Comparison of the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT gas chromatography columns for the qualitative analysis of synthetic cannabinoids

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Abstract

As popularity of synthetic cannabinoids and the prevalence of their harmful side effects grow, so does the need to control such substances. For high throughput labs such as the West Virginia State Police (WVSP) Drug Identification Laboratory, the high molecular weight and low volatility of synthetic cannabinoids poses a problem for analysis as not all synthetic cannabinoids elute within the parameters of their standard GC-MS method. This study compares the Restek Rxi®-1ms and Rxi®-1HT GC columns to the Restek Rtx®-5 GC column (standard method in the WVSP Drug Laboratory) to determine if either column could improve the efficiency of synthetic cannabinoid detection and analysis using the standard GC-MS method. A total of 53 synthetic cannabinoid standards were analyzed and the results indicated a dramatic decrease in retention time (average of 2.106 minutes) when using the Restek Rxi®-1HT GC column for analysis and a slight decrease in retention time (average of 0.488 minutes) when using the Restek Rxi®-1ms GC column for analysis. Data from both the Restek Rxi®-1ms and Rxi®-1HT columns were determined to be statistically significantly different from data obtained using the Restek Rtx®-5 column based on paired t tests with 95% confidence intervals. Both columns demonstrated adequate reproducibility of retention time for the qualitative analysis purposes of the West Virginia State Police Drug Identification Laboratory. In conclusion, the Restek Rxi®-1HT and Rxi®-1ms columns have proved to be a promising possibility for the qualitative analysis of synthetic cannabinoids in high throughput laboratories.

Introduction

There are three groups of cannabinoids which act on CB1 and CB2 receptors in the body[1] including natural cannabinoids such as Δ9-tetrahydrocannabinol (THC) found in marijuana; endogenous cannabinoids which are made in the body; and synthetic cannabinoids which are made in a laboratory and are the focus of this study. Synthetic cannabinoids were first developed in the 1960s and 70s as "potential pharmaceutical agents"[2] and were first reported in the United States as recreational drugs in December 2008 "when a shipment was seized and analyzed by United States Customs and Border Protection in Dayton, Ohio"[3]. These compounds are typically made in noncommissioned or clandestine laboratories and dissolved into a solution, which is then applied onto a variety of vegetation. The

modified vegetation is then packaged and sold legally in drug paraphernalia or "head shops", convenience stores, or on the internet[4,5,6]. The products are typically marketed as potpourri and labeled "not for human consumption." Synthetic cannabinoid products are often referred to as "spice" or "K2" but go by many names such as Fake Weed, Yucatan Fire, Genie, Skunk, Black Mamba, Bombay Blue, Chill Out, Cultured Weed Ahia, Dream, Encense Gorilla, Forest Humus, Jamaican Gold, Joker, King B, Mojo, Krypton, Nirwana, Relax, Space Diamond, Space Gold, Spice Diamond, ZoHai, Smoke XXX, Heaven, Bonzai Cuba, Jamaican Spirit, Ivory Wave, Dragon Herbal Incense, Ivory Wave, Tai Fun, Chaos, Chill Zone, Aztec Thunder, Zen, Nuke, Kronic, Magic Dragon, and Karma to name a few[1,7,8,9]. Synthetic marijuana products have been easily available internationally since the early to mid-2000's[10] and became available in the United States in 2008, quickly gaining popularity[3,8]. The growing popularity of synthetic cannabinoids can be attributed to "the desire for a 'legal high' and the ability to avoid detection on standard drugs-of-abuse testing such as those for THC"[10].

Synthetic cannabinoids are most often smoked, or otherwise ingested, by people seeking a marijuana-like high while evading the risk of detection by common drug screens[10]. Before 2010, synthetic cannabinoids were not controlled by any state or at the federal level[3], providing another advantage for the use of such substances over marijuana. Currently, there are no preliminary screening or toxicological tests for these substances because little is known about the metabolites produced through biotransformation of synthetic cannabinoids. Also, the large variability in the structure of synthetic cannabinoids has prevented the development of an immunoassay capable of screening for a large portion of the drugs at once[11].

