Optimization and Exploration of Sampling Methods Affecting Ionization Efficiency for Direct Sample Analysis (DSA)

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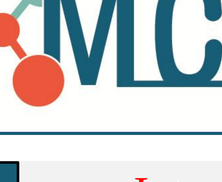
1 Introduction

- DSA is a recent innovation by PerkinElmer that streamlines the way samples are ionized for mass spectrometric analysis while providing rapid, continuous sampling with high throughput .
- With an expanding scope of applications, there is a need to explore and optimize conditions affecting sample ionization using the DSA.
- The purpose of this project is to develop a set of optimal conditions by studying instrumental parameters and environmental variables predicted to affect ionization.

2 Background

- DSA provides high resolution and accurate mass data analysis without chromatographic separation and requires minimal sample preparation.
- There is currently no known set of conditions that are considered to be optimal for running samples using DSA
- Caffeine standards were used to test how uncharged compounds ionize.
- Rhodamine-6G standards were used to test how charged compounds ionize.



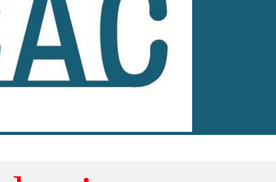


Figure1: Structures of caffeine[1] (left) and rhodamine-6G [2] (right).

3 Sample Preparation

- Standards were obtained from Sigma-Aldrich and Fisher.
- Varying concentrations of the standards were prepared using a 50/50 (v/v) water-methanol solution by serial dilutions.
- Each concentration was optimized based on signal intensity to determine which concentration would be used for experimental trials.

4 Methods

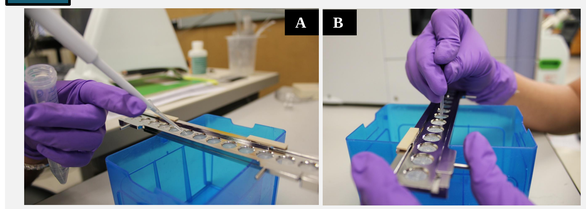
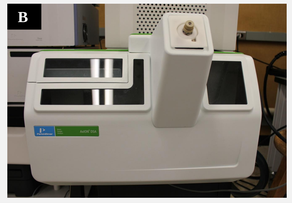


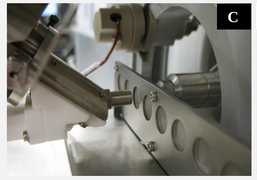
Figure 2: A) Spotting samples onto the sample screen. **B)** Denting the screen with a pipette tip to change its shape.

- The factors tested include: dryness of a sample, distance between the screen and source, and shape of the screen.
- Caffeine and rhodamine-6G standards were applied to ten spots on a sterile sample screen of the DSA and exposed to the ionization source.
- To test dryness, samples were either spotted and immediately run while still wet or allowed to dry completely and then run.
- To test distance, the source was physically moved to 1.0 cm, 1.5 cm, or 2.5 cm from the screen.
- To test the shape of the screen, the screen was pushed in or out with a sterile pipette tip or left flat.

5 Instrumentation

- The DSA source was attached to a PerkinElmer AxION Time-of-Flight (TOF) mass spectrometer.
- The entire source was enclosed to protect the operator from the sample as well, as the sample from contamination.





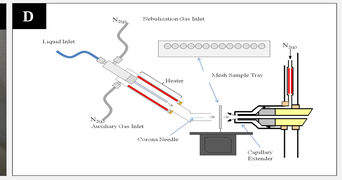


Figure 3: A) TOF spectrometer used to detect caffeine and rhodamine-6G. **B)** DSA source that is attached to the TOF. **C)** Close up view of the DSA sample tray inlet to the AxION TOF. **D)** Schematic of the DSA ionization source.

Table 1: Parameters used with the DSA-TOF setup.

Parameter	DSA-MS for Rhodamine-6G	DSA-MS For Caffeine
Capillary Exit (Volts)	800	800
Low m/z	410	170
High m/z	470	230
Ion Polarity	Positive	Positive
Ion Source Type	DSA	DSA
Needle (Volts)	2100	2200
Endplate (Volts)	-100	-100
APCI Vaporizer Temperature (°C)	300	300
Drying Gas Heater (°C)	25	25
Nebulizer Gas Pressure (PSI)	80	80
Humidity (%)	20-30	50-55

6 Results

Once samples were applied and ionized, a total ion chromatogram (TIC) and mass spectra were obtained from each trial and compared using multiple factors including signal-to-noise (S/N) ratio, relative abundance, and peak area.

