High Throughput Screening and confirmation of 41 Pain Panel Drugs in Oral Fluid by an Integrated On-Line Extraction UHPLC-MS/MS System

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Introduction

Saliva test is one of the easiest, cost-effective and most accurate ways to measure the presence of drugs in the body. Collecting saliva sample is relatively non- invasive, easier to procure and reduced risk of sample adulteration. However, saliva matrix display much lower levels of drug compounds compared to urine samples, making the need to test at lower cut-off levels more important. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a technique of choice for both screening and confirmation lower levels because it is sensitive, specific, and accurate.

Solid Phase Extraction (SPE) is widely used for sample clean up before LC-MS/MS analysis. It is costly and time consuming. Here we present a high throughput, cost effective and sensitive procedure for screening and confirmation of Pain Panel Drugs (PPDs) in Synthetic Saliva using Thomson filter vial for sample preparation and using an integrated On-Line Extraction (OLE)-UHPLC-MS/MS System for sample analysis. The lower limit of quantitation (LLOQ) was 0.01-0.2 ng/mL and upper limit of quantitation (ULOQ) was 100 ng/mL. The linearity regression coefficient R^2 was >0.99. The blanks show no interference of the analysis at the LLOQ level. The sub ng/mL level PPDs detection with about three orders of dynamic detection range will cover the clinical research needs.

Fig. 1 EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker integrated On-Line Extraction-UHPLC and CTC Autosampler

Sample Preparation

- Transfer 200 µL of 60% Methanol/water containing 5 ppb internal standard into Thomson vial.
- Add 200 µL of drug standard in synthetic saliva (Immunalysis Corp., p/n NOFC-0500) to the vial and mix.
- Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials-eXtreme/FV 0.2 um PVDF, w/Pre-Slit Red Cap (p/n #85531)
- Press filter plunger down approximately ¼ of the way into each of the Thomson Vial outer shells.
- Vortex for 10 sec
- Press Filter plunger the rest of the way down using Thomson Vial Filter Press.

Methods

Instruments:

EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker Integrated On-Line Extraction-UHPLC and CTC Autosampler (see Fig. 1)

LC Parameters:

Trap Column: YMC-Pack Pro ODS-AQ, 3 µm, 10 mm x 3.0 mm I.D.
Mobile Phase C: 0.1%formic acid (FA), 0.05% TFA in water
Equilibration flow: 600µL (3.0 min)
Loading Flow:600 µL
Analytical Column: YMC-Triart pfp, 1.9 µm, 50mm × 2.0 mm (I.D.)
Column Temperature: 40 °C
Injection Volume: 30 µL
Mobile Phase A: 0.1% FA in water
Mobile Phase B: 2 mM Ammonium formate and 0.1% FA in MeOH/Acetonitril=50/50

Gradient:

Time	%A	%B	Flow (µL/min)
0.0	80	20	350
0.2	80	20	350
3.5	5	95	350
3.9	5	95	350
4.0	80	20	350
6.0	80	20	350

MS Parameters:

Spray Voltage (ESI positive): 4000 v
Cone Gas Flow: 30 units
Cone Temperature: 350 °C
Heated Probe Gas Flow: 40 units
Heated Probe Temperature: 400 °C
Nebulizer Gas Flow: 65 units
Exhaust Gas: on
q2 pressure: 2.0 mTorr (Argon)

Name	Linear Range (ng/mL)	R^2	Response Factor % RSD	Name	Linear Range (ng/mL)	R^2	Response Factor % RSD
6-MAM	0.02-100	0.999	13.3	Meprobamate	0.05-100	0.998	9.1
Alprazolam	0.01-100	1.000	3.5	Methadone	0.01-100	1.000	4.7
Amphetamine	0.02-100	0.999	7.2	Methamphetamine	0.10-100	1.000	8.0
Benzoylecgonine	0.02-100	1.000	10.3	Midazolam	0.01-100	0.999	10.0
Buprenorphine	0.02-100	0.999	8.0	Morphine	0.02-100	1.000	5.0
Carisoprodol	0.05-100	0.999	9.0	Naloxone	0.02-100	0.999	11.2
Clonazepam	0.05-100	1.000	5.7	Naltrexone	0.02-100	1.000	11.0
Codeine	0.02-100	1.000	6.6	Norbuprenorphine	0.20-100	1.000	3.6
Diazepam	0.02-100	0.998	8.1	Nordiazepam	0.02-100	1.000	9.1
EDDP	0.01-100	0.997	6.5	Norfentanyl	0.01-100	1.000	6.1
Fentanyl	0.01-100	1.000	5.0	Normeperidine	0.05-100	0.999	5.8
Flunitrazepam	0.02-100	1.000	5.8	Norpropoxyphene	0.02-100	0.999	8.7
Flurazepam	0.01-100	1.000	2.0	Oxazepam	0.02-100	1.000	12.6
Hydrocodone	0.02-100	0.997	6.3	Oxycodone	0.02-100	0.996	13.8
Hydromorphone	0.02-100	1.000	4.9	Oxymorphone	0.01-100	1.000	4.4
Hydroxyalprazolam	0.02-100	1.000	4.3	PCP	0.01-100	1.000	7.4
Lorazepam	0.10-100	1.000	14.6	Propoxyphene	0.01-100	0.999	4.9
MDA	0.02-100	0.996	9.9	Sufentanil	0.01-100	0.998	9.1
MDEA	0.05-100	0.998	14.4	Temazepam	0.01-100	1.000	6.1
MDMA	0.02-100	1.000	4.3	Tramadol	0.01-100	1.000	6.2
Meperidine	0.02-100	1.000	2.9				

Table 1. 6MAM-d_6, Alprazolam-d_5, Buprenorphine-d_4, Clonazepam-D_4, Codeine-d_6, Fentanyl-d_5, Meperidine-d_4, Methadone-d_3, Morphine-d_6, Norbuprenorphine-d_3, Norfentanyl-d_5, Oxymorphone-d_3, Tramadol ^{13}C-d_3 were used as internal standard for above data.

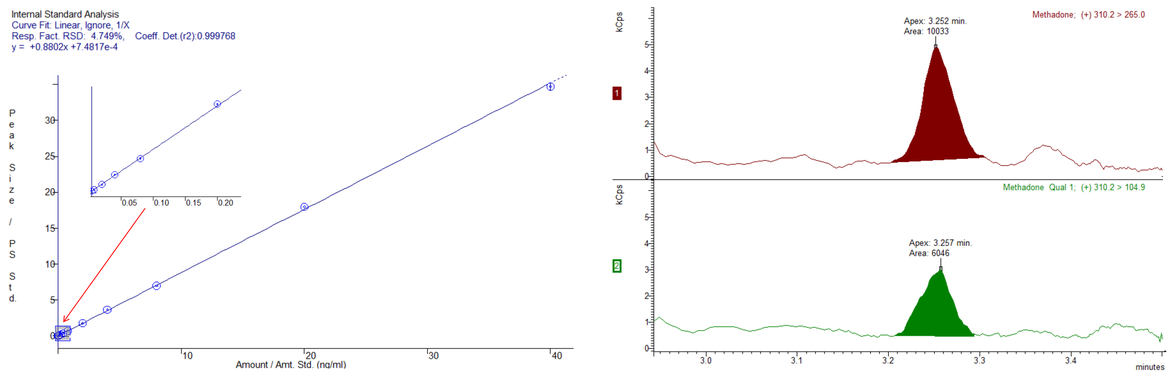

Fig. 2. The curve on the left was plotted as response ratio vs concentration ratio of Methadone/ Methadone-d_3 (Concentration 0.01-100 ng/mL with 2.5ng/mL IS). The chromatograms on the right was 0.01 ng/mL Methadone in Synthetic Saliva.

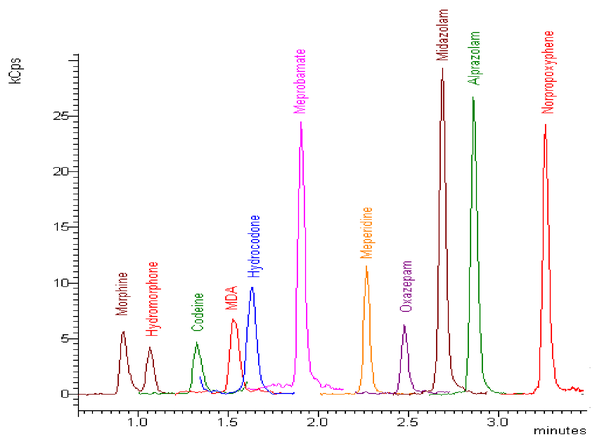

Fig. 3. Selected chromatograms at 0.2 ng/mL PPDs in Synthetic Saliva.

Results & Discussion

The sample preparation time was less than a minute by transferring saliva sample to filter vial and diluting with same volume of 60% methanol/water containing internal standard (IS) followed by mixing and press filtering.

Forty one pain drugs were evaluated. Two MRM transitions were used for each compound. The first peak and last peak were eluted at 0.9 minutes and 3.3 minutes, respectively. Thirteen isotope labeled drugs were used as IS that had retention time spreading from 0.9 minutes to 3.27 minutes. The total method run time was 8.5 min including re-equilibration. The time for the entire procedure was less than 10 minutes.

Conclusions

- **Simple** (diluted, filter and shoot), **Fast** (less than 10 min) and **Sensitive** (LOQ at 0.01-0.2 ng/mL)
- Bruker LC/MS/MS coupled with integrated On-Line Extraction-UHPLC is a system of choice for high throughput PPDs analysis for clinical research needs.