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Review

Determination of drugs of abuse in meconium

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Abstract

Fetal exposure to drugs has many adverse effects upon the neonate including low birthweight, small head size and an increased risk of miscarriage and death. Correct diagnosis of drug use during pregnancy is essential if the child is to receive specialized treatment and care, which will aid in learning and behavioral development. Diagnosis will also help in the prevention of subsequent drug-exposed children being born to the same mother. Meconium is the first fecal material excreted by the newborn and is an excellent depository for drugs to which the fetus has been exposed. Its analysis is widely accepted in the scientific and medical communities since it has several advantages over urinalysis, including providing a longer historical record of drug exposure and easier collection. Various drugs and their metabolites have been detected in meconium, however, the metabolic profile of drugs in meconium differs from that of neonatal and/or maternal urine. This article addresses the determination of cocaines, amphetamines, opiates, cannabinoids, phencyclidine, nicotine and methadone in meconium using several analytical procedures including immunochemical and chromatographic methods. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Various neonatal birth defects are thought to be related to fetal exposure to drugs, alcohol, chemical agents or other xenobiotics. The vast majority of research in the USA has focused on the effects of maternal cocaine use upon the newborn. Cocaine use has been implicated in many cases of placental abruption, maternal hypertension, subarachnoid and intracerebral hemorrhage, premature labor, small head size, reduced birth weight, ruptured uterus and fetal death [1–3]. Behavioral consequences as neonates reach childhood have also been studied particularly in cocaine exposed babies. Maternal methamphetamine abuse has similar effects upon the fetus as cocaine, including complications during pregnancy, medical problems in early life and increased rates of premature birth [4,5]. Neonates exposed to opiates often display withdrawal symptoms such as irritability, tremors, hyperactivity and seizures [3]. An early diagnosis of drug exposure is highly desirable in order to provide aid for the long-term development of the child and may help in the prevention of subsequent children from the same mother being exposed to drugs.

Meconium is the first fecal matter passed by a neonate. It begins to form between the 12th and 16th week of gestation and usually accumulates thereafter until birth. It represents the intestinal contents of the fetus and is a complex matrix, consisting mainly of water, but also containing mucopolysaccharides, lipids, proteins, vernix caseosa, bile acids and salts, epithelial cells, cholesterol and sterol precursors, blood group substances, squamous cells, residual amniotic fluid and enzymes. The contents of meconium provide a history of fetal swallowing and bile excretion, therefore, it is considered a more accurate history of drug use in the latter half of pregnancy than is neonatal urine. Meconium is usually passed by the neonate 1–5 days after birth.

To date, urine is the most widely tested biological fluid for the determination of drug exposure during pregnancy. However, it is a difficult sample to collect from newborns, and is only indicative of recent drug exposure (occurring within a few days of birth). Therefore the false negative rate is high when urine drug testing is used. Many authors have concluded that meconium is a superior sample to neonatal urine for the purposes of determining drug use in pregnancy [6–10]. However, others disagree with these findings stating that meconium offers no significant advantage over urinalysis and is in fact a more difficult specimen to process for analysis [11,12].

Regardless, meconium testing is now widely accepted as the procedure of choice for the determination of fetal drug exposure. The major advantage of meconium analysis is that it extends the window of detection of drug use to approximately the last 20 weeks of gestation. Meconium is easy to collect and is non-invasive to the child. Drugs are stable in meconium for up to 2 weeks at room temperature and for at least a year when stored frozen. The main disadvantage to meconium analysis is that it is not a homogenous sample because it forms layers depending upon the time of deposition in the intestine. Therefore it must be mixed as thoroughly as possible before analysis to help diffuse the drug throughout the matrix. Also, the testing is more demanding than urinalysis and it is difficult to prepare proficiency or control materials to assess laboratory quality assurance.

2. Methods of analysis

2.1. Screening meconium for drugs of abuse

Radioimmunoassay (RIA), fluorescence polarization immunoassay (FPIA) and enzyme multiplied immunoassay technique (EMIT) have all been described as useful analysis methods for screening meconium specimens. Overall, FPIA and RIA have been shown to be more sensitive than EMIT for the detection of cocaine metabolite (benzoylecgonine) in spiked meconium samples. Other comparative research has shown that the CAC Cocaine RIA (DPC Corporation, CA, USA) is the most sensitive assay for meconium screening. Presumably this is because there is significant cross reactivity with cocaine which is often present in meconium, compared to various other immunoassays which are specific for benzoylecgonine.

2.1.1. Radioimmunoassay (RIA)

The original work carried out on meconium used radioimmunoassay for the detection of drugs. In the

1980s, Ostrea became the first researcher to publish and patent procedures for the screening of drugs of abuse in meconium [13,14]. In his original method, for each analysis 0.5 g of meconium was collected directly from the diaper. The sample was mixed with distilled water (10 ml), then concentrated hydrochloric acid (1 ml) and this homogenate was filtered through glass wool. The filtrate was centrifuged and an aliquot of the supernatant was tested for morphine (heroin metabolite) and benzoylecgonine (cocaine metabolite) using Abuscreen RIA kits. The recovery from drug-free meconium for benzoylecgonine and morphine was 70 to 105% and 84 to 97%, respectively. For cannabinoids, methanol (0.4 ml) was added to meconium (0.1 g). The sample was mixed and allowed to stand at room temperature for 10 min then centrifuged. An aliquot of the supernatant was tested for the cannabinoid metabolites by RIA.

A modification of this method reported that meconium (0.5 g) was suspended in 0.1 *M* phosphate buffer–methanol (4:1), mixed and centrifuged [14]. The ultrafiltrate was analyzed for morphine, cocaine and cannabinoid by EMIT using cutoff values of 60, 50 and 50 ng/ml, respectively. The modified method provided more accurate results than the original and was adapted for mass meconium screening. Of 61 samples analyzed, opiates were detected in 13% by the original method and in 15% by the modified method. Cocaine was detected in 64% by both original and modified methods. A high prevalence rate (38% of total positive results) was reported from Hutzel Hospital, Detroit, MI, USA, and a very low rate (1–4% of total positive results) in three other rural communities. However, these results were screen only numbers and positives were not confirmed using any chromatographic technique.

In another study, Schutzman et al. [15] analyzed meconium samples collected from 500 infants. Meconium (0.2 g) was added to 2 ml of sterile water. The mixture was homogenized and subsequently analyzed using RIA. The lower limit for positive test was 15 ng/0.1 g of meconium. The meconium of 59 babies (11.8%) from socioeconomically mixed suburban settings tested positively for cocaine. Again, these results are screen only, without confirmation.

Nair et al. [16] applied radioimmunoassay to analyze meconium samples collected from 141 infants. Acid extraction was used for heroin metabolite (morphine), for cocaine metabolite (benzoylecgonine) and methanol extraction (both according to Ostrea and described above [13,14]) for cannabinoid metabolite. The cut-off values were 15, 25 and 50 ng/ml for cocaine, morphine and marijuana metabolite, respectively. Cocaine was present in 31% of analyzed specimens, opiates in 18% and cannabinoids in 17%. In 38 infants urine was obtained and analyzed for drugs. The comparative results show that meconium was more sensitive than urine in detecting drug exposure (55.3% vs. 31.5%).

2.1.2. Enzyme multiplied immunoassay technique (EMIT)

Screening of meconium without some degree of extraction is difficult and results in many false positive results especially when enzyme or fluorescence polarization immunoassays are used, due to turbidity in the meconium extract. Chen and Raisys [17] described the detection of cocaine and benzoylecgonine in meconium using EMIT following solid-phase extraction. A known amount of cocaine and benzoylecgonine was added to drug-free meconium. Samples were extracted with methanol, evaporated to dryness and reconstituted in 0.1 *M* potassium phosphate buffer, pH 6. The suspension was centrifuged and filtered onto a conditioned Varian Bond Elut Certify 1 LRC solid phase column. The extracts were dried and resuspended in saline solution for analysis by EMIT. The limit of detection established for this method for benzoylecgonine, even when the drugs were extracted from the specimen, was high: 300 to 400 ng/g of meconium.

Moriya et al. [11] developed a reliable and sensitive screening procedure for amphetamines, cocaine metabolites, opiates and phencyclidine (PCP) in meconium. Meconium (0.5 g) was mixed with water (2 ml) and sodium bicarbonate–sodium carbonate (5:1; 50–100 mg) powder. The mixture was extracted with chloroform–2-propanol (3:1, 6 ml). A drop of concentrated HCl was added and the mixture was evaporated to dryness under a gentle stream of air at 50–70°C. The residue was reconstituted with buffer, pH 6 (0.5 ml), and the mixture was added to ethyl acetate (0.5 ml) to remove lipids. The aqueous phase was tested by EMIT. To evaluate recovery for the EMIT screening a solution containing 3000 ng/ml of benzoylecgonine, 3000 ng/ml of d-metham-

phetamine, 1000 ng/ml of morphine, 75 ng/ml of phencyclidine and 1 ml of water was added to 0.5 g of meconium. For the negative control 1.5 ml of water was added to the drug-free meconium. The mixtures were extracted as described above. The concentration of each drug in meconium extract was determined from the change in absorbance using a calibration curve obtained from a solution of known drug concentration. The lower detection limits of the EMIT for benzoylecgonine, d-methamphetamine, morphine and PCP were 250, 730, 110 and 100 ng/g, respectively. Meconium samples from 50 infants born to mothers suspected of using drugs of abuse during the pregnancy were analyzed. Twelve (24%) were positive for benzoylecgonine, seven (14%) for opiates and one (2%) for PCP. The presence of benzoylecgonine and PCP was confirmed by GC–MS, while opiates were confirmed by thin-layer chromatography. This method for meconium screening has better sensitivity for benzoylecgonine than others previously described.

2.1.3. Fluorescence polarization immunoassay (FPIA)

In 1994 Lewis patented and published a new method for screening meconium samples for cocaine, cannabinoids, amphetamines and opiates [18]. Meconium was mixed with glacial acetic acid (3 ml) and homogenized. Diphenylamine in acetone (1.67 mg/ml) was added, mixed and centrifuged. The top layer was decanted and one drop of 1% sulphuric acid was added. After evaporation to dryness the residue was reconstituted in buffer–methanol (50:50, v/v), centrifuged and analyzed for drugs using FPIA (Abbott TDx). Using this method, 54 analyses of paired neonatal urine and meconium specimens were performed for cocaine detection. 9.3% of urine specimens tested positive and 25.9% of meconium samples for cocaine metabolites (including GC–MS confirmation).

Franssen et al. [19] used Abbott TDx and HPLC for the determination of morphine and amphetamine in meconium. To meconium (0.5 g), water (5 ml) and one drop of 0.5 *M* hydrochloric acid were added. The mixture was vortex-mixed for 1 min, then mixed ultrasonically for 5 min, vortex-mixed for 1 min and finally centrifuged for 10 min. The TDx opiate and amphetamine assays were performed. For the FPIA screening of meconium the lower limit of detection for amphetamine was 1.35 μg/g and for morphine 0.35 μg/g.

The procedures described above essentially considered the major drugs of abuse in the USA, i.e. cocaine, opiates, amphetamines and cannabinoids. Of course, there are many other drugs both licit and illicit which can be abused during pregnancy. Screening methods have been described for the determination of methadone, barbiturates, benzodiazepines and nicotine metabolites in meconium.

Wingert et al. [20] screened meconium for the presence of cocaine, marijuana metabolites, opiates and methadone using EMIT. Two of 344 samples tested were positive for methadone. Dahlem et al. [21] analyzed meconium and urine samples using RIA for barbiturates, benzodiazepines, methadone, cannabinoid, cocaine, LSD, phencyclidine and morphine. Of 20 newborn infants from drug-dependent mothers, meconium was positive for methadone in 9 cases, for morphine in 9, for cocaine in 6 and for cannabinoid in 4 cases. In 9 cases urine was positive for methadone and in 1 case newborn urine was positive for barbiturates. Ostrea et al. [22] analyzed meconium samples from 55 infants whose mothers were nonsmokers, passive smokers, light or heavy active smokers. Meconium (0.5 g) was emulsified in 0.1 mol/l phosphate buffer, pH 7.0 (5 ml). The suspension was mixed and centrifuged. The supernatant was ultrafiltered and was analyzed for nicotine metabolites (cotinine, *trans*-3′-hydroxycotinine) using double-antibody radioimmunoassay. For the recovery study of cotinine, drug-free meconium was spiked with cotinine at a concentration of 0.74 and 764 ng/ml and samples were analyzed as above. The mean recovery from meconium samples was 72%. The presence of nicotine metabolites was confirmed by GC–MS.

2.1.4. False positives and false negatives in meconium screening

In some publications described above, results were confirmed using chromatographic techniques, but in the vast majority, data is dependent on screen only results; a practice which was examined by Moore et al. in 1995. The group published data regarding the

incidence of false positive and negative results in meconium screening [23].

2.1.4.1. *False negatives*. It was reported that the method of isolating the drugs from the meconium substantially affects the outcome of the screen. When an essentially clean extract (i.e. drugs are isolated from the matrix using solvent or solid-phase methods) is not used, a high rate of false negative results is observed. The immunoassay technique does not substantially affect the outcome of the analysis, but the sample preparation procedure does.

2.1.4.2. *False positives*. Five hundred and thirty-five (535) meconium samples which screened positively for at least one of the following drugs: cocaine metabolite, opiates, amphetamines or marijuana metabolite, were chosen. The screening cut-off levels were 25 ng/g for all drugs except amphetamines (100 ng/g). Of these screen positive specimens, 285 (53.3%) were subsequently confirmed using gas chromatography–mass spectrometry (GC–MS) for one or more of the drugs at cut-off levels of 5 ng/g for all except marijuana metabolite (2 ng/g) (Table 1).

According to these results, immunoassay screening is falsely positive 46.7% of the time at the cut-off levels used, assuming that the correct drug metabolites are identified in the confirmatory procedure. It is possible that the immunoassay results are not in fact false positives but that there are drug metabolites present in meconium which are contributing to the immunoreactive response. These compounds are subsequently not determined in the confirmatory method, producing false negative results. Probably the most significant advance, to date, in the determination of drugs in meconium was reported by Steele et al. in 1992 [24] who determined that for cocaine analysis, there was a contributing compound in meconium to immunoreactive response which was not being confirmed on GC–MS. The research group were unable to confirm a significant number of cocaine positive screens using a standard GC–MS assay which identified cocaine, cocaethylene, benzoylecgonine, ecgonine methyl ester and norcocaine. Subsequently, the authors determined that the significant contributor to the immunoassay was *m*-hydroxybenzoylecgonine (*m*-OH-BZE), a previously unreported metabolite of cocaine in meconium. The authors noted some difficulties with the construction of a standard curve for *m*-OH-BZE using meconium as the matrix. Following hydrolysis of meconium, the authors also concluded that *m*-OH-BZE glucuronide has approximately the same immunoreactivity as unconjugated *m*-OH-BZE.

The presence of this metabolite in significant concentrations in meconium demonstrated that the metabolic profile of newborns differs between urine and meconium, therefore simple application of urine protocol to meconium specimens will cause false negative results.

In another study, inspired by Steele's publication, Lewis et al. [25] used FPIA to screen meconium samples for cocaine metabolites at a cut-off value of 50 ng/g. Of 208 samples which screened positively, the confirmation rates were 132 (63%) for cocaine, 161 (77%) for benzoylecgonine and 197 (95%) for *m*-hydroxybenzoylecgonine using N-methyl-N-(*tert.*-butyldimethylsilyl)-trifluoroacetamide containing 1% *tert.*-butyl-dimethylchlorosilane (MTBSTFA) to form *tert.*-butyldimethylsilyl derivatives. This derivative gives higher masses for the *m*-hydroxy metabolite making it simpler to analyze. In 23% of the cases, *m*-hydroxybenzoylecgonine was the only cocaine metabolite present. Ethically, the authors concluded, it is mandatory to confirm all positives from the preliminary screening by GC–MS and

Table 1
Positive screening vs. positive confirmation by GC–MS for THC metabolite, cocaine metabolite, opiates and amphetamines [23]

	Positive screen	Positive confirmation	%
THC metabolite	173	97	56.1
Cocaine metabolite	228	135	59.2
Opiates	60	34	56.7
Amphetamines	74	19	25.7

screen only meconium results should be interpreted with some caution.

