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Use of ammonium bicarbonate to increase instrument sensitivity to low levels of cannabinoid metabolites in urine

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ABSTRACT

Synthetic cannabis products have been categorized as dangerous mind-altering substances and thus have been deemed illegal for use by military members by the Department of Defense. The need for a validated testing method to be used by the Air Force Drug Testing Laboratory had to be developed in response. Previous research has produced an accurate method of detection using isocratic conditions on an Ultra Performance Liquid Chromatography (UPLC) instrument coupled with a Mass Spectrometer (MS). Initial testing proved that the limit of quantitation (LOQ) for the current method is 4 ng/mL of cannabinoid metabolites. The purpose of continued research on these metabolites is to lower the LOQ through manipulation of testing parameters. Research conducted by Phenomenex Inc. indicated that use of higher pH mobile phases, such as ammonium bicarbonate, in reverse phase HPLC separations produced sharper analyte peak shapes and a significant increase in instrument sensitivity. Additional research presented in March of 2012 indicated that addition of the ammonium bicarbonate post column showed signal enhancement of at least two-fold for each substance tested. Using this information as a foundation, part one of the research aimed to determine the optimal method for introduction of ammonium bicarbonate solutions to the system to maximize the desired effect. Experiments using both addition methods were conducted using a control lacking ammonium bicarbonate. Results reflected that infusion of ammonium bicarbonate solution into the MS post column had the best signal enhancement. From here, part two manipulated the rate of the infusion to find the optimal signal enhancement. Triplicate analysis proved that a flow rate of 20 µL/min increased the analyte signal by 206-428%.

PROJECT OBJECTIVES

The primary objective of this project is to:

1. Determine the best method of addition of ammonium bicarbonate solution
2. Determine flow rate of addition as to maximize sensitivity in UPLC/MS instrument

IMPORTANCE & APPLICATION

The United States Air Force needs the ability to detect airmen violating orders prohibiting use of synthetic cannabis products. Maximizing the sensitivity of the instruments so that even lower levels of cannabinoid metabolites can be detected accurately will ensure that all violators test positive and are handled accordingly. Additionally this will increase the perceived threat of punishment, therefore deterring drug use.

EXPERIMENTAL

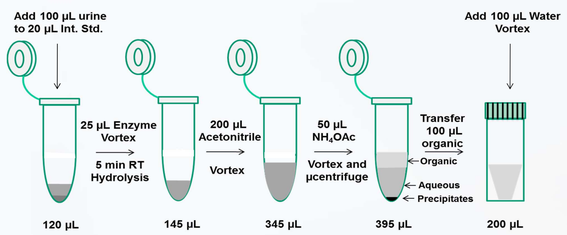


Figure 1. Salting-out Assisted Liquid-Liquid Extraction

Negative urine samples were spiked with a 2000 ng/mL metabolite spiking solution. Solution was serially diluted to give desired 0.5 and 2 ng/mL urine samples. These samples were then processed with the Salting-out Assisted Liquid-Liquid Extraction process detailed in Figure 1.

Extracts were then run in the UPLC/MS under 50/50 isocratic conditions for both parts one and two.

Condition 1:
Mobile Phase A – 24 mmol ammonium bicarbonate in water
Mobile Phase B – Acetonitrile
Method – 50/50 isocratic

Condition 2:
Mobile Phase A – Water/0. 1% formic acid
Mobile Phase B – Acetonitrile/0.1% formic acid
Method – 50/50 isocratic
Infusion specifications – 250 mM ammonium bicarbonate solution, post column infusion at flow rates from 5 – 30 µL/min in increments of 5 µL/min

The four metabolites of interest were detected by monitoring the MRM (multiple reaction monitoring) transitions from the parent compounds to the common daughter ion, the naphthoyl cation (Figure 2).

