Expanded Uncertainty Assessment of GC-MS Chromatographic Peak Area and Retention Time

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ABSTRACT

Gas chromatography-mass spectrometry (GC-MS) is a technique widely utilized for forensic disciplines, including seized drug analysis. Retention times and peak areas are often used to help with the identification of unknowns or quantitative measurements. Therefore, understanding the uncertainty associated with retention time and peak area measurements is essential. This study investigates the uncertainty associated with retention time and peak area measurements of commonly encountered seized drugs over 5 months.

INTRODUCTION

Seized drug analysis commonly utilizes GC-MS as a confirmatory technique to identify unknown casework samples. GC-MS provides retention time and mass spectral information, which can help identify unknown compounds. In addition, chromatographic peak areas are utilized for qualitative and quantitative purposes. Regardless of the measurement, it is important to understand the associated uncertainty of measurement, especially for forensic science casework.

Previous studies have assessed the uncertainty of retention time and mass spectral ion abundances [1,2]. These studies have great importance, as many forensic laboratories utilize retention time or ion abundances for acceptance criteria. Therefore, understanding the uncertainty of retention time or ion abundance measurements can help inform the community about appropriate acceptance criteria. However, there is a lack of research surrounding the uncertainty of peak area measurements.

This longitudinal study explores the uncertainty of measurement associated with retention time and peak area measurements for 16 common seized drugs or metabolites. The factors studied were elution order, instrument, concentration, and usage. Mixtures were analyzed about twice per week for 5 months with an internal standard to evaluate retention time, relative retention time, peak area, and relative peak area uncertainty.

MATERIALS & METHODS

Chemicals & Sample Preparation

The 16 compounds analyzed in this study were dimethyl sulfone, amphetamine, methamphetamine, MDMA, pentylone, PCP, xylazine, cocaine, codeine, 4-ANPP, Δ^9-THC, 6-MAM, heroin, fentanyl, clonazepam, and bromazolam. A basic extraction was performed on amphetamine, methamphetamine, and MDMA to improve peak shape. A low and high concentration mixture of all compounds was prepared in methanol, although the concentration of each compound varied within the mixtures. The internal standard, PCP, was present at the same concentration in both mixtures.

RESULTS & DISCUSSION

Table 1. Peak area (PA) combined expanded uncertainty (U_c) for both concentrations analyzed with each instrument.

	2021 GC-MS		2014 GC-MS		2009 GC-MS	
	Low PA U_c	High PA U_c	Low PA U_c	High PA U_c	Low PA U_c	High PA U_c
Within-day	30.22%	22.72%	28.34%	19.92%	26.56%	18.47%
Within-week	34.92%	27.36%	32.25%	21.81%	28.08%	20.44%
Within-month	56.02%	54.96%	44.75%	30.05%	35.85%	27.51%

- Peak area U_c was generally 18-56%, whereas relative peak area U_c was generally 13-27%.

Table 2. Retention time (RT) combined expanded uncertainty (U_c) for both concentrations analyzed with each instrument.

	2021 GC-MS		2014 GC-MS		2009 GC-MS	
	Low PA U_c	High PA U_c	Low PA U_c	High PA U_c	Low PA U_c	High PA U_c
Within-day	0.03%	0.03%	0.05%	0.07%	0.03%	0.04%
Within-week	0.03%	0.04%	0.06%	0.07%	0.03%	0.04%
Within-month	0.06%	0.06%	0.09%	0.10%	0.05%	0.06%

- Retention time U_c was 0.03-0.10%, and relative retention time U_c was 0.03-0.08%.

Table 3. Within-day combined expanded uncertainty (U_c) by compound for peak area (PA) and relative peak area (RPA) for the 2009 GC-MS.

	Low PA U_c	High PA U_c	Low RPA U_c	High RPA U_c
Dimethyl sulfone	18.96%	14.80%	10.21%	6.12%
Amphetamine	21.67%	18.71%	10.39%	10.49%
Methamphetamine	25.75%	21.40%	16.49%	14.29%
MDMA	32.72%	21.02%	23.32%	11.86%
Pentylone	35.23%	14.32%	25.56%	5.16%
PCP (ISTD)	16.99%	13.73%	-	-
Xylazine	34.40%	14.23%	24.43%	7.62%
Cocaine	22.36%	12.89%	11.23%	6.32%
Codeine	21.51%	16.58%	12.77%	11.70%
4-ANPP	21.77%	14.25%	11.95%	9.54%
Δ^9-THC	19.57%	15.89%	11.22%	11.13%
6-MAM	22.80%	18.62%	15.70%	14.87%
Heroin	17.77%	16.20%	11.98%	11.77%
Fentanyl	42.07%	30.25%	33.54%	24.36%
Clonazepam	31.31%	24.18%	23.98%	19.52%
Bromazolam	30.54%	23.67%	24.69%	19.12%

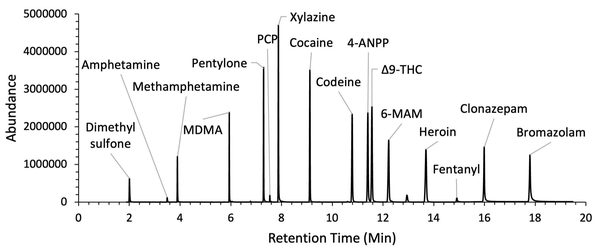

Figure 1. Exemplar TIC for the high concentration Mixture 1 analyzed on the 2009 GC-MS.

- Dimethyl sulfone was added to Mixture 2 to address the confounding variables of low abundance and early elution.

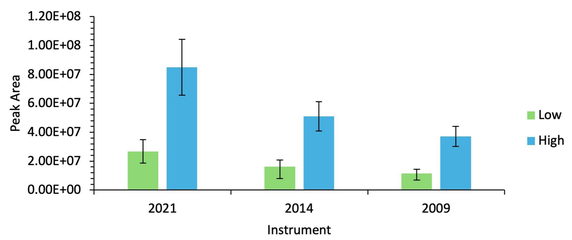

Figure 2. Comparison of the within-day peak areas for each instrument utilized in this study. The error bars represent the U_c.

- A significant difference ($p < 0.001$) was found for peak area measurements between all three instruments, and between both concentrations.

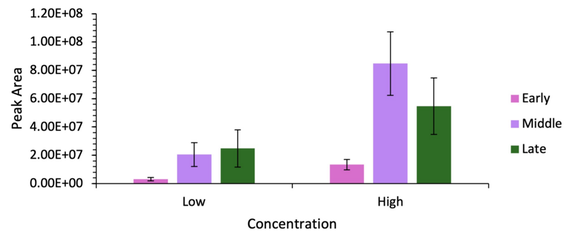

Figure 3. Exemplar within-month peak areas for the 2014 GC-MS, showing the low and high concentration mixtures based on elution order. The error bars represent the U_c.

Table 4. Summary of the ANOVA and η^2 results used to assess the impact of elution order, concentration, instrument, and usage on the peak area (PA), relative peak area (RPA), retention time (RT), and relative retention time (RRT) measurements. Significant differences at the 95% confidence level (i.e., α = 0.05) are bolded and italicized.

	PA		RPA		RT		RT	
	p-value	η^2	*p*-value	η^2	*p*-value	η^2	*p*-value	η^2
Elution order	***<.001***	.205	***<.001***	.330	***<.001***	.834	***<.001***	.835
Concentration	***<.001***	.145	***<.001***	.244	.964	<.001	.959	<.001
Instrument	***<.001***	.068	***<.001***	.018	.465	<.001	.892	<.001
Usage	***<.001***	.042	***<.001***	.007	.042	.003	.046	.003

MATERIALS & METHODS

Instrumentation

Three Agilent GC-MS instruments were utilized for this study: an Agilent 8890-5977B from 2021, an Agilent 7890-5977C from 2014, and an Agilent 6890-5975B from 2009. The temperature program for all instruments was as follows: 100 °C (1 min hold), 15 °C/min ramp to 130 °C, 30 °C/min ramp to 250 °C (1 min hold), 1 °C/min ramp to 255 °C (1 min hold), 30 °C/min ramp to 300 °C (4 min hold), for a total run time of 19.5 minutes. The low and high concentration mixtures were analyzed with 5 replicates per day, ~2 days per week for a total of 5 months.

Data Analysis & Processing

Agilent MSD ChemStation was used for obtaining all retention time and peak area measurements through the ChemStation integrator. Further processing was completed in Microsoft Excel and the SPSS suite for statistical analysis. Expanded uncertainty (U) was defined as 2 * %RSD to approximate a 95% confidence interval. However, to account for within-day, within-week, and within-month uncertainty values, a combined U (U_c) was chosen to best represent the data. U_c was calculated as shown, where U = 2 * %RSD and w = number of injections.

$$U_c = \frac{\Sigma wU}{\Sigma w}$$

CONCLUSIONS

- The use of an internal standard decreased both relative peak area and relative retention time U_c values.
- All four factors (i.e., elution order, instrument, concentration, and usage) had a significant effect ($p < 0.001$) at the 95% confidence level on peak area and relative peak area measurements.
- Elution order had the largest effect size on peak area measurements, at 20.5%.
- Fentanyl had the highest peak area uncertainty out of all compounds.

REFERENCES

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