2.2. Confirming meconium for drugs of abuse

2.2.1. Cocaine

There are several published confirmation methods for the determination of cocaine and its metabolites in meconium. Reported analytes include cocaine, norcocaine, benzoylnorecgonine, cocaethylene [26], ecgonine methyl ester, *m*-hydroxybenzoylecgonine, and more recently, *p*-hydroxybenzoylecgonine, anhydroecgonine methyl ester and ecgonine ethyl ester [27].

2.2.1.1. *Gas chromatography–mass spectrometry* (*GC–MS*). The most commonly reported confirmatory procedures involve gas chromatography–mass spectrometry (GC–MS). Clark et al. [28] used trimethylsilyl derivatives to determine cocaine metabolites in meconium. Following methanolic extraction, the solvent was evaporated to dryness and reconstituted in phosphate buffer before solid-phase extraction. Chromatography was performed isothermally at 260°C on a HP-5 phenylmethyl capillary column. The assay was linear up to 10 μg/g with regression coefficients of 0.99 and 0.97 for cocaine and benzoylecgonine, respectively.

A different confirmatory technique was described by Abusada et al. [29] who used a similar extraction procedure to generate linear quantitative response curves for cocaine, ecgonine methyl ester, benzoylecgonine and cocaethylene on GC–MS over the concentration range 0–1000 ng/g. The regression coefficients ranged from 0.85 to 0.946. To produce the PFPA derivative, pentafluoro propionic anhydride (PFPA; 50 μl) and 2,2,3,3,3-pentafluoro-1-propanol (PFP; 50 μl) were added to the extract and incubated for 20 min at 60°C. The excess reagent was evaporated and the residue re-suspended in ethyl acetate (75 μl). The column used was a Hewlett–Packard Ultra 2 crosslinked phenylmethyl silicone (12 m×0.2 mm I.D.×0.33 μm film thickness) and the carrier gas was helium (40 cm/min). The injector temperature was 260°C and a split injection of 50:1 was used. The oven was held at 145°C for 4.5 min, then increased by 60°C/min to 210°C for 8 min. Then the temperature was increased to 280°C at 60°C/min and held for 0.25 min. The transfer line was maintained at 280°C. The selected ions monitored were *m*/*z* 182, 303 and 272 for cocaine; 300, 272 and 316 for the derivative of benzoylecgonine; 182, 345 and 314 for the ecgonine methyl ester derivative, 196, 272 and 317 for cocaethylene. The deuterated internal standards for each compound were three mass units higher.

2.2.1.2. *High-performance liquid chromatography* (*HPLC*). Browne et al. [30] reported the presence of cocaine (COC), norcocaine (NC) and cocaethylene (CE) in meconium using solid-phase extraction and HPLC ($n=106$). Any presumed positives were subsequently confirmed using GC–MS. The same protocol was adopted by Dusick et al. [31] where the meconium of 323 very low birth weight babies was tested. The HPLC procedure consisted of pumping mobile phase (flow-rate 1.5 ml/min) onto a μBondapak C_{18} column (300×3.9 mm i.d.). A C_{18} guard column and a Rheodyne 20 μl loop were incorporated. A Spectra-Physics forward optical multiwavelength detector was connected and the eluent was monitored at 230, 254 and 275 nm. Full spectra were recorded over the range 190–400 nm. The mobile phase consisted of 0.025 *M* potassium dihydrogen phosphate–acetonitrile–butylamine (500:125:12.5, v/v/v) adjusted to pH 2.9 with 85% phosphoric acid. The assay was linear over the range 0.1–10 mg/kg, the quantitation limit was 0.1 mg/kg and the limit of detection was 0.03 mg/kg.

