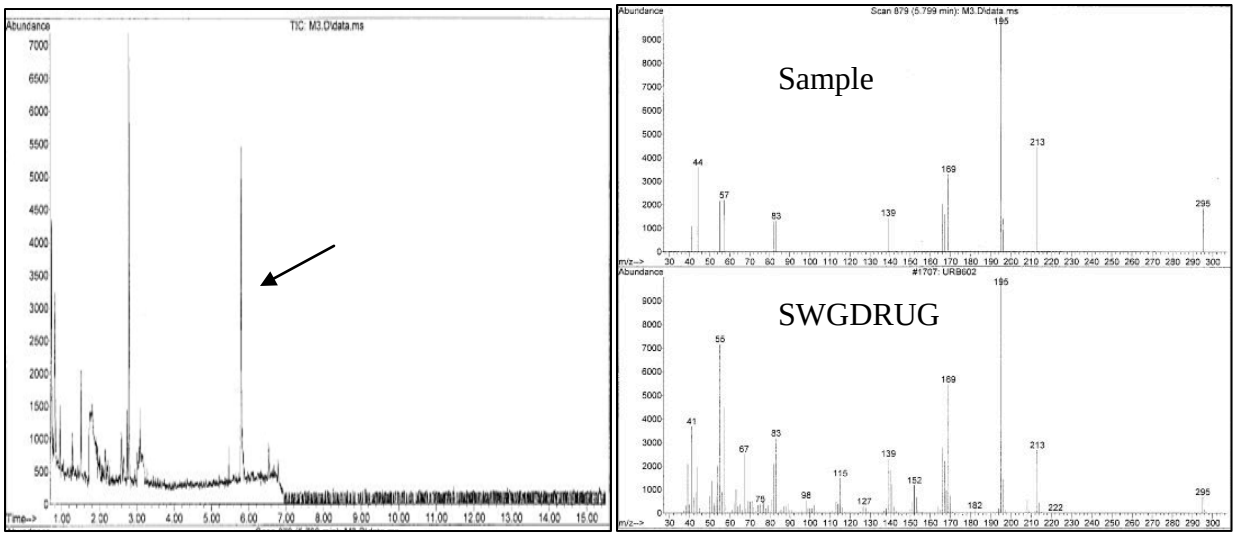


Figure 15. Identified 602 peak in M3 and library match

Table 1. Summary of detected compounds in mock evidence samples

Sample	Detected match
M1	None
M2	None
M3	URB-602 (QMS=87)
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
Ma	None
Mb	None
Mc	URB-602 (QMS=89)
Md	None
Me	None
Mf	URB-602 (QMS=99)

Conclusions

Method Development

This developed "HOT" method has shown to be sufficient for standards in methanol, however degradation and breakdown must constantly be regarded as significant issues. The degradation observed in all standards may have to be considered as part of a compromise in

determining a detection method for the URB series. Breakdown and degradation were seen in nearly all spectra of all standards. A suggested breakdown product of URB-597 involves the cleavage of the ester bond [17] to form two byproducts (Fig 16).

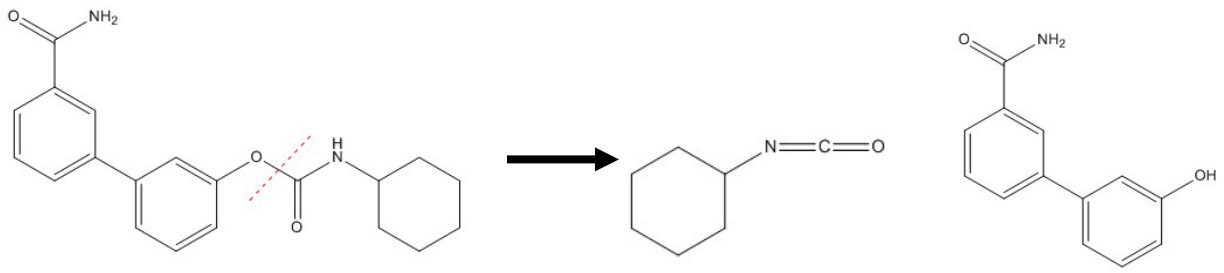


Figure 16. Proposed cleavage of URB-597

Because of the similarities in structure between URB-597 and -937, these two drugs may breakdown by the same mechanism. Both show two distinguishable peaks whose ratio changes depending on the state of degradation. As such, the breakdown of both may be solved by the same method, specifically a single derivatization. In any seized sample the identity of any URB present would not likely be readily known and the ability to use a single solution to the problems faced in each individual drug would be greatly advantageous.

