Introduction

Pregnant woman with a history of drug abuse often deny or report only minimally the true extent of their drug exposure. The rapid analysis of a broad-spectrum of drugs in neonatal meconium (Fig. 1) - a complex, heterogeneous composition consisting of e.g. water, epithelial cells, bile acids, enzymes, sugars, lipids and proteins [1] - has a significant impact on the prompt diagnosis (e.g. neonatal abstinence syndrome - NAS) and to facilitate treatments.

Our aim was to develop a rapid drug screening method for the identification of toxicologically relevant substances in human meconium based on automated on-line extraction, HPLC, QqTOF and software assisted library search (XLC-QqTOF).

Meconium specimens (n=35) were sent from delivery hospitals from infants of mothers who were suspected (or to be known) to be drug consumers and analyzed via XLC-QqTOF.

Sample Preparation

Meconium (250 mg +/-10%) was weighed into a 1.5-ml plastic cup. Four glass balls and internal standard buffer solution (containing: diazepam-d5, 17-nor-17-decarboxy-THC-d3, benzoylcegonine-d3, EDDP-d3, ecgonimethylester-d3, MDMA-d5, morphine-d3, paracetamol-d4, trimipramine-d2) were added. The mixture was vortexed (5 min) and centrifuged for 5 min at ~16.000 g. The supernatant was transferred into a 1.5-ml plastic cup and evaporated to a volume of approx. 100 µL/N in the TurboVap. After adding 0.2 ml acetate buffer (pH=6), the mixture was hydrolyzed with beta-glucuronidase (50 µL) at 60°C for 15 min. A volume of 0.1 ml was diluted with 1 ml acetate buffer (pH=6) prior to analysis.

Analytical Method (XLC-QqTOF)

Online-SPE (X):

- **Symbiosis PicoTOX Analyzer** (Shap Holland)
- **SPE solvent:** Oasis WCX 10×1 mm (Waters) /sample
- **SPE solvents:** NH4Ac solution (pH 6), MeOH

Liquid chromatography (LC):

- **Analytical column:** Luna PFP(2), 150 x 2 mm, 5 µm (Phenomenex)
- **Over temperature:** 50°C
- **Mobile phase A/B:** 1% formic acid/w70MeOH
- **Flow rate:** 0.3 ml/min
- **Gradient elution time:** 13 min analytical run time - 4 min equilibration time

QqTOF:

- **Instrument:** Triple TOF 5600 (AB Sciex)
- **Source/ionization mode:** TurbolIonSpray (+ESI)
- **Ion spray Temp.:** 5500 V / 500°C
- **Resolution:** 25,000 - 35,000 @ 300 m/z
- **Mass accuracy:** <= +/− 3 ppm
- **Acquisition mode:** MS TOF/MS/MS TOF (m/z 101/50 – 950) +AD/DBS

Data management:

- **Software:** Analyst TF 1.5.1.1, Peak View 2.1.0.3 (ABSciex)
- **Libraries:** 796 data entries (MSTOF/MSS/MS/RT/RT)

Results

A total of 35 meconium samples (17/16 neonates) were analyzed and 47 compounds (in total: 193 matches, range: 0–15 compounds maximum per analysis – IS’s, caffeine and theophylline not included) were identified by XLC-QqTOF.

Following drugs of abuse were detected (no of positive cases): methadone/EDDP/EMDP (11/11), morphine/codeine/nor/ (6/3/1), nor/ cocaine, benzoylcegonine, ecgonimethylester, levamizole (2/2/2/2), THC-COOH/11-OH-THC/THC-COOH-glucuronide (2/2/2), nor/buprenorphine (3/2), amphetamine/metamphetamin (2/1), nor/ketamine (1/1).

Following medications were identified (no of positive cases):

- Lidocaine (11), paracetamol (8), norfentanyl (6), metoclopramide (5), O-desmethyl- tramadol (4/4), hydrocortisone (3), ranitidine (3), ampicillin (2), bupivacaine (2), lopinavir (2), nortizepam/naxepam, temazepam (2/1/1), quetiapine/N-desallyl-7-OH-, (2/2/2), antipyrine (1), atazanavir (1), chlorproxiene (1), haloperidol (1), nalbuphine (1) nor-/ olanzapine(1/1) and phenobarbital (1).

The majority (68.6%) of mothers were smokers (nicotine/cotinine: 24/22).

Case Example

A mother with a history of tildine abuse was brought to the hospital. During hospitalization she was substituted with buprenorphine. Before she gave birth, she received a laxative (macrogol/polyethylene glycol (PEG) 3350). During labour N-butyloxyclopamine, tramadol (via suppositories), nalbuphine and paracetamol were administered.

The meconium analysis (XLC-QqTOF/PeakView) identified a number of PEG-peaks resulting from the administration of the laxative/suppositories (Fig. 2).

In the present case, no tildine but eight other compounds (metoclopramide, O-desmethyl- tramadol, nicotine, nalbuphine, paracetamol, nor-buprenorphine) and the nine IS’s were automatically identified by XLC-QqTOF/SmileMS analysis (Fig. 3). Nalbuphine-glucuronide and pantoprazole-glucuronide (M=176.0320 Da) were additionally detected after deactivating of pre-filters (mass tolerance, δRT-off).

The results of our cohort disclosed that 31.4% of newborns were tested positive for methadone, 25.7% for opiates, 11.4% for tramadol/2.9% for ketamine (opioids), 5.7% for amphetamines, 5.7% for cocaine and 5.7% for THC.

Conclusion

The described screening procedure based on automated on-line extraction, HPLC and triple TOF technology offers a rapid and accurate identification of drugs of abuse, medications and their metabolites in neonatal meconium to detect fetal drug administration and to help the pediatrician to support antenatal interventions.

Intrapartum drug exposition cannot be differentiated from gestational drug use (e.g. tramadol or fentanyl).

References: