The use of LC/MS/MS has become an important but routine tool in the screening and confirmation of drugs in biological samples. LC/MS/MS offers excellent specificity and sensitivity with limited sample preparation for the detection and analysis of the majority of drugs. The method utilizes dynamic MRM in order to dramatically reduce MS cycle times for complex multi-analyte assays while increasing the detection capability of the method. The fast polarity switching capabilities of the 6400 were required along with the robustness, high speed and high resolution separations of the Poroshell 120 columns.

Therefore in this case, LC/MS/MS analytical methods and evaluated various columns and solvent combinations in order to demonstrate the chromatographic separation, detection and quantification of compound classes in one method. The compound classes included opiates and opioids, benzodiazepines, anti-depressants, tricyclic antidepressants, barbiturates, cannabinoids, anti-psychotics, anti-convulsants, amphetamines and stimulants, hallucinogens, sedatives. The sample preparation choices were kept simple and included dilute and stored urine for oral fluid and protein crash for blood and the methodologies were developed on an Agilent 1200 LC and 6460 Time of Flight Mass Spectrometer. The method was a 10 minute analytical gradient in positive and negative ionization modes in order to quantify a minimum of 250 drugs and their metabolites along with 180 of their corresponding internal standards.

**Experimental**

**Reagents, Standards, Calibrators and Controls**

**Standard/Calibrators**

- **Sample Preparation - Urine**: 200 µL of urine sample, calibrators, controls was taken and 10 µL of IST at 1000 ng/mL were added to each followed by 40 µL IMCSzyme hydrolysis enzyme, 50 µL IMCSzyme buffer and mix gently the heat for 30 minutes at 55°C.
- **Sample Preparation - Oral Fluid**: 100 µL of oral fluid sample (25 µL of oral fluid and 75 µL of buffer), calibrators controls was taken and 2.5 µL of IST at 1000 ng/mL were added to each tube and vortexed briefly.
- **Sample Preparation - Blood**: 100 µL of blood sample, control and 10 µL of IST at 1000 ng/mL were added to each tube and vortexed briefly.
- **Sample Preparation - Drug-free**: 200 µL of 0.2M Zinc sulphate solution was added to each tube and vortexed for 1 min prior to centrifugation for 10 minutes at 13000 rpm.
- **Sample Preparation - Drug-free**: The supernatant was transferred to an MS vial.

**Method**

**HPLC Conditions**

- **Agilent 1260 Infinity HPLC series binary pump, well plate, thermostatted column compartment**
- **Column**: Agilent Technologies Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm
- **Column Temperature**: 55°C
- **Injection Volume**: 10 µL (Urine, Oral Fluid), 5 µL (Blood)
- **Autosampler Temperature**: 4°C
- **Needle Wash**: Flush (50% Methanol:50%Water) 10 seconds
- **Mobile Phase A**: 0.01% Formic Acid + 5mM Ammonium Formate in Water
- **Mobile Phase B**: 0.01% Formic Acid in Methanol
- **Flow Rate**: 0.5 mL/min
- **Gradient**: 0 min: 95 A:5 B, 0 min: 90 A:10 B, 5 min: 95 A:5 B
- **Run/Stop time**: 10 min/3 min

**Results and Discussion**

**Precision/ Specificity**

The intra-assay precision was determined over 5 days with LOD and LOQ being determined as 3.1 and 10.1 of signal to noise respectively where the mean and the coefficient of determination (R²) for each calibration point were all <10%.

**Accuracy**

The accuracy was determined by the analysis of the In-house control material as the percentage deviation from the target mean and the results were <10% for all levels in each matrix. Therefore, the analytical method in can achieve the required levels for the analysis of a comprehensive and extended drug panel in Urine, Oral Fluid, and Blood.

**Conclusions**

- Baseline separation of the majority of the drugs in 10minutes with good LOD/LOQ in positive mode was achieved in three different matrix types.
- Simple sample preparation in three matrices (Urine, Oral Fluid and Blood) achieved desirable LOD/LOQ but Oral Fluid and Blood may require basic sample cleanup to achieve lower sensitivity and to avert the effect of the blue buffer contained in the oral fluid collection device and endogenous interferences.
- Excellent linearity of calibration curves with acceptable accuracy, precision and reproducibility in positive mode was achieved in all matrices <10% for CV.
- Further evaluate different sample preparation techniques to determine which gives the best results while maintaining low cost and ease of use.
- DMRM allowed for fast MS cycle time while not diminishing sensitivity and the next step is to extend the amount of drugs to over 300 and to evaluate faster run time for large multi-analyte applications.