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Life in the Fast Lane: A Rapid Screening Method for the SAMHSA Urine and Oral Fluid Drug Lists by RapidFire-MS/MS

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Introduction

The Substance Abuse and Mental Health Services Administration (SAMHSA) is responsible for defining the guidelines for workplace drug testing programs for those employed under federal regulations. Because the guidelines are based on research from leaders in the toxicology field, many other laboratories follow these recommendations. While the landscape of workplace drug testing is changing, there are still many employers who rely on it, leading to numerous samples that make their way to labs for testing. Because of this large specimen volume, there is a need for a rapid and simple screening method that is not tied to the cost of reagents. To minimize cost and to increase efficiency, the compound list defined by the SAMHSA guidelines was screened by RapidFire-MS/MS (RF/TQ).

Agilent RapidFire 400.

Experimental

Two sample matrices were tested, one for each of the SAMHSA guidelines, using the appropriate compound list. Urine and synthetic negative oral fluid (OF) prediluted with extraction buffer were spiked with drug standards from the working stock solution corresponding to matrix type. Each matrix was diluted further prior to injection on the RF400-MS/MS system. For both matrices, an online SPE method with a C18 cartridge was used and samples were reverse-eluted into the mass spectrometer. The total cycle time was about 10.5 seconds sample to sample, and two transitions per compound were monitored via ESI in positive mode.

Experimental

Sample prep was a straightforward dilute and shoot, with differing dilutions for each matrix. Urine required a 200-fold dilution, while the OF samples used a 5-fold dilution, given their lower concentrations.

Cartridge Type	C18, type C	
Injection Volume	10 µL	
Buffer A	Water + 10 mM ammonium formate + 0.1% formic acid	
Buffer B	MeOH	
Buffer C	75:25 MeOH:IPA + 0.1% formic acid	
Wash Solvents	**Aq**: Water **Org**: MeOH	
State Timings	**State** 1 (aspirate) 2 (load/wash) 3 (extra wash) 4 (elute) 5 (reequil)	**Time (ms)** 600 3000 0 5000 1500

Table 1. RF parameters.

The RF/TQ system consisted of a RapidFire 400 front end with a 6495C triple quadrupole mass spectrometer for detection. The RF method is shown in Table 1, with MS source conditions summarized in Table 2.

Gas Temp	290 °C
Gas Flow	14 L/min
Nebulizer Pressure	50 psi
Sheath Gas Temp	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	3500 V
Nozzle Voltage	500 V
RF high	90 V
RF low	60 V
Delta EMV	400 V

Table 2. Agilent JetStream ESI source parameters.

Urine Matrix

Method development work started with the urine drug list. Several cartridges were tested during the development process, with the C18 cartridge showing the best overall results across the compound list. Solvent optimization showed good responses with both ACN and MeOH, but the final elution solvent utilized MeOH due to its cleaner baseline. IPA was added to the mix to help with peak shape and to help minimize carryover.