While the chromatograms were improved under the “HOT” methods, URB-602, -754, and -937 were all validated using the Eastern Regional Laboratory’s own lower-temperature methods. Though it is apparent that a higher temperature is beneficial for clean and complete elution of the standards, notably URB-447, it may also be considered that a lower temperature method may be used if a particular lab has no need to detect the compounds that require higher temperatures such as URB-447 and -597. URB-447 and -597 were detected and matched with the lower-temperature methods, but the spectra were not of a sufficiently high quality to be

validated. The chromatograms improved under the “HOT” method, however as the method was not validated, the standards could not be validated.

Derivatization studies should be the next step in developing a good, consistent detection method for the URBs. The lack of library matches to URB-602 stems mainly from the difference in the appearance of the 166 v. 169 ion peak, as well as the ratio of this peak to the 195 and 213 peaks. These are three of the four most abundant ions of the URB-602 mass spectra and therefore it presents as a good candidate for derivatization to improve these ratios and the ability to match to a database. URB-597, -754, and -937 show significant breakdown over multiple runs as analyte signal decreases compared to the breakdown signal. Derivatization may be employed to keep the molecules intact to improve signal strength and quality.

Mock Evidence

Preparation of the mock evidence samples was comparable to seized, suspected synthetic marijuana samples and therefore testing for URBs could be easily incorporated into existing cannabinoid protocols for a lab. However under these conditions, the detection of any of these drugs is inconsistent. The most prominent cause of inconsistency is the degradation of the samples at room temperatures which is exacerbated by thermal decomposition at inlet and column temperatures. Any derivatization procedure used to improve the detection method should also be tested on these mock evidence type samples. Additional studies may be done to determine viability of recovery from burnt samples that may be encountered as residue on seized paraphernalia. The thermal degradation problems observed in the standards may have an impact on how the compounds would be found in these types of residual samples.

Currently many states and jurisdictions do not consider the URB series illegal and are not yet concerned with their detection. Use may increase as other synthetic cannabinoids become illegal and jurisdictions may see a rise in the prevalence of the URB series. Having a validated detection method compatible with current standard operating protocols will allow a faster transition between legislation and detection.

Acknowledgments

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Appendix 1

Standard Method Parameters

GCFID10

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	1.0 µL	Final temperature	280° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	8.50 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	FID Parameters		Ramp 2	150.00 psi/min
Split ratio	10:1	Temperature	250° C	Final pressure	40.00 psi
		Hydrogen flow	40.0 mL/min	Final hold time	0.50 min
		Air flow	450.0 mL/min	Initial flow	0.2 mL/min
		Helium flow	45.0 mL/min	Avg velocity	15 cm/sec

GCFID50

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	1.0 µL	Final temperature	280° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	8.50 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	FID Parameters		Ramp 2	150.00 psi/min
Split ratio	50:1	Temperature	250° C	Final pressure	40.00 psi
		Hydrogen flow	40.0 mL/min	Final hold time	0.50 min
		Air flow	450.0 mL/min	Initial flow	0.2 mL/min
		Helium flow	45.0 mL/min	Avg velocity	15 cm/sec

GCFID50LG

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	1.0 µL	Final temperature	280° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	10.00 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	FID Parameters		Ramp 2	150.00 psi/min
Split ratio	50:1	Temperature	250° C	Final pressure	40.00 psi
		Hydrogen flow	40.0 mL/min	Final hold time	0.50 min
		Air flow	450.0 mL/min	Initial flow	0.2 mL/min
		Helium flow	45.0 mL/min	Avg velocity	15 cm/sec

DRUGS10

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	1.0 µL	Final temperature	280° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	8.50 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	MS Parameters		Ramp 2	150.00 psi/min
Split ratio	10:1	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

DRUGS25LG

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	1.0 µL	Final temperature	280° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	15.00 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	0.60 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

DRUGS50

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	1.0 µL	Final temperature	280° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	8.50 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	MS Parameters		Ramp 2	150.00 psi/min
Split ratio	50:1	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

Custom Method Parameters

GCFID50xLG

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	FID Parameters		Ramp 2	150.00 psi/min
Split ratio	50:1	Temperature	250° C	Final pressure	40.00 psi
		Hydrogen flow	40.0 mL/min	Final hold time	0.50 min
		Air flow	450.0 mL/min	Initial flow	0.2 mL/min
		Helium flow	45.0 mL/min	Avg velocity	15 cm/sec

DRUGS25xLG

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

GCFID50HOT

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	3.0 µL	Final temperature	300° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	10.00 min	Intermediate hold time	6.00 min
Pressure	5.00 psi	FID Parameters		Ramp 2	150.00 psi/min
Split ratio	50:1	Temperature	250° C	Final pressure	40.00 psi
		Hydrogen flow	40.0 mL/min	Final hold time	0.50 min
		Air flow	450.0 mL/min	Initial flow	0.2 mL/min
		Helium flow	45.0 mL/min	Avg velocity	15 cm/sec

DRUGS25HOT

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	3.0 µL	Final temperature	300° C	Intermediate pressure	15.00 psi
Inlet temperature	250° C	Final hold time	10.00 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