Murphey et al. [32] were the first to report an analytical HPLC procedure for the quantitation of benzoylnorecgonine (BN) in meconium, and their method is also applicable to the determination of COC, benzoylecgonine (BZE) and NC. Extraction was carried out on a mixed mode column with the sample adjusted to pH 2.0. Separation of the compounds was achieved on a Microsorb C_{18} column (100×4.6 mm I.D., 3 μm particle size), using a mobile phase of 0.01 *M* NaH_2PO_4 pH 2.0 with 58 μ*M* of tetrabutylammonium hydroxide and 13% (v/v) acetonitrile pumped at a rate of 1 ml/min. The injection volume was 200 μl and the eluent was monitored at 233 nm. Standard curves were linear over the range 0.05–5.0 μg/g of meconium and the limit of quantitation was 0.05 μg/g. BN was detected in the meconium of seven out of eleven

neonates whose mothers were known to have used cocaine during pregnancy. BZE was found in ten out of the eleven specimens. The authors comment on the heterogeneity of meconium stating that... ''in some infants benzoylnorecgonine, cocaine or benzoylecgonine could be detected in some aliquots but not all''... They further comment upon the possibility that this lack of homogeneity could lead to false negative results.

2.2.2. Opiates

2.2.2.1. *GC–MS*. There are very few published procedures for the confirmation of opiates in meconium. There are no reports of heroin or 6-acetylmorphine being present in the matrix. Wingert et al. [20] confirmed morphine using a methanolic extract of meconium. The extract was hydrolyzed with concentrated hydrochloric acid, cooled and after adjusting the pH to 9.0, was extracted using a mixed-mode solid-phase extraction column. The final eluent was evaporated to dryness and the trimethylsilyl derivatives were formed using BSTFA. Chromatography was performed using either a HP-1 or a HP-5 capillary column. The initial temperature was 80°C for 1 min followed by a 40°C/min ramp to 290°C. The ions monitored were m/z 417 and m/z 432 for the deuterated morphine (internal standard) and m/z 401, 414 and 429 for morphine. The authors confirmed two out of 344 meconium specimens (0.6%).

A recent report by Moore et al. [33] describes the GC–MS confirmation of hydrocodone and hydromorphone as well as codeine and morphine in meconium. The authors describe the analysis of the trimethylsilyl derivatives of the opiates using a GC–MS procedure. The meconium was homogenized in 2.4 *M* hydrochloric acid and centrifuged. The supernatant was treated with 11.8 *M* potassium hydroxide, a buffer salt and extracted with *tert*.-butyl methyl ether. Following acidification and back extraction the final solvent was evaporated to dryness and reconstituted in ethanol. After transfer to autosampler vials, the ethanol was re-evaporated and BSTFA derivatives were formed. For analysis, a DB5-MS (25 m×0.2 mm I.D.×0.33 μm film thickness) column was used and helium was the carrier gas. The oven was programmed from 100°C for 1 min to 230°C at a rate of 25°C/min, then at 3°C to a final temperature of 260°C. The injector was set at 250°C and the detector at 270°C. The instrument was operated in splitless injection and selected ion monitoring mode. The limit of detection for the assay was 5 ng/g for each drug. Codeine and hydrocodone were quantitated using D3-codeine as the internal standard; morphine and hydromorphone were quantitated using D3-morphine. The selected ions were: codeine m/z 343, 371, 234; hydrocodone 371, 372, 234; morphine m/z 429, 430, 401; hydromorphone m/z 429, 430, 414.

In the two cases discussed, the levels of codeine, hydrocodone and hydromorphone in meconium increased markedly following acid hydrolysis of the sample. The level of morphine detected also increased but not as significantly as the levels of the other opiates. Research by Becker et al. [34] has shown that the hydrolysis of meconium does not significantly increase the amount of morphine detected. There are very low levels of glucuronyl transferase in all fetal and placental tissues, suggesting that the absence of morphine glucuronide is not an unusual finding, but the other opiates do seem to be significantly bound.

As with cocaine, opiate investigations also signify that the metabolic profile of meconium is different from that of neonatal urine. Specifically, (a) meconium does not need to be hydrolyzed to produce measurable levels of morphine, and (b) to date, no reports of the presence of mono-acetylmorphine in meconium have been published.

2.2.2.2. *HPLC*. A report by Franssen et al. [19] describes the HPLC analysis of meconium for morphine and amphetamines. The meconium was mixed with water and one drop of 0.5 *M* hydrochloric acid. After mixing, ultrasonication and centrifugation, borax buffer (pH 9.0) was added and the sample was extracted using an Extrelut® column. The final eluent was evaporated to dryness and reconstituted in mobile phase. The HPLC system consisted of a C_{18} stationary phase (LC-18 DB), 150×4.6 mm I.D. (5 μm particle size). The mobile phase comprised potassium dihydrogen phosphate (0.077 *M*) in water – methanol – acetonitrile – tetrahydrofuran – triethylamine (600:100:25:7:1.5, v/v/v/v/v) and the flow-rate was 1.0 ml/min. A diode array detector with

Millennium software was used and both morphine and amphetamine were monitored at 204 nm. The assay was linear from 0.25 to 1.0 μg/g of meconium and the recovery for morphine was 65.6%. The lower limit of detection for both analytes was 0.5 μg/g which is one hundred times less sensitive than the splitless injection GC–MS published methods.

HPLC procedures, while useful for some research projects, lack the sensitivity and specificity of GC–MS, particularly when ultraviolet detection is employed. The analysis of meconium requires sensitive procedures for routine application to avoid false negative results.

2.2.3. Amphetamines

2.2.3.1. *GC–MS*. A report on the confirmation of methamphetamine and amphetamine in the meconium of guinea pigs was published by Nakamura et al. [35]. Their hypothesis was that gestational age would affect the quantitative recovery of methamphetamine in urine, meconium and amniotic fluid. They tested this hypothesis by making an intraperitoneal injection of methamphetamines (1 mg/kg) at various time intervals during gestation then analyzing meconium and amniotic fluid by GC–MS. The analytical procedure for meconium involved homogenization in 2.4 *M* hydrochloric acid, centrifugation and solvent extraction of the supernatant using *n*-heptane–methylene chloride–ethylene dichloride–2-propanol (50:17:17:16 v/v/v/v). The final dried extracts were reconstituted in 25 μl of mesitylene and derivatized by adding 20 μl of bis-N-methylheptafluorobutyramide (MBHFBA). The samples were heated at 85°C for 15 min. A 5% phenyl, 95% methylsilicone phase capillary column (Hewlett–Packard HP Ultra 2) 25 m×0.2 mm I.D.×0.33 μm film thickness was used with helium as the carrier gas. The oven was held at 100°C for 30 s then programmed to a final temperature of 220°C at a rate of 20°C/min. The injection temperature was 225°C and the injector was operated in splitless mode. The limit of quantitation for amphetamine and methamphetamine in meconium samples was 1 ng/g. The authors concluded that the gestational age may be an important consideration in interpreting quantitative methamphetamine recovery in perinatal samples and that the timing of sample collection affects detection rate in amniotic fluid and meconium.

2.2.3.2. *HPLC*. Franssen et al. [19] included amphetamine but not methamphetamine in their procedure described in the opiate section above. The recovery of amphetamine was 91.8%, and the detection limit was high (500 ng/g). The authors did not confirm any meconium ($n=14$) or urine samples for amphetamines.

2.2.4. Marijuana

The determination of cannabinoid metabolites in meconium poses difficult analytical problems principally because of the small amounts present. Some authors (e.g., Wingert et al. [20]) found it difficult to confirm screen THC positive samples using their reported procedure. A publication by Moore et al. [36] describes the determination of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC–COOH) in meconium. Following homogenization of the meconium in methanol and the addition of 11.8 *M* potassium hydroxide, the sample was allowed to stand for 15 min. Following centrifugation, deionized water was added to the supernatant and the specimen was extracted with hexane–ethyl acetate (9:1, v/v). After back extraction into concentrated hydrochloric acid, the solvent was evaporated to dryness and reconstituted in ethanol (50 μl). The *tert*.-butyldimethylsilyl derivative of THC–COOH was formed by using MTBSTFA as the derivatizing agent. The selected ions were 575, 416 and 518 for the deuterated (D3)THC–COOH; 572, 413 and 515 for THC–COOH. The column used was a DB-5 MS (25 m×0.2 mm I.D.×0.33 μm film thickness) and the oven was held at 100°C for 1 min, then programmed at 30°C/min to a final temperature of 310°C where it was held for 6.6 min to give a run time of 14.6 min. The injector and detector were set at 270°C and 310°C, respectively, and helium was used as the carrier gas. The limit of detection and quantitation was 2 ng/g. The method included a base hydrolysis of the meconium which suggests that THC–COOH is significantly glucuronide bound in meconium. The authors suggested, as others have, that an alternative THC metabolite may be present in meconium, although no specific reports of other metabolites have yet been published.