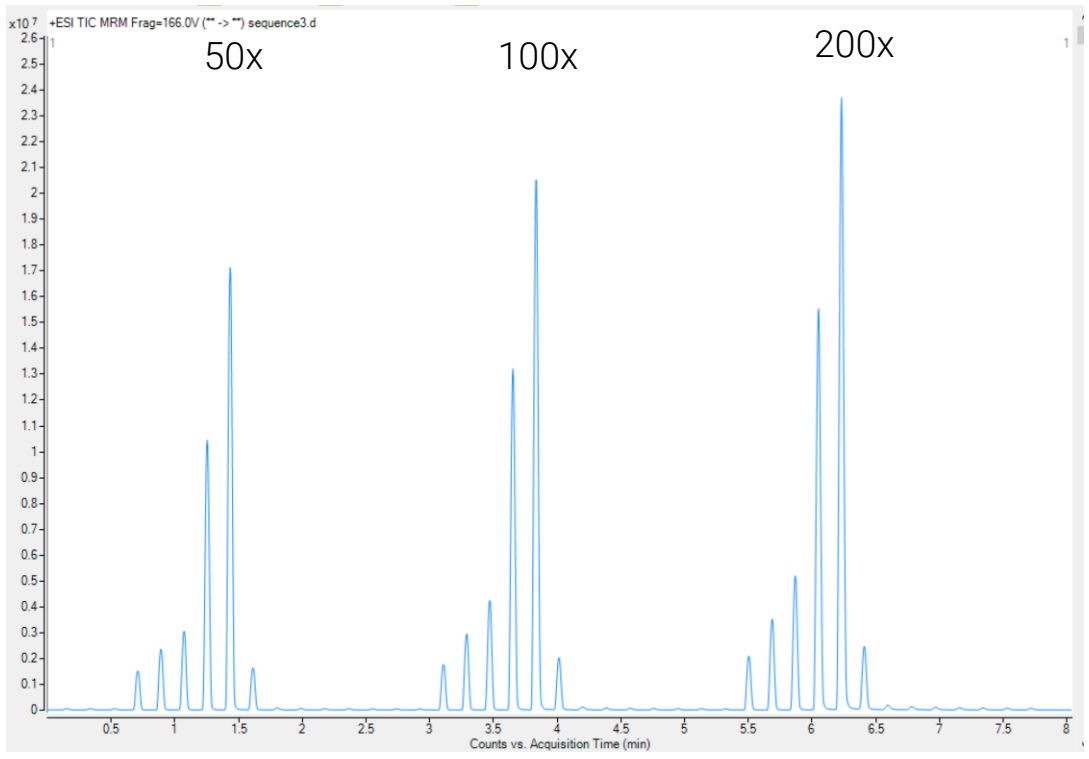

Figure 1. Total ion chromatogram showing the effects of dilution on the response of the analytes. The same curve was diluted three different ways to determine the optimal sample prep for sensitivity using the RF/TQ.

Dilution studies were performed to determine the optimal dilution factor for sensitivity for all compounds. With the varied chemistries represented on the SAMHSA list, as well as the wide range of concentrations required, optimizing the sample prep was critical to prevent saturation of the highly concentrated compounds while still allowing for enough sensitivity for the very low concentration analytes. With the sensitivity of the mass spectrometer, a 200-fold dilution was determined to be optimal for all compounds (Figure 1). This dilution factor has the benefit of minimizing sample volumes required for analysis while also increasing analyte response due to the lower amount of matrix present when loading the sample onto the cartridge.

The calibration concentrations for each analyte were 10% of the screening LOQ to 1000% of the LOQ, which ranged from 1 ng/mL (10% of LOQ of 6MAM) to 5000 ng/mL (1000% of LOQ of amphetamines). Representative curves are shown in Figure 2.