Dangers associated with the use of these compounds became apparent as emergency department and poison control cases related to synthetic cannabinoids exploded after their appearance in the United States. The associated statistics lead to the conclusion that the drugs were a threat to public health,[9,12] which provided grounds for emergency scheduling of the compounds. While the

desired effects of synthetic cannabinoids for most users are relaxation, elevated mood, and altered perception[8], overdosing on synthetic cannabinoids has become commonplace where symptoms may include anxiety, disorientation, profuse sweating, agitation, nausea, vomiting, hallucinations, dependence, withdrawals, palpations, tachycardia, bradycardia, hypertension, myocardial infarction, ischemic stroke, kidney injury, psychosis "including aggressive, violent, and self-injurious behavior,"[13] tremors, seizures, and in rare cases even death[2,10,12,13].

While these side effects can be quite dangerous, they do not appear to deter synthetic cannabinoid use. In 2009, there were 14 calls to poison control centers associated with synthetic cannabinoid use in the United States. Those numbers skyrocketed in the next two years reaching 2,906 in 2010 and 6,968 in 2011 before falling to 5,230 in 2012 and 2,668 in 2013[1,14]. However, the trend appears to be on the rise again. Preliminary data indicates that 3,682 calls associated with synthetic cannabinoids were made to poison control centers in 2014 and 4,377 calls were made from January 1, 2015 to July 6, 2015[14]. Of the 2,961 poison control center calls related to synthetic cannabinoids between January 2015 and April 2015 resulting in a reported medical outcome, 47.5% experienced "moderate effects", 11.3% experienced "major adverse effects", and 0.5% resulted in a fatality[15]. In 2010, a significant number of emergency department visits involving synthetic cannabinoids was detected by the Drug Abuse Warning Network[16] (DAWN) providing yet another statistic to depict the growing popularity and danger of synthetic cannabinoids in the United States. According to DAWN, there were 11,406 emergency department visits involving synthetic cannabinoids in 2010 and 28,531 in 2011[16]. Despite these disturbing statistics and side effects, synthetic cannabinoids were reported to be the second most used illegal drug, behind marijuana, among twelfth graders in 2012[3] and the third most used illegal drug, behind marijuana and inhalants, among eighth graders in 2013[5].

Why is it so easy to overdose and experience adverse effects with synthetic cannabinoids in comparison to *cannabis*? One explanation is that without the control of the Food and Drug Administration (FDA),

the "purchaser [of synthetic cannabinoids] has no information on the real composition"[9] or the concentration of the drug they are buying. Research suggests that several synthetic cannabinoids are much more potent due to their higher affinity for CB1 and CB2 receptors[7,9,12,17] than THC and that the concentration of synthetic cannabinoid compounds on "spice" products is very inconsistent from sample to sample, packet to packet, and brand to brand[7,9,17]. These two conditions in combination make it difficult for a user to know how strong a product will be before using it. Studies have also shown that there is little correlation between brand name and the type of synthetic cannabinoid present on plant material[7,9,11] making it hard to determine what products to avoid based on past experience. Research carried out by Zuba *et. al*. suggests there is a correlation between flavor and type of synthetic cannabinoids present[9], however, given the previous information, flavor should not be relied upon by a user to gauge quality or concentration. Lastly, plant materials listed as ingredients on the packaging are often inaccurate[9,17]. Further, the plants used to produce smoking mixtures could themselves be psychoactive[10] or be a source of adverse reactions.

The first attempts to schedule synthetic cannabinoids on a federal level in the United States occurred in November of 2010 when the United States Department of Justice (USDOJ) began the process of emergency scheduling[10,12]. In 2011, the DEA had successfully emergency scheduled five synthetic cannabinoids under the Controlled Substances Act based on their demonstrated risk to public health. These 5 compounds were scheduled, with intentions of banning several classes of synthetic cannabinoids based on the Controlled Substance Analogue Enforcement Act of 1986 which states that a compound can be treated as a controlled substance if it is chemically or pharmacologically similar to a compound that is already a schedule I or II drug[3,5,18]. In 2012, the FDA passed the Safety and Innovation Act of 2012 which included the Synthetic Drug Abuse Prevention Act. This act served to define "cannabiminetic," extend the length of time a substance can remain emergency scheduled, and permanently schedule select synthetic drugs[3,5,18]. This Law also led to "Operation Log Jam" in 2012

which “yielded arrests of more than 90 individuals and the seizure of more than 5 million packets of finished synthetic designer drugs [including synthetic cannabinoids] and the ingredients to produce 13.6 million more packets”[5]. Additional synthetic cannabinoids were scheduled in 2013, 2014, and 2015[5,18] leading to over 25 synthetic cannabinoids being scheduled as of January 2015[18,19-22] including their salts, isomers, and salts of isomers according to the Synthetic Drug Abuse Prevention Act of 2012. Other large scale enforcement operations include “Project Synergy” which began in December of 2012 and yielded over 227 arrests, search warrants served in 35 states, 49 cities, and five countries, seizure of over $51 million in cash and assets, and 1,252 kilograms of synthetic cannabinoid drugs among other drugs[5,23] and “Project Synergy Phase II” in 2014 yielding over 150 arrests and the seizure of “hundreds of thousands of individually packaged, ready-to-sell synthetic drugs as well as hundreds of kilograms of raw synthetic products to make thousands more”[5].

There are some factors that complicate the enforcement of these laws and analysis of the drugs however. Beyond the formerly mentioned fact that there are no presumptive tests developed thus far, manufacturers of the drugs are making efforts to evade the law with their products. For example, manufacturers will label their products “not for human consumption” in an effort to evade the Controlled Substance Analogue Enforcement Act of 1986 which only applies to substances intended for human consumption[5,18]. Also, new synthetic cannabinoid compounds are emerging all the time in order to bypass current bans on specific compounds. As new synthetic cannabinoids are developed there is a delay in scheduling them and creating the standards needed for analysis in the laboratory. In addition to manufacturer’s efforts to circumvent the law, synthetic cannabinoids tend to be larger molecules compared to other drugs, and by nature, less volatile. Because gas chromatography- mass spectrometry (GC-MS) is one of the main instruments used in many forensic laboratories, low volatility of synthetic cannabinoid compounds can interfere with throughput capabilities.

At the West Virginia State Police Drug Identification Laboratory, the low volatility of synthetic cannabinoids pose a problem, as not all synthetic cannabinoids will elute using their standard GC-MS drug method. For this reason, the laboratory employs a method with a longer elution time for synthetic cannabinoid analysis, decreasing their possible throughput of synthetic cannabinoid cases. The goal of this study is to determine if a different column could be used to modify their standard GC-MS method to reduce the retention time and improve the efficiency of synthetic cannabinoid analysis in the laboratory.

Materials and Methods

Standards of 53 synthetic cannabinoids listed in Table 1 were purchased from Cayman Chemical (Ann Arbor, Michigan). In a gas chromatography (GC) vial, approximately 1 mg of each standard was dissolved in 1.5 mL of methanol. Using an Agilent Technologies 7890A GC instrument equipped with an Agilent Technologies 5975C inert mass spectral detector (MSD) with triple-axis detector, each sample was run at least once on the Restek Rxi ®-1ms and Rxi®-1HT columns. Seven of the standards (JWH-018, JWH-022, JWH-073, JWH-073 6-methoxyindole analog, CP-47,497 C8 homolog, AM-2233, and AB-PINACA) were analyzed ten times on each column to provide insight with respect to retention time reproducibility. All synthetic cannabinoid runs began and ended with a standard compound mix including methamphetamine, cocaine, and hydromorphone to ensure the instrument was working properly throughout the run and all spectra were library-matched to determine that identification by MS was not altered by either column. One methylene chloride blank and two methanol blanks were also run between each sample to prevent carryover. Once all data was collected, statistical analyses, including Anderson-Darling tests and paired two-tailed t-tests, were performed using the program MaxStat to determine if there was a significant difference in retention times on different columns.

GC-MS Parameters

The GC inlet was operated at 225°C. The method used a 1 µl injection volume. The column flow was held at 1.2 mL/minute. The oven was set at 115°C at the beginning of each run. The temperature was then ramped to 290°C at a rate of 20°C per minute and held for 4 minutes. The MS solvent delay was 0.60 minutes and the scan parameters were as follows: 40.00-500.00 AMU, 25 count threshold, and 3.15 scans/second.

Results

The raw data collected for basic retention time analysis can be viewed in Table 2 of the Appendix. The number of synthetic cannabinoid compounds tested using the intended method on the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT columns were 38, 53, and 53 respectively. The synthetic cannabinoids analyzed on the Rtx®-5 GC column had retention times ranging from 6.826 minutes to 13.621 minutes. The synthetic cannabinoids run on the Rxi®-1ms GC column had retention times ranging from 6.522 minutes to 9.736 minutes. The synthetic cannabinoids run on the Rxi®-1HT GC column had retention times ranging from 5.288 minutes to 9.314 minutes. There was no sample carryover detected on either GC column.

An Anderson-Darling test was performed to determine if the data for each GC column was normally distributed before performing two-tailed paired t-tests. The paired t-tests indicated that the retention times obtained on the Rxi®-1ms and Rxi®-1HT GC columns were significantly decreased from those obtained using the same method on the Rtx®-5 GC column. A p value of 0.05 was considered statistically significant. When comparing the Rtx®-5 column to the Rxi®-1ms column the t_{calc} value was 7.378 (t_{crit} = 2.028). When comparing the Rtx®-5 column to the Rxi®-1HT column the t_{calc} value was 19.688 (t_{crit} = 2.028). A two-tailed paired t test also determined that the retention times on the Rxi®-

1ms and Rxi®-1HT columns were significantly different from each other (t_{calc} = 29.589, t_{crit} = 2.007). These results are outlined in Figure 1 and Tables 3 and 4 of the Appendix.

For the 37 synthetic cannabinoid compounds analyzed on both the Rtx®-5 and Rxi®-1ms columns the average decrease in retention time when switching to the Rxi®-1ms GC column was 0.409 minutes with a standard deviation of 0.335 minutes. When examining the 37 synthetic cannabinoid compounds analyzed on both the Rtx®-5 and Rxi®-1HT GC columns, there was an average decrease in retention time of 2.023 minutes with a standard deviation of 0.623 minutes when switching to the Rxi®-1HT GC column. Comparing the 57 synthetic cannabinoid compounds run on both the Rxi®-1ms and Rxi®-1HT GC columns, retention times were decreased an average of 1.616 minutes using the Rxi®-1HT GC column. The standard deviation for the retention time shifts between the Rxi®-1ms and Rxi®-1HT GC columns was 0.396 minutes. These results are outlined in Figure 2 and Table 4 of the Appendix.

The raw data collected for the retention time reproducibility portion of this study can be viewed in Table 5 of the Appendix. For the seven compounds involved in the reproducibility study, standard deviations ranged from 0.030 to 0.085 minutes when eluting on the Restek Rxi®1-ms column and from 0.030 to 0.061 minutes when eluting on the Restek Rxi®-1HT column. With this level of reproducibility, requiring retention times to be within 0.100 minutes of the expected average should leave plenty leeway for identification.

Discussion and Conclusion

While a decrease in retention time was observed with both columns, the Restek Rxi®-1HT column yielded the best results with a larger average decrease in retention time and more reproducible retention times based on standard deviation in the reproducibility study. The results of this study indicate that the implementation of the Restek Rxi®-1HT column for synthetic cannabinoid analysis would prove the most fruitful for the West Virginia State Police Drug Identification Laboratory. The

average retention time decrease of 2.023 minutes observed with the Rxi®-1HT column is an adequate decrease to ensure all synthetic cannabinoid compounds currently tested will elute in the allotted time. With the large volume of cases received the value added will be significant based on the time saved during analysis. While the change in column will not necessarily impact quantification abilities in the laboratory, it will be beneficial for the qualitative analyses performed by the drug identification laboratory. Further studies would need to be done to determine whether the application of such columns would be realistic in a quantitative analysis setting.

