Confirmation of multiple drugs in oral fluid using LC-MS/MS

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Abstract

• In 2011 the Drug Testing Advisory Board recommended to the Substance Abuse and Mental Health Service Administration (SAMHSA) that oral fluid be allowed as a specimen in the Federal workplace drug-testing program.

• The Board had previously considered oral fluid, but had determined it was not an allowable matrix due to specific disadvantages such as inadequate or unknown volume for analysis, drug absorption to collection pads, and low drug concentrations.

• The new recommendation is recognition that technology has largely overcome these problems.

• It was further recommended that the synthetic opioids oxycodone and hydrocodone be added to the test panel.

Objectives

• To develop and validate a simple rapid method to allow the simultaneous analysis of amphetamines, PCP, opioids, cocaine and benzoylecgonine.

•To provide reliable quantitative results with the use of appropriate internal standards while limiting the amount of sample utilized for analysis, a distinct advantage in oral fluid testing.

• To perform a single solid-phase extraction to encompass all the drugs in the SAMHSA panel, except THC, and analyze extracts using LC-MS/MS (Agilent Technologies 6430).

Oral Fluid

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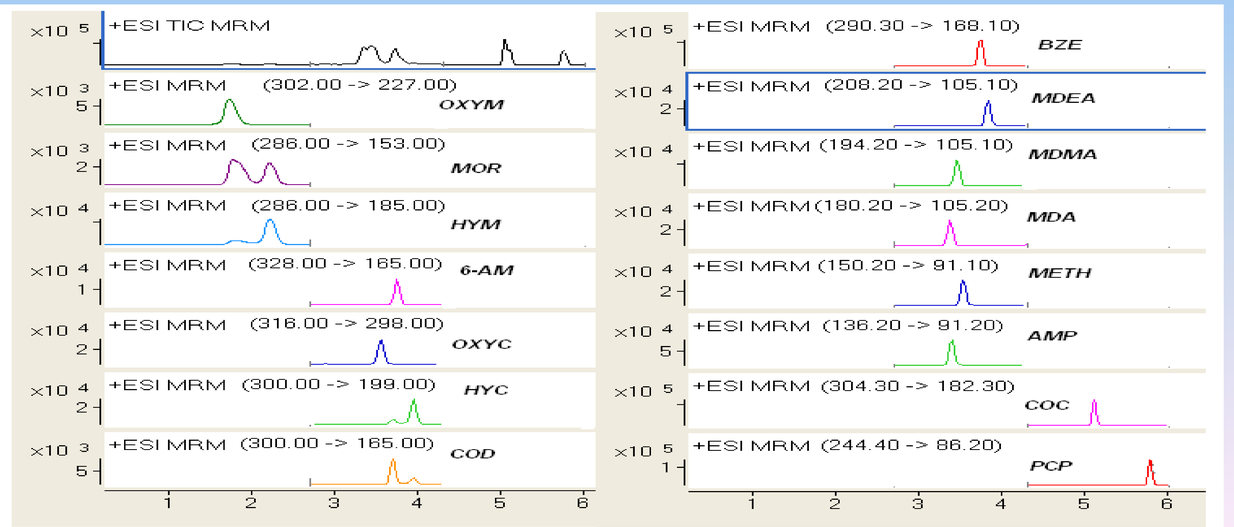
Methods

• Oral fluid samples collected with the Quantisal™ collection device are routinely received in our laboratory.
• Screened by immunoassay with positives carried on to confirmation
• Calibration range: 1 - 200ng/mL encompassed the SAMHSA proposed cut-off concentrations
• 1mL of sample (750µl buffer + 250µl oral fluid) + deuterated internal standards added; buffered to pH 6.0
• Clin II (SPEWare) SPE columns conditioned: methanol (2mL); 0.1M phosphate buffer (pH 6.0; 2mL)
• Samples loaded and washed: DI water (2mL); 0.1M HCl (2mL); methanol (1mL); ethyl acetate (1mL).
• Dried under nitrogen (5 min)
• Analytes eluted: methylene chloride: methanol: ammonium hydroxide (78:20:2 v/v; 2mL)
• Eluent evaporated; reconstituted: 20mM ammonium formate (pH 6.4); methanol (50:50; 50µL)
• Mobile phase: 85% ammonium formate pH 6.4 (A) : 15% methanol (B); after 6 min 80%B
• Constant flow rate: 0.7mL/min; Stop time: 6min; Post time: 3min
• Two transitions were monitored for each analyte at optimized fragmentation voltage and collision energies

20 ng/mL Extracted Calibrator



Transitions

Analyte	*Precursor*	*Quant*	*Qualifier*
OXYM	302	227	198
MOR	286	153	165
HYM	286	185	157
MDA	180	105	135
AMP	136	91	65
MDMA	194	105	135
METH	150	91	65
OXYC	316	298	241
COD	300	165	215
6-AM	328	165	211
BE	290	168	105
MDEA	208	105	135
HYC	300	199	165
COC	304	182	82
PCP	244	86	91

Results

• The method was optimized using calibrators which covered the concentration range proposed by SAMHSA

• A low volume of oral fluid was used for extraction of multiple drugs

•The procedure was validated according to standard protocols and applied to routine specimens

Conclusion

• A simple extraction and analysis procedure has been developed for the SAMHSA proposed drugs of abuse in oral fluid with the exception of THC

• Consolidation of methods can circumvent the limitations of sample volume; and lower laboratory costs; as well as extending the life of the instruments and columns