Femtogram Sensitivity for Difficult Drug Analytes in Oral Fluids Using Dual Ion-Funnel LC/MS/MS

Peter JW Stone, Agilent Technologies Inc, Santa Clara, CA, USA

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Introduction

Oral Fluid drug analyses offer potential advantages for forensic toxicology and research laboratories over urine and other common matrices. Saliva is relatively clean in comparison, its' collection is non-invasive and importantly the collection process can be witnessed.

The disadvantage of analyzing oral fluid for common prescribed or illicit drugs lies with the analyte concentrations which can be factors of 10-20 times less than the corresponding urine matrix.

Increased ion capture and transmission via dual ion-funnel tandem mass spectrometry was investigated for this purpose since it offers the potential to reach the desired analytical sensitivity levels for measuring drugs in saliva samples.

The results outlined herein are focused on only five of the most difficult drug analytes that were analysed using an established comprehensive LC/MS method which screens and quantitates over 70 analytes with their internal standards in one 6 minute analysis (figure 1.)

We focus only on these five analytes to illustrate the feasibility of ion-funnel tandem MS for oral fluid analyses. Furthermore, minimal oral fluid pre-treatment was undertaken and the results described herein were obtained via direct-injection of neat (filtered) oral fluids.

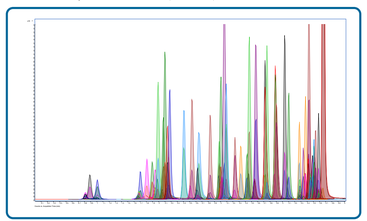


Figure 1. Six-minute comprehensive analysis of >70 drug analytes

Sample Information

Five challenging forensic analytes that respond in positive MS polarity were spiked at various concentrations into synthetic oral fluid solution obtained from immunalysis. (morphine, 6-monacetyl morphine, buprenorphine, norbuprenorphine, and THC.) These were filtered using 3KDa centrifuge eppendorf filters and the supernatent transferred to injection vials.

Instrument Parameters

HPLC Parameters
Agilent 1290 HPLC binary pump, well plate sampler with thermostat, temperature-controlled column thermostat

Parameter	Value
Column	Zorbax Poroshell EC-C18, 2.1 x 100mm, 2.7µm
Column Temp	55°C
Injection Volume	1µl
Autosampler	4°C
Needle Wash	Flushport, 5 seconds
Mobile Phase A	NH_4OH + Formic Acid in H_2O
Mobile Phase B	Formic Acid in Acetonitrile
Flow Rate	0.5 ml/min

Table 1. LC parameters

Mass Spectrometer Parameters
Agilent 6490 QqQ Mass Spectrometer

Ion Source Conditions	
Ion Mode	ESI +
Capillary Voltage	3000 V
Drying Gas (N_2)	14 L/min
Drying Gas Temp	250°C
Nebulizer Gas (N_2)	45 psi
Sheath Gas Tempreature	380°C
Sheath Gas Flow	11 L/min
Nozzle Voltage	0V
Δ EMV	0 V
Dwell Time	dynamic

Table 2. Mass spectrometer parameters

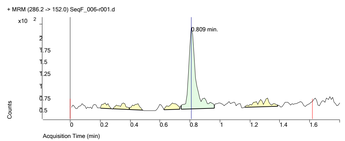
Dynamic MRM
Dynamic MRM allows a mass spectrometer to acquire select MRM data during a specified retention time window, decreasing the number of ion transitions monitored simultaneously. Cycle time is consistent to keep an even distribution of data points and ensure accurate quantitation.

Parameter	Value
Cycle Time	330 ms
Total MRMs	174
Max Concurrent MRMs	31
Retention Time Window	30 sec
Min/Max Dwell Time	7.5/161.5 ms
Q1/Q2 Resolution	0.3 amu

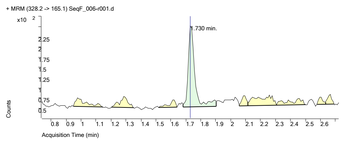
Table 3. Dynamic MRM parameters

Results and Discussion - Chromatography

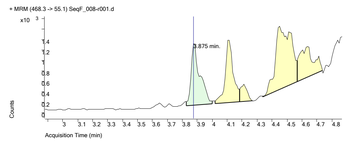
(A) Morphine (50 fg on-column)



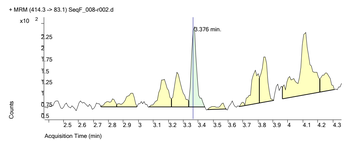
(B) 6-monoacetyl morphine (50 fg on-column)



(C) Buprenorphine (500 fg on-column)



(D) Norbuprenorphine (500 fg on-column)



(E) Delta9-THC (500 fg on-column)

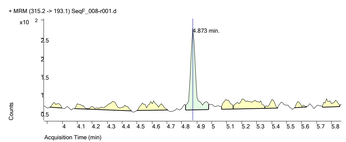


Figure 2. LOQ chromatograms for each analyte (A-E.)

