ANALYSIS OF MECONIUM FOR AMPHETAMINES AND OPIATES. OUR FIRST EXPERIMENT

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ABSTRACT: The aim of the study was to carry out a the laboratory diagnosis of fetal drug exposure. We focused on the analysis of meconium, as a specimen widely accepted for this purpose. In the preliminary study on meconium samples spiked with methamphetamine or morphine we tested the conditions for homogenization and isolation. Emit was used as a screening method and two dimensional HPTLC, GC/ECD, NPD and exceptionally also GC/MS as confirmation methods. The limit of detection was 0.5 μg/g meconium for both drugs. 39 meconium samples from babies newly born to mothers suspected of drug abuse were analyzed for methamphetamine and/or morphine. 18 of them were positive. In 29 cases both meconium and urine were available. The results and their possible interpretation are discussed.

KEY WORDS: Meconium; Methamphetamine; Opiates.

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INTRODUCTION

The aim of the study was to diagnose of fetal drug exposure.

Peri- and post-natal complications in infants born to drug dependent mothers have recently appeared as a new problem connected with drug abuse. Meconium is widely accepted material for the diagnosis of fetal drug exposure, mostly preferred to urine [2, 3, 5, 7, 8, 9]. Therefore we focused on its analysis and the detection of methamphetamine and morphine, as methamphetamine and heroine are the most frequently abused drugs in our country.

EXPERIMENTAL

Drug free meconium spiked with methamphetamine or morphine at concentrations of 4.0, 3.0, 2.0, 1.0, 0.75, 0.5, 0.25 and 0.00 mg/g was used for the pilot study.

Homogenization and preparation of spiked samples: 6 ml of the tested fluid – water, phosphate buffer pH 6.8, methanol, water-methanol 4:1 – were added to 1 g of

meconium, shaken vigorously and spiked with tested drug. The mixture was vortexed for 1 min. and sonicated for 15 min.

The same homogenization procedure was used for the real samples.

Isolation

Liquid-liquid extraction (LLE) with different solvents (chloroform, butylchloride, cyclohexane, ethylacetate-2-propanol 9:1) from alkaline medium was tested for supernatant after centrifugation and for the homogenate.

Solid phase extraction (SPE) on Chem Elut and alkaline Tox Elut columns for 20 ml of the sample was tested for supernatant. After centrifugation the supernatant was tapped up to 20 ml with phosphate or borate buffer pH 8.05/9.0 for Chem Elut and pH 6.2/7.4 for Tox Elut. Dichloromethane-2-propanol 90:10 and butylchloride were used for the elution from the column. 2 x 15 ml of chloroform and 10 ml of other solvents were used for LLE and 2 x 15 ml for SPE.

Organic solvent was evaporated to dryness on a water bath under the stream of air. A drop of hydrochloric acid was added to prevent the volatility of amphetamines.

Analytical methods

Immunoassays

EMIT on ACA Du Pont analyzer was used for screening. 1ml of centrifuged supernatant after homogenization in water or a phosphate buffer was centrifuged once more in an Eppendorf microcentrifuge prior to analysis. The methanolic supernatant was evaporated to dryness (a drop of HCl was added) and reconstituted in water or buffer used in the analyzer.

Chromatography

High performance thin layer chromatography (HPTLC) on HPTLC aluminium sheets or glass plates, Kieselgel 60 10 x 10 cm (Merck) was used for the identification of both drugs, mostly in two dimensional modification (2-HPTLC).

Mobile phases for 2-HPTLC: A_1 – ethylacetate-methanol-ammonia 85:10:5; A_2 – methanol-ammonia 99:1 [4]; B_1 – ethylacetate-methanol-ammonia 85:10:5; B_2 – acetone-water-ammonia 20:20:1.

Mobile phase for one dimensional chromatography of methamphetamine: chloroform-2-propanol-ammonia 20:20:1.7.

Methamphetamine was detected with Fast Black K salt [6], morphine with Marquis reagent and as dansyl derivative, prepared in situ prior development. Dansylchloride derivatization: prepare 0.1% solution of dansylchloride in acetone and 8% water solution of sodium bicarbonate. Apply 1 μl of dansylchloride solution on a spot of the sample. Dry briefly with hot air blower. Then apply 2 μl of sodium bicarbonate and dry for 7

min at 80°C. Derivatize standard and blank sample in the same way. After development observe under UV-light 254 nm.

GC/NPD, SPD on HP-gas chromatograph 5890 with capillary columns HP-5 for ECD and HP-17 for NPD was used for the confirmation of methamphetamine as pentafluorobenzoyl derivative. Internal standard cyclohexylamine.

Temperature programme: HP-5 80–290°C, HP-17 60–290°C, injector 300°C, detectors – NPD 300°C, ECD 330°C. Time of the analysis 35 minutes [1].

GC/MS on Finnigan Mat Magnum was used for the confirmation of morphine as trimethyl silylderivative. Analysis condition of the: capillary column Alltech Econo-Cap 30 m x 0.25 mm x 0.25 mm, phase EC-5 (SE-54), temperature programmable injector SPI 85–255°C, temperature of MS transfer line 270°C, temperature programme 85–275°C, analysis time 25 minutes.

RESULTS AND DISCUSSION

Water proved to be the best medium for the homogenization of meconium. The homogenization with methanol was very difficult and the results were irreproducible, even when the extracts were clearer.

The detection limits of the respective extraction procedures for methamphetamine and morphine are presented in Table I.

The choice of the suitable organic solvent is crucial for the isolation. The differences in the detection limit between LLE and SPE are not significant, but SPE can be recommended for the isolation, because of clear extract residues in comparison with LLE. When using Tox Elut columns, the opalescent extract often appeared and the results were inconsistent. Therefore Chem Elut columns were preferred for SPE.

Only in several cases was a concentration of 0.25 μg/g detected ocassionally. Therefore we take as the lowest detection limit 0.5 μg/g, as a value detected unambiguously.

Emit response for methamphetamine and morphine in meconium eluted with water is shown in Figure 1 together with the cut off level we use for urine. The response of 0.02 QUAL was taken as positive. The brown-green colour of supernatant does not interfere. However some specimens remain too dense after centrifugation and cannot be measured by Emit. The dilution and repeated centrifugation may help to solve this problem.The extraction of meconium with organic solvent, evaporation and reconstitution in buffer, water or urine did not improve Emit measurement.

TABLE I. DETECTION LIMIT MECONIUM [μg/g]

Method	Methamphetamine		Morphine	
	Homo-genate	Superna- tant	Homogenate	Supernatant
LLE				

Butylchloride	0.50	0.75	0.00	0.00
Cyclohexane	0.75	1.00	–	–
Chloroform	0.75	0.75	0.75	0.50
Ethylacetate-2-propanol 9:1	–	–	–	1.00
SPE				
Butylchloride	–	1.00	–	–
Dichloromethane-2-propanol 9:1	–	0.50	–	0.50
Chloroform	–	1.00	–	–

* Chem Elut column, supernatant diluted with phosphate buffer pH 8.05.

HPTLC proved to be sufficiently sensitive for the detection of low concentrations of both drugs. The detection limit of Fast Black K for methamphetamine was 0.05 μg/spot, for morphine as dansyl derivative 0.01 μg/spot. 2-HPTLC removes or restricts the background interference and enables an unambiguous identification of analyzed drugs in lower concentrations than commonly used one dimensional chromatography. An example of methamphetamine detection is presented in Figure 2.

The lowest concentration of methamphetamine detected by GC/NPD, ECD was the same as by 2-HPTLC. However this method is more sensitive for the amphetamine group. In 4 cases amphetamine was detected together with methamphetamine, whereas by 2-HPTLC only once. Chromatograms for both detectors are presented in Figures 3 and 4.

GC/MS was used only 3 times for technical reasons.

An analysis of meconium samples was requested by 3–4 Prague hospitals, usually when abstinence syndrome appeared in the newborn, the birth weight was too low or the mother was suspected of taking drugs. No epidemiological study was requested.

In a period of 20 months we analyzed 39 cases. In 29 of them both meconuim and urine were available. An overview of the analyses requested and number of positive meconium findings is presented in Table II. A comparison of positive findings in meconium and urine when both specimens were available is shown in Table III. In other positive samples only meconium was delivered for analysis.

TABLE II. POSITIVE MECONIUM FINDINGS IN REQUESTED ANALYSIS

Requested analysis	Number	Positive
Methamