K25 The Detection of Oxycodone in Meconium Samples

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After attending this presentation, attendees will learning a procedure for the extraction of oxycodone from meconium and its detection using gas chromatography-mass spectrometry (GC/MS) is described for the first time. The abuse of oxycodone (OxyContin™) has been widely discussed in the mainstream media and it is often described as a cheap form of heroin. Following the presentation, attendees will understand the abuse potential of oxycodone and be able to analyze the drug in meconium.

The described procedure can be used by researchers to determine the exposure of newborns to oxycodone, a drug with high abuse potential. The detection of exposed neonates will aid in their treatment, and allow mothers to be counseled and assisted with their drug use problems. Identification of drug exposed newborns may assist in the prevention of further drug-addicted children being born to the same mother, since services and assistance can then be provided.

Introduction: Oxycodone is a semi-synthetic opioid derived from the opium alkaloid thebaine.Oxycodone (14-hydroxy-7,8-dihydrocodeinone) marketed as OxyContin™ and Roxicodone™ is a strong opioid agonist that is available alone or in combination with mild analgesics. It is suitable for oral and nasal administration due to high bioavailability (50-65%), which makes it a good candidate for nasal abuse. In analgesic potency, oxycodone is comparable to morphine and with the exception of hallucinations, which may occur more rarely after oxycodone than after morphine, the side effects of these drugs are closely related. The abuse potential of oxycodone is equivalent to that of morphine

Oxycodone has been reported as having a high degree of abuse and potential complications in neonates from maternal drug use. Using a standard enzyme multiplied immunoassay (EMIT) screening technology, the cross-reactivity of oxycodone to the morphine antibody is only 5-6%. A positive screening value would require a high concentration of drug to be present, so an assay for the detection of oxycodone in meconium using gas chromatography-mass spectrometry was developed. Hospitals employ routine testing of neonatal and/or maternal specimens for the determination of drug and alcohol use during pregnancy. While neonatal urine is widely tested, it gives only a short history of maternal drug use. Meconium, the first fecal material passed by a newborn, extends the window of drug detection up to 20 weeks and has become widely accepted as an alternative to urinalysis. In addition to morphine and codeine, there have been reports of heroin metabolites in meconium, hydrocodone and hydromorphone, but to date there are no reports of the metabolism, deposition or detection of oxycodone or its metabolites, in either meconium or neonatal urine. Since oxycodone has been increasingly identified as a potent narcotic resulting in drug dependence, overdose and death, its use during pregnancy may result in withdrawal symptoms in the newborn.

Sample Preparation: Deuterated internal standard (50 µL) was added to an aliquot (0.5 g) of each calibrator, control or meconium specimen. The internal standard concentration of deuterated oxycodone was 200 ng/g. Methanol (3 mL) was added and the specimens were homogenized, centrifuged, and the supernatant was decanted into a small glass tube. The supernatant was evaporated to dryness at 40°C, and refrigerated overnight. The next day, 0.1M hydrochloric acid (3 mL) was added with 250µL of 10% methoxyamine hydrochloride (aqueous). The mix was incubated at room temperature for 1 hour and mixed. 0.1M phosphate buffer (pH 6.0; 3mL) was added.

Extraction Procedure: Solid-phase mixed mode extraction columns were placed into a vacuum extraction manifold. Each column was conditioned with methanol (3 mL), deionized water (3 mL) and 0.1 M phosphate buffer (pH 6.0; 3 mL). The sample was allowed to flow through the column using no vacuum. The sorbent bed was dried for one minute at full vacuum. The column was washed with deionized water (3 mL), 0.1M hydrochloric acid (3 mL) and methanol (3 mL). The column was allowed to dry after each wash stage. Glass collection tubes were placed in the manifold and the opiates were finally eluted fresh methylene chloride: isopropanol: ammonium hydroxide (80:20:5; 3mL). The extracts were evaporated to dryness under nitrogen at 17psi at 60°C. Ethanol (100µL) was added, the specimens were mixed and transferred to autosampler vial inserts and re-evaporated to dryness.

Derivatization: The vials were capped and the residue was reconstituted with 50 µL iso-octane and 10 µL of BSTFA + 1% TMCS. The extracts were heated for 30 minutes at 80°C in dry heating block prior to analysis by GC/MS.

Analytical Procedure: An Agilent 6890 gas chromatograph coupled to a 5973 mass selective detector (MSD) operating in electron impact mode was used for analysis. The gas chromatographic column was 5% phenyl -95% methyl silicone DB-5 MS, 0.20 mm ID, 0.33 µm film thickness, 25 m length and the injection temperature was 280°C. The injection mode was splitless and the injection volume was 3 µL. The oven was programmed from 150°C for 1 minute; ramped at 20°C/min to 245°C and held for 8 minutes. Then, it was ramped at 50°C/min to 290°C. The source was held at 230°C and the quadropole at 150°C. The ions monitored were 419.4, 420.4 for d_3 Oxycodone and 416.4, 417.4 for Oxycodon.

Results and Discussion: Since inception of this procedure, three meconium specimens received into our

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laboratory have been determined as being positive for oxycodone. The concentrations detected were 117 ng/g, 150 ng/g and 2279 ng/g. There are no published reports of oxycodone concentrations in meconium samples, so correlation with maternal use or abuse is not possible.

While no specific reports of oxycodone on neonatal outcome have been reported, there are many papers studying the effects of heroin, buprenorphine, methadone or other opiates. It is estimated that 55-94% of infants born to opioid-dependent mothers in the USA show signs of withdrawal. Newborns exhibiting neonatal abstinence syndrome (NAS) generally have longer hospital stays and symptoms include tremors, irritability, sleep abnormalities, feeding problems, low birth weight and seizures. It has further been shown that methadone maintenance treatment during pregnancy is associated with more consistent prenatal care, more normal fetal growth and reduced fetal mortality. However, neonatal withdrawal from methadone appears to be more severe than from heroin, as judged by amount of medication required to control symptoms and duration of treatment. The analytical procedure described details the determination of oxycodone in meconium specimens and may provide useful information to neonatologists and researchers studying the effects of opiates on newborns.

Oxycodone, Meconium, Neonatal Abstinence Syndrome

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