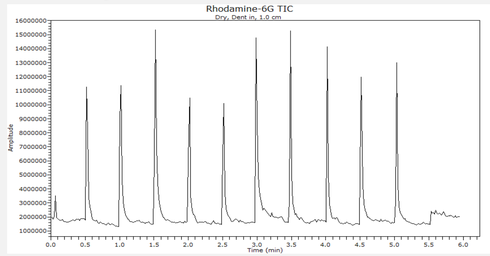


Figure 4: Example of a TIC of a rhodamine-6G trial

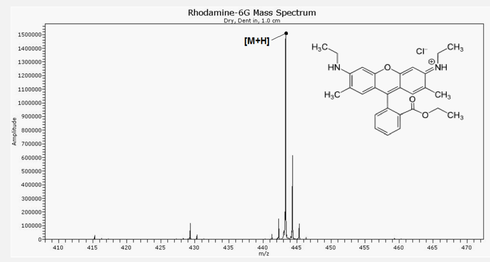


Figure 5: Example of a mass spectrum of a peak of a rhodamine-6G trial.

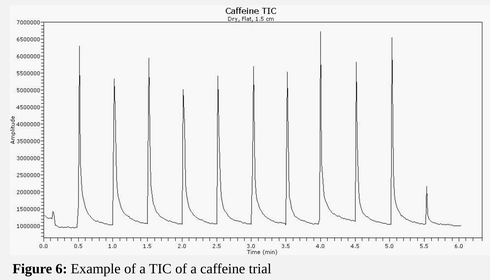


Figure 6: Example of a TIC of a caffeine trial

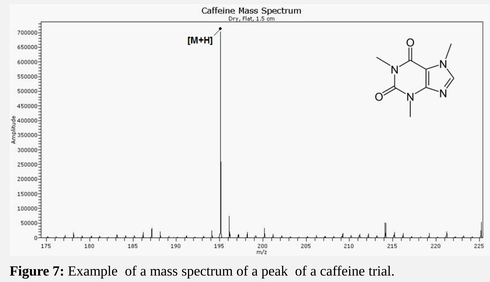


Figure 7: Example of a mass spectrum of a peak of a caffeine trial.

Table 2: Conditions and results for rhodamine-6G trials.

Trial	Concentration (mg/mL)	Wet or Dry	Distance (cm)	Screen Shape	Average TIC Peak Area	Average TIC S/N Ratio
1	0.10	Dry	1.0	Dent in	32345108.11	81.13
2	0.10	Dry	1.0	Dent out	24228977.83	78.55
3	0.10	Dry	1.0	Flat	25228685.48	45.03
4	0.10	Dry	1.5	Dent in	13399472.52	26.46
5	0.10	Dry	1.5	Dent out	15366716.76	24.92
6	0.10	Dry	1.5	Flat	16775385.72	22.17
7	0.10	Dry	2.5	Dent in	17458922.09	9.42
8	0.10	Dry	2.5	Dent out	17095559.37	11.29
9	0.10	Dry	2.5	Flat	14770084.50	9.42

Note: Numbers are not intended to be significant. and are represented as taken from the software..

Table 3: Conditions and results for caffeine trials.

Trial	Concentration (mg/mL)	Wet or Dry	Distance (cm)	Screen Shape	Average TIC Peak Area	Average TIC S/N Ratio
1	0.001	Dry	1.0	Dent in	8224502.61	92.62
2	0.001	Dry	1.0	Dent out	13868566.62	28.42
3	0.001	Dry	1.0	Flat	18959056.63	27.46
4	0.001	Dry	1.5	Dent in	6323332.85	63.64
5	0.001	Dry	1.5	Dent out	7643293.09	42.05
6	0.001	Dry	1.5	Flat	14057162.31	58.19
7	0.001	Dry	2.5	Dent in	3284980.13	21.31
8	0.001	Dry	2.5	Dent out	4914845.59	21.55
9	0.001	Dry	2.5	Flat	5055543.33	17.83

Note: Numbers are not intended to be significant. and are represented as taken from the software..

7 Conclusions

General trends observed are as followed:

- Caffeine and rhodamine-6G standards were detected with high resolution mass accuracy.
- Samples allowed to dry completely showed better peak intensities and S/N ratios compared to wet samples.
- Dried samples applied on a screen stretched away from the source showed higher peak intensities and signal to noise ratios than samples placed on a screen dented away from the source.
- Increasing the current in the needle by increasing the voltages applied resulted in spectrum with higher resolution and signal to noise ratios.

Acknowledgements: This work was funded, in part, by the Molecular Characterization Analysis Complex (MCAC) and PerkinElmer.

Sources: 1. http://upload.wikimedia.org/wikipedia/commons/thumb/5/5e/Caffeine-2D-skeletal.svg/220px-Caffeine-2D-skeletal.svg.png
2. http://upload.wikimedia.org/wikipedia/commons/thumb/d/dd/Rhodamine_6G.svg/200px-Rhodamine_6G.svg.png