An interesting observation during the reproducibility study, which can be viewed in Table 5, is that the retention times of different compounds appeared to steadily decrease with column use. While the decreases in retention time were not typically large, it does indicate a continuous shift. This could be due to the fact that the column was not properly conditioned before collecting data but rather samples were run and data collected immediately after column installation. If this was the case, the retention times should become more consistent over time. However, if a range of reproducibility is defined in a protocol for identification based on standard deviation and variance, it may be worthwhile to maintain a running log of retention times in order to maintain the most accurate mean retention times and standard deviations if this is not already a standard practice.

In future studies, the cleanliness of casework spectra using the different columns should be examined. Unlike spectra of standards, the spectra obtained the extraction of evidentiary synthetic cannabinoid samples is often cluttered with many extra peaks related to the vegetation. The West Virginia State police Drug Identification Laboratory's policy requires that every peak with an abundance of 5,000 units above the baseline be identified by mass spectrometry library match. If one of the columns can help reduce the number of non-controlled substance peaks by allowing them to elute during the MS solvent delay while maintaining detection of those that are scheduled it could greatly

reduce an analysts time spent looking at spectra as well as reduce the number of spectra included in reports and thus reducing paper expenditures for the lab.

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APPENDIX

Table 1. List of the synthetic cannabinoid standards analyzed.

25B-NBOMe	CP 47,497 C8 HOMOLOG	JWH-203
25C-NBOMe	CP 55,940	JWH-203-3 chlorophenyl
25D-NBOMe	EAM2201	JWH-203-4 chlorophenyl
25H-NBOMe	FUB-PB-22	JWH-210
5-fluoro-AB-PINACA	HU-210	JWH-250
5-fluoro PB-22	HU-211	JWH-302
AB-CHMINACA	JWH-018	JWH-398
AB-FUBINACA	JWH-018	MAM-2201
AB-PINACA	ADAMANTYLCARBOXAMIDE	PB-22
AKB-48	JWH-019	RCS-4
AKB-48 N-(5-fluoropentyl)	JWH-022	RCS-4-C4 homolog
AM-1220	JWH-073	RCS-8
AM-2201	JWH-073 6-methoxyindole	STS-135
AM-2233	JWH-081	THJ-2201
AM-694	JWH-122	UR-144
CB-25	JWH-122 N(4-pentenyl)	UR-144-N-(5chloropentyl)
CB-52	JWH-200	URB-597
CP 47,497	JWH-201	XLR-11

Table 2. Raw retention time data collected for synthetic cannabinoid standards run on the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT GC columns. Gray boxes indicate that a retention time was not obtained.

Synthetic Canabinoid	R_t on Rtx-5	R_t on Rxi-1ms	R_t on Rxi-1HT
25C-NBOMe	7.330	7.330	5.964
25B-NBOMe	7.691		
25D-NBOMe	7.047	6.872	5.522
25H-NBOMe	6.869	6.678	5.339
AB-FUBINACA	9.202	8.954	7.539
AB-CHMINACA	9.327	8.960	7.524
AB-PINACA	8.047	7.962	6.751
AKB-48	9.973	9.315	7.778
AKB-48 N-(5-fluoropentyl) analog	9.972	9.742	8.232
AM-694	9.862	9.018	7.701
AM-1220	12.118	11.577	9.161
AM-2233	10.640	10.323	8.612
CB-25	11.149	10.859	8.884
CB-52	11.367	11.119	9.014
CP 55,940	9.286	8.747	7.486
EAM2201	11.657	11.046	8.950
5-fluoro-AB-PINACA	8.387	8.287	6.950
5-fluoro PB-22	11.422	10.796	8.850
FUB-PB-22	13.621	12.243	9.314
JWH-018 adamantylcarboxamide	10.659	10.430	8.492
JWH-019	10.794	9.851	8.208
JWH-073 6-methoxyindole analog	11.289	10.182	8.552
JWH-122 N(4-pentenyl) analog	10.409	10.084	8.327
JWH-203-3 chlorophenyl isomer	8.856	8.733	7.383
JWH-203-4 chlorophenyl isomer	8.801	8.732	7.307
JWH-250	8.676	8.612	7.239
JWH-302	8.883	8.724	7.353
JWH-201	9.027	8.910	7.532
JWH-398	11.150	10.346	8.505
MAM-2201		10.566	8.598
PB-22	10.469	10.209	8.436
RCS-4-C4 homolog	9.048	8.414	7.045
RCS-8	11.291	10.284	8.452
STS-135	11.476	10.992	8.790
THJ-2201	9.350	9.119	7.702
UR-144	7.401	7.041	5.697
UR-144-N-(5chloropentyl) analog	8.209	8.157	6.782
URB-597	6.826	6.522	5.288
XLR-11	7.603	7.408	6.062
JWH-122		10.286	8.505
RCS-4		8.736	7.546
JWH-073		9.232	7.960
JWH-200		12.028	9.185
JWH-081		10.981	8.792
JWH-210		10.637	8.679
JWH-018		9.559	8.190
JWH-203		8.634	7.242
JWH-022		9.568	8.138
AM-2201		10.010	8.364
HU-211		8.972	7.642
HU-210		8.919	7.616
CP 47,497 C8 HOMOLOG		7.806	6.644
CP 47,497		7.476	6.189
JWH-122		10.021	8.429