The individual extracted ion chromatograms (EIC) for each analyte at their respective observed LOQs are displayed and outlined in figure 2 (A-E.)

Furthermore, the overlaid EICs at a concentration of 5 ng/ml are illustrated in figure 3 and show excellent chromatographic separation and peak shape and signal/noise.

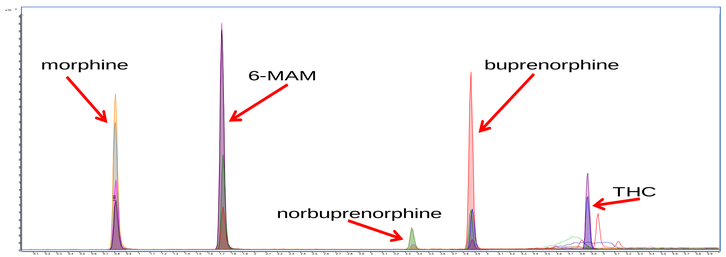


Figure 3. Overlaid EICs of each analyte at a concentration of 5 ng/ml (actual).

Results and Discussion

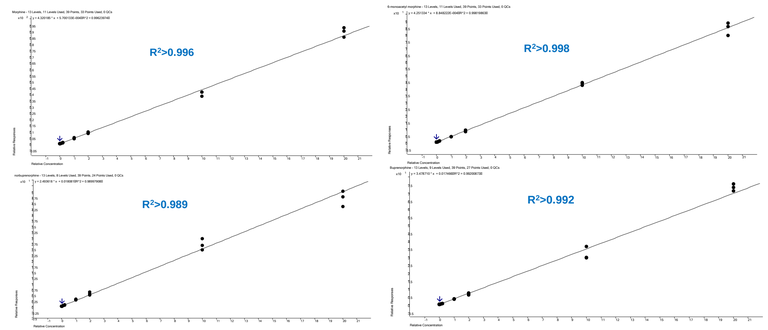


Figure 4. Calibration curves for (a) morphine (b) 6-monoacetyl morphine (c) norbuprenorphine and (d) buprenorphine.

The dual ion-funnel QQQ mass spectrometer yields on-column lower limits of quantitation values of 50 femtogram for morphine and 6-monoacetyl morphine with directly injected filtered saliva samples. THC, buprenorphine and norbuprenorphine each exhibited a lower limit of quantitation of 500 femtogram on-column with direct saliva injection. The lower limit of quantitation was defined as the secondary qualifier ion transition having at least a 5:1 signal to noise ratio, RMSx3. Complete source optimization inclusive of temperatures, gas flows and voltage potentials together with optimal radio frequency values for low and high vacuum ion funnels were optimised for this panel.

Linear ranges of quantitation were observed over 4 orders of magnitude for all analytes in this study and each with correlation coefficients of higher than 0.9700 over the linear range. Precision data for each analyte was typically below 6% for each analyte over 5 batches tested.

Analyte	LOQ (S/N<10)	Linearity	Average %RSD at LOQ (N=5)
morphine	50 fg/on-column	R2>0.996	6
6-MAM	50 fg/on-column	R2>0.998	5
norbuprenorphine	500 fg/on-column	R2>0.989	6
buprenorphine	500 fg/on-column	R2>0.992	5
Delta9-THC	500 fg/on-column	R2>0.916	10

Table 4. Executive summary of LOQ results for each analyte.

Conclusions

- Synthetic spiked saliva was filtered using 3KDa centrifuge and the supernatant injected directly on to the column;
- Optimal source and MRM conditions were determined to obtain the most sensitive results prior to analysis;
- Femtogram on-column sensitivity levels were obtained for the most difficult responding analytes in a typical drug screen;
- Dynamic MRM methodology allowed simultaneous analysis of over 70x drug analytes in a relatively fast 6 minute method;
- Linearity coeficients of above 0.9700 were obtained for each analyte over four orders of magnitude;
- Precision data was typically below 6% RSD, except THC which was averaged at 10% over 5x batches.