2.2.5. Phencyclidine (PCP)

Phencyclidine users also tend to abuse other drugs, so the teratogenic effects of PCP alone are not well understood. However, PCP does cross the placenta and is present in neonatal urine and plasma. Moriya et al. [11] reported PCP determination using D5-PCP as the internal standard. The drug was extracted from meconium using a chloroform–isopropanol mixture (3:1, v/v); the organic phase was evaporated to dryness, reconstituted and re-extracted using a mixed mode solid-phase column. The final eluent was dried, reconstituted in ethyl acetate (50 μl) and analyzed by GC–MS. The column was a HP-1 capillary (25 m×0.02 mm I.D.) and the injector was set at 250°C. The initial temperature of 140°C was increased to 280°C at 25°C/min; the final temperature was held for 3.4 min. The ions monitored were 186, 200 and 243 for PCP; 205, 206 and 248 for D5-PCP. The sensitivity was 20 ng/g. Of 50 meconium samples analyzed, only one confirmed positively for PCP.

Confirmation of PCP screen positive specimens is a problem. In 1996, Moore et al. [37] lowered the detection limit of their assay to 5 ng/g using selected ion storage functions of an ion trap mass spectrometer in an attempt to confirm more screens. However, this change did not significantly increase the number of confirmed positives. It is possible that other metabolites of PCP are present in meconium which are contributing to the immunoassay screens but are not being monitored in the confirmation procedure.

2.2.6. Other drugs

2.2.6.1. *Methadone*. Methadone and its principal metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were recently reported in meconium by Stolk et al. [38] using FPIA screening followed by HPLC confirmation. A reversed-phase (C_{18}) stationary phase was employed, and a mobile phase of 530 ml distilled water, 146 μl triethylamine and 350 μl of phosphoric acid at pH 3.3 using 10% potassium hydroxide and 470 ml of acetonitrile was pumped at a rate of 0.6 ml/min. Methadone and EDDP were monitored at 204 nm. The meconium samples were extracted using Extrelut® columns and eluted with dichloromethane–2-propanol (80:20, v/v). The assay was linear over the range 460–3680 ng/g for methadone and 1000–6000 ng/g for EDDP. The limits of detection were 99 and 113 ng/g, respectively. The authors report a positive correlation between the maternal methadone dose and the concentrations detected in meconium.

Wingert et al. [20] carried out the confirmation of underivatized methadone in meconium using a mixed phase solid-phase extraction followed by GC–MS using the system described previously for morphine. The ions monitored were 226 and 297 for the deuterated internal standard; 223, 294 and 295 for methadone. The authors confirmed two meconium samples for methadone out of a total number of 344 (0.6%). One of the specimens had a corresponding urine sample which was negative.

2.2.6.2. *Nicotine*. Ostrea et al. [22] measured the nicotine metabolites cotinine and *trans*-3′-hydroxycotinine in the meconium from infants of passive and active smokers in order to determine nicotine exposure in utero. Their confirmatory GC–MS procedure used a cross-linked methyl silicone gum phase column (no details of the dimensions or GC temperature program were given) and helium was the carrier gas. The ions at m/z of the trimethylsilyl derivatives of cotinine m/z 98, 176, 119 and m/z 249, 144, 75 of 3-hydroxycotinine were monitored and deuterated cotinine was used as the internal standard. The authors state that . . . ''the presence of the target ion and at least one qualifier in the ion spectrum was required to confirm the identity of either drug in the test samples'' . . . The authors claim that the concentrations of nicotine metabolites in meconium are directly related to the degree of active smoking by the mother.

3. Conclusions

Meconium is considered to be a useful and viable specimen in the determination of drug abuse in pregnancy, since it gives a longer history of drug exposure than neonatal urine. Publications concerning drug testing of meconium are becoming a significant part of medical, toxicological and forensic literature. Screening procedures exist for a number of drugs and confirmatory methods are increasing in number. An early and correct diagnosis of drug exposure is the newborn's best chance of receiving

treatment and the development of good scientific procedures to determine drugs in meconium is of great benefit to society.

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