Table 3. Average retention time and standard deviation obtained when analyzing synthetic cannabinoid standards on the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT GC columns.

	Rtx®-5	**Rxi®-1ms**	**Rxi®-1HT**
n	38	53	53
Average Retention Time (Minutes)	9.693	9.359	7.744
St. Deviation of Retention Time (Minutes)	1.63	1.367	1.046

Table 4. Average decrease in retention time and standard deviation resulting from the use of different GC columns.

	Rtx®-5 to Rxi®-1ms column	**Rtx®-5 to Rxi®-1HT column**	**Rxi®-1ms to Rxi®-1HT column**
n	37	37	53
Average decrease in retention time (min.)	-0.488	-2.106	-1.614
St. Deviation of decrease in retention time (min.)	0.585	0.758	0.397
Statistically significant difference in retention times (according to T test)	Yes	Yes	Yes

Table 5. Raw retention time data collected to test reproducibility of retention times obtained.

Rxi-1ms column

JWH-018 File Name	R_t	JWH-073 6-methoxyindole analog	R_t	JWH-022	R_t	JWH-073	R_t	CP-47,497-C8 HOMOLOG	R_t	AB-PINACA	R_t	AM-2233	R_t
052815/30.D	9.559	052815/10.D	10.182	060815/34.D	9.568	052915/09.D	9.232	052915/40.D	7.824	052915/21.D	7.984	052910/32.D	10.363
060915/10.D	9.706	052815/13.D	10.204	061615/10.D	9.533	061210/42.D	9.210	060815/38.D	7.806	060815/18.D	7.962	060810/30.D	10.323
061210/26.D	9.520	061210/10.D	10.155	061615/66.D	9.526	061615/22.D	9.123	061015/22.d	7.845	061210/30.D	7.959	061615/42.D	10.284
061615/30.D	9.630	061615/26.D	10.109	061715/14.D	9.517	0611715/18.D	9.115	061210/58.D	7.802	061615/46.D	7.947	061715/26.D	10.269
061716/30.D	9.616	061715/14.D	10.097	061715/46.D	9.517	061715/54.D	9.114	061615/34.D	7.825	061715/34.D	7.939	061715/62.D	10.267
061715/66.D	9.612	061715/50.D	10.091	061815/34.D	9.429	061815/26.D	9.104	061715/22.D	7.819	061715/70.D	7.937	061815/10.D	10.251
061815/22.D	9.485	061815/30.D	10.077	061815/54.D	9.456	061815/58.D	9.166	061715/58.D	7.818	061815/38.D	7.926	061815/46.D	10.249
061815/42.D	9.468	061815/62.D	10.090	061815/82.D	9.428	061815/86.D	9.105	061815/18.D	7.766	061815/90.D	7.923	061815/74.D	10.246
061815/70.D	9.538	061815/78.D	10.074	062315A/014.D	9.376	062315A/018.D	9.122	061815/66.D	7.784	062315A/022.D	7.884	062315A/026.D	10.171
062315A/030.D	9.432	062315A/053.D	10.008	062315A/057.D	9.375	062315A/049.D	9.121	062315A/034.D	7.745	062315A/065.D	7.884	062315A/061.D	10.168
Average	9.557	Average	10.109	Average	9.473	Average	9.141	Average	7.803	Average	7.935	Average	10.259
St. Deviation	0.085	St. Deviation	0.057	St. Deviation	0.069	St. Deviation	0.046	St. Deviation	0.030	St. Deviation	0.032	St. Deviation	0.060