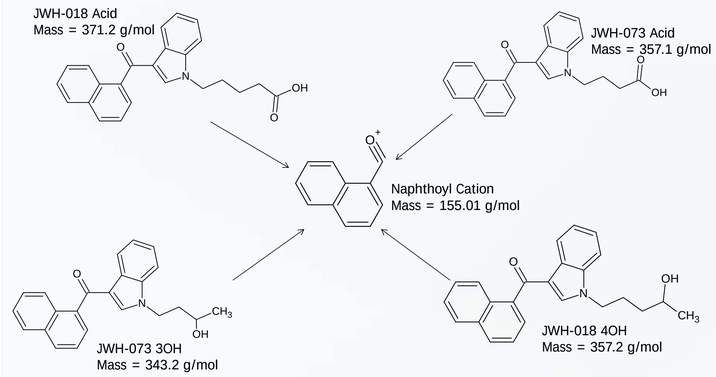
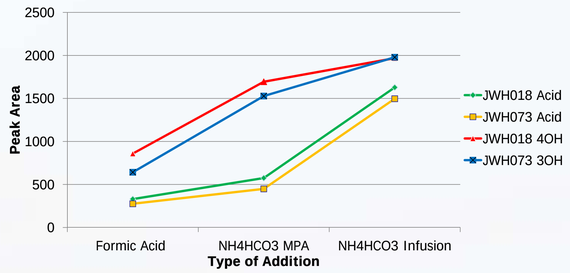


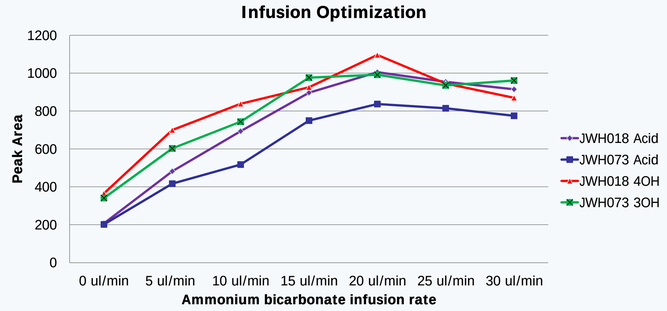
Figure 2. Fragmentation of metabolites to common daughter

RESULTS

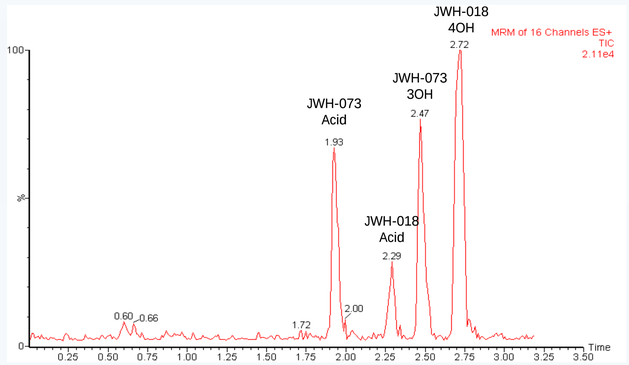


The post column infusion of 250 mM ammonium bicarbonate solution had a much greater effect on the instrument sensitivity increasing peak area two-five fold for each metabolite.

	No additive		NH_4HCO_3 Mobile Phase		NH_4HCO_3 Infusion	
Metabolite	Peak Area	% increase	Peak Area	% increase	Peak Area	% increase
JWH018 Acid	330	0%	575	74%	1628	393%
JWH073 Acid	276	0%	449	63%	1497	442%
JWH018 4OH	859	0%	1694	97%	1968	129%
JWH073 3OH	642	0%	1528	138%	1979	208%



Infusion method was then optimized for flow rate. Flow rate was increased from 5 µL/min to 30 µL/min. Plot of peak areas shown in plot below validates that a flow rate of 20 µL/min maximizes instrument sensitivity.



CONCLUSION

Addition of 250 mM ammonium bicarbonate via post column infusion at a flow rate of 20 µL/min yielded the greatest increase in instrument sensitivity. Resulting chromatograms show superior peak shape and justify lowering the limit of quantitation (LOQ) to 0.5 ng/mL for actual specimen testing.

FUTURE RESEARCH

Further optimization of instrument parameters to increase peak area and instrument sensitivity can be conducted. One such possibility would vary concentration of infusion solution can be altered.

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