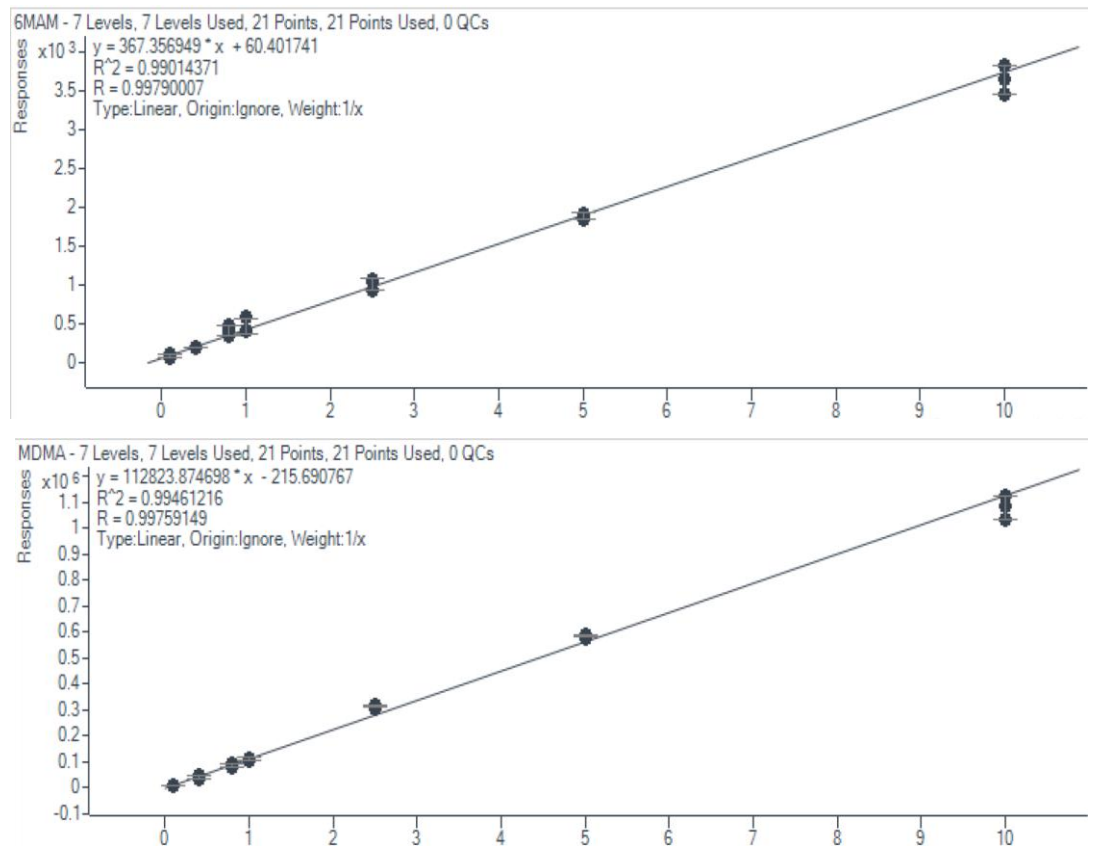

Figure 2. Representative calibration curves in urine. The lowest and highest concentration analytes are shown. Concentrations are shown as percentages, with 1 equaling 100% of LOQ.

Carryover was also assessed as part of the method development process, as shown in Figure 3. High calibrators were injected, followed by matrix blanks, and the carryover was determined to be negligible, due in part to the high dilution factor employed.

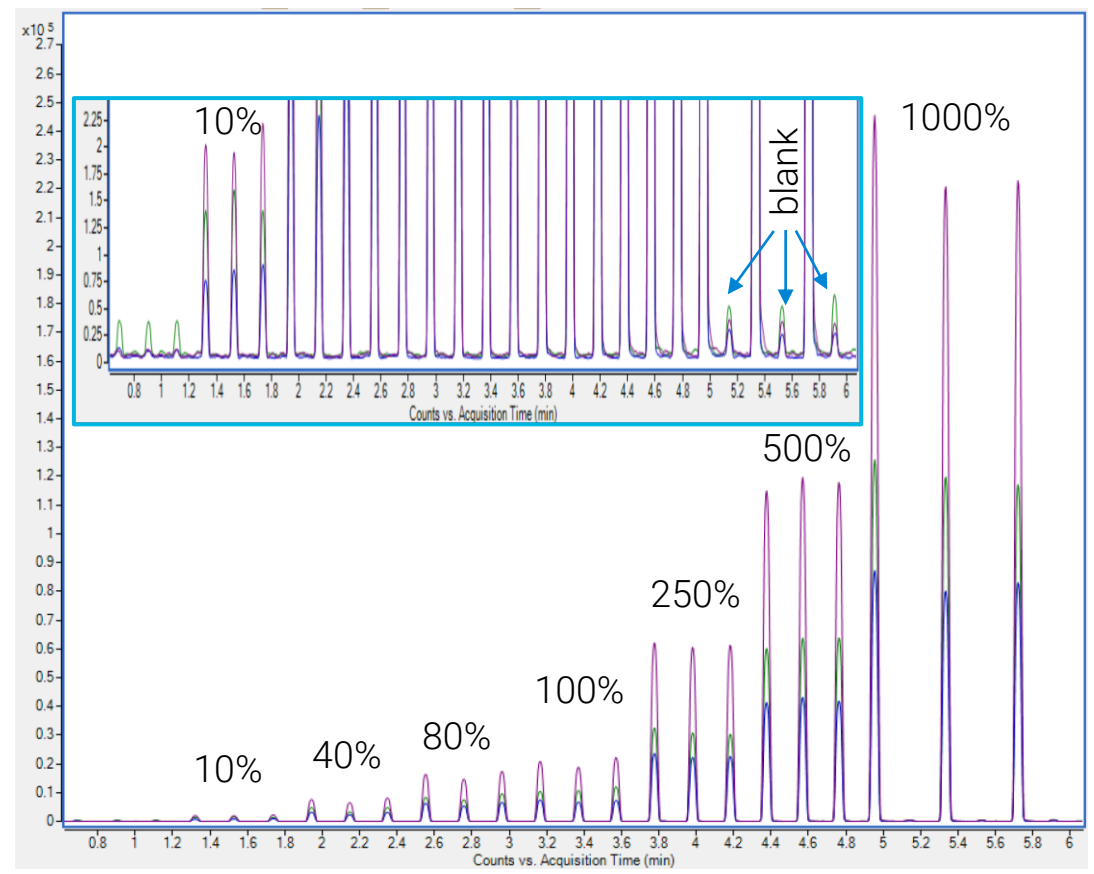

Figure 3. Example of carryover assessment during RapidFire method development. A curve was injected in triplicate, with a matrix blank following every high calibrator. The inset shows the 10% calibration point (first set of true peaks), as compared to the blanks after the highest cal, showing negligible carryover.

Results and Discussion

Oral Fluid Matrix

The primary difference for the OF compounds was the inclusion of parent THC and exclusion of the carboxy metabolite; otherwise, the compounds were identical. Because the analytes were mostly the same, the RF method was also mostly the same. The cartridge and mobile phases matched, as did the MS parameters. The only change made between the urine and OF methods was the elution flow rate on pump 3. To enhance sensitivity with the more complex OF matrix, the flow rate was dropped from the default setting to 0.6 mL/min, as shown in Figure 4.

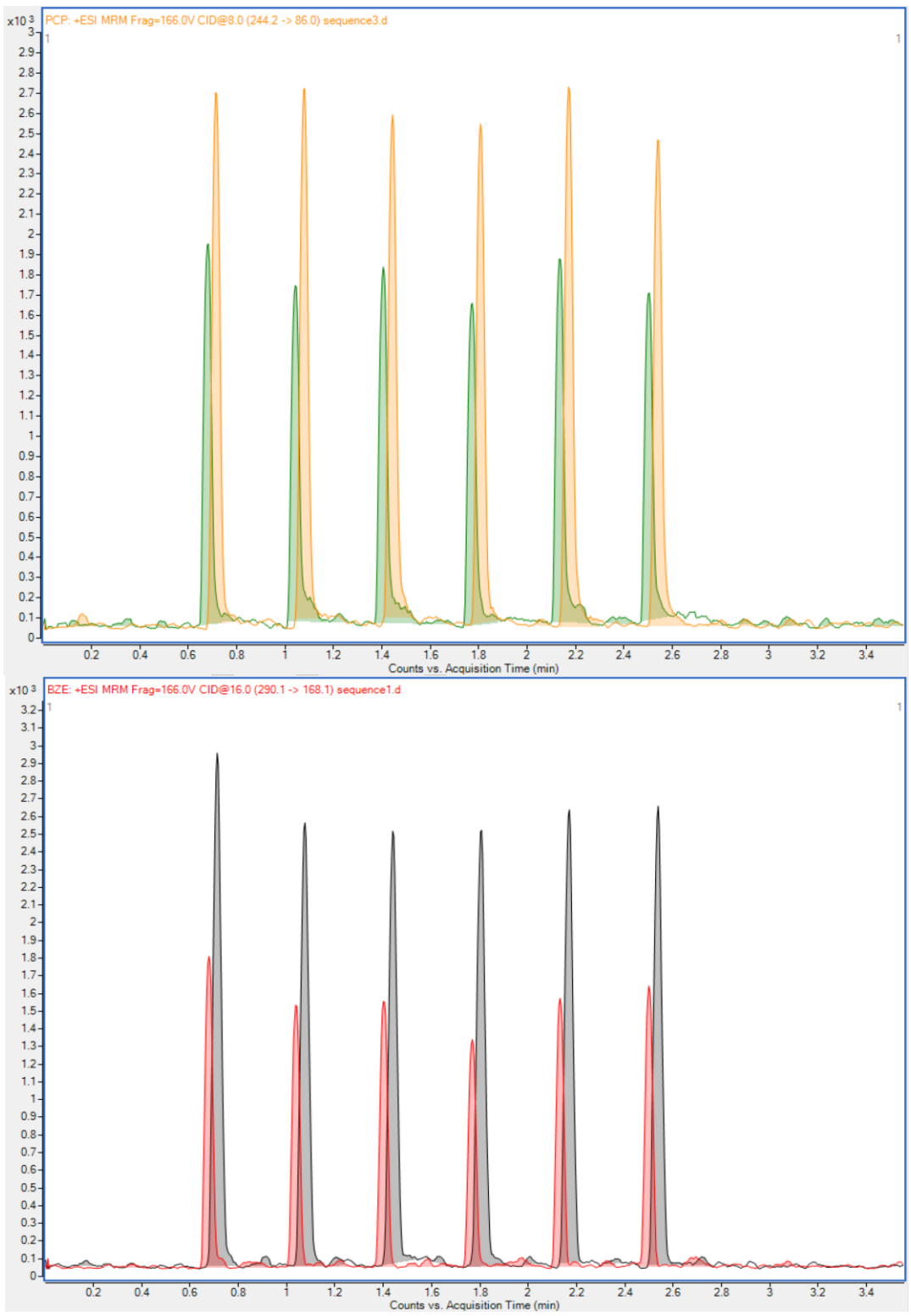

Figure 4. Chromatograms for PCP (top) and benzoylecgonine (bottom) showing signal enhancement when the flow rate is dropped to 0.6 mL/min for the elution pump. The green (top) and red (bottom) peaks show the default flow rate responses, while the orange (top) and black (bottom) peaks show the slower rate.