Rxi-1HT column

JWH-018 File Name	R_t	JWH-073 6-methoxyindole analog	R_t	JWH-022	R_t	JWH-073	R_t	CP-47,497-C8 HOMOLOG	R_t	AB-PINACA	R_t	AM-2233	R_t
063015/65.D	8.138	063015/57.D	8.499	063015/53.D	8.090	070615/16.D	7.724	063015/49.D	6.600	063015/45.D	6.712	063015/41.D	8.562
070615/40.D	8.031	070615/20.D	8.410	070615/24.D	8.035	070615/76.D	7.722	070615/28.D	6.487	070615/32.D	6.618	070615/36.D	8.465
070715/30.D	7.978	070715/14.D	8.408	070715/10.D	7.974	070715/18.D	7.691	070715/22.D	6.458	070715/34.D	6.615	070715/26.D	8.463
070815/42.D	7.976	070815/50.D	8.405	070815/66.D	7.973	070815/58.D	7.683	070815/62.D	6.454	070815/46.D	6.613	070815/54.D	8.460
070915/30.D	7.974	070915/34.D	8.404	070915/22.D	7.942	070915/26.D	7.686	070915/10.D	6.453	070915/18.D	6.610	070915/14.D	8.460
071015/10.D	7.974	071015/18.D	8.402	071015/34.D	7.941	071015/26.D	7.683	071015/30.D	6.451	071015/14.D	6.609	071015/22.D	8.457
071015/49.D	7.973	071015/53.D	8.403	071015/31.D	7.940	071015/57.D	7.683	071015/73.D	6.451	071015/65.D	6.609	071015/69.D	8.454
071415/10.D	7.943	071415/18.D	8.371	071415/22.D	7.908	071415/14.D	7.651	071415/26.D	6.423	071415/30.D	6.587	071415/34.D	8.424
071515/18.D	7.931	071515/30.D	8.360	071515/22.D	7.898	071515/26.D	7.642	071515/34.D	6.412	071515/10.D	6.572	071515/14.D	8.415
071515/53.D	7.929	071515/65.D	8.358	071515/57.D	7.898	071515/61.D	7.639	071515/69.D	6.410	071515/49.D	6.570	071515/73.D	8.412
Average	7.985	Average	8.402	Average	7.960	Average	7.680	Average	6.460	Average	6.612	Average	8.457
St. Deviation	0.061	St. Deviation	0.040	St. Deviation	0.062	St. Deviation	0.030	St. Deviation	0.055	St. Deviation	0.040	St. Deviation	0.042

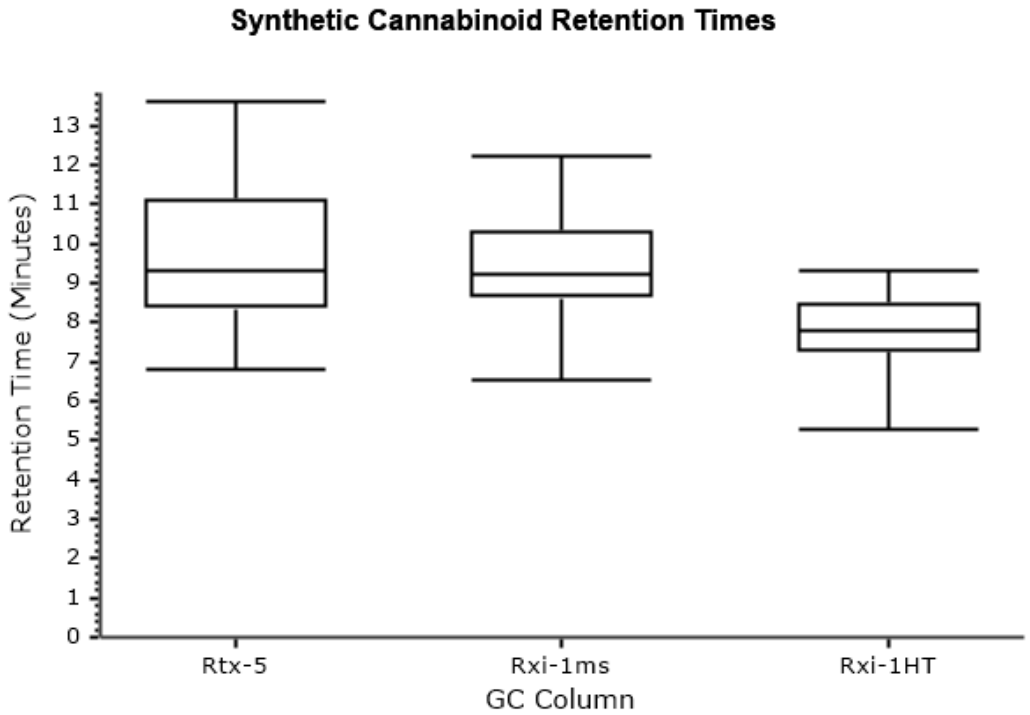

Figure 1. Box and whisker plot depicting the synthetic cannabinoid retention times obtained on the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT GC columns.

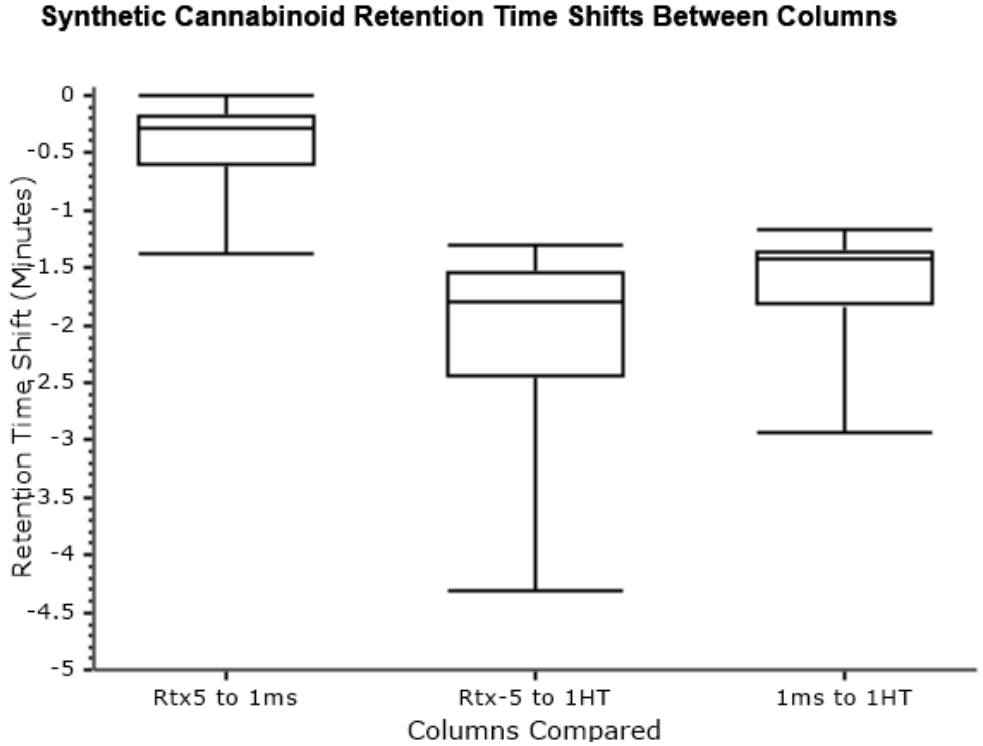

Figure 2. Box and whisker plot depicting the retention time shifts between the GC columns being compared.