The OF samples were also tested using a dilute and shoot sample prep workflow in an effort to minimize up-front sample workup. However, given the much lower LOQs required, as well as the more complex matrix, the dilution factor utilized was significantly lower than that for urine samples. Testing revealed that a dilution of 5x was the best option, balancing sensitivity with minimizing matrix complexity and the potential for carryover.

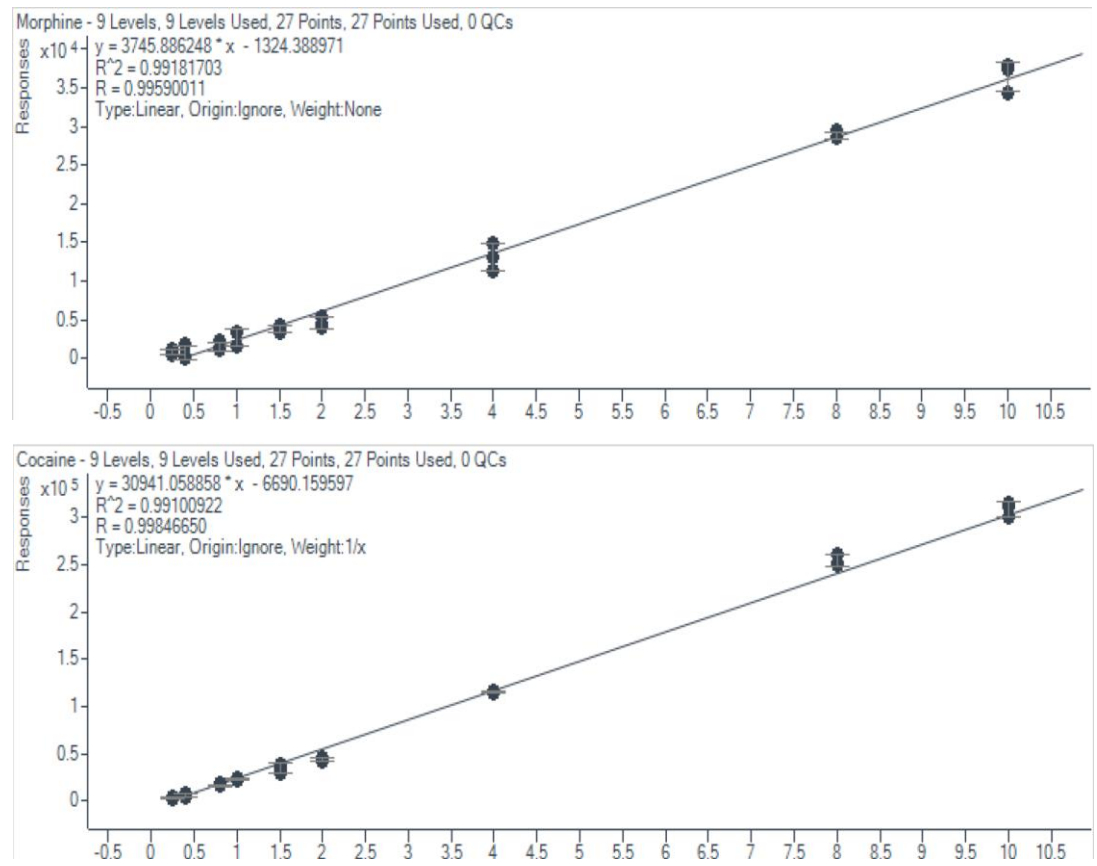

Figure 5. Representative calibration curves in oral fluid. Concentrations are shown as percentages, with 1 equaling 100% of LOQ.

The calibration concentrations for each analyte were 25% of the screening LOQ to 1000% of the LOQ, which ranged from 1 ng/mL in mouth to 500 ng/mL in mouth (examples shown in Figure 5).

Conclusions

A rapid mass spec-based screening workflow for samples tested under the SAMHSA guidelines for federal workplace drug testing programs was developed. This study demonstrated a simple and efficient method for both matrices tested under these guidelines while minimizing cost. Future work will determine if a single method is viable for all compounds at the required analytical levels or if a second injection has to be utilized to capture all compounds at all LOQs.

References

"Mandatory Guidelines for Federal Workplace Drug Testing Programs." Federal Register 88:70814 (October 10, 2023) p. 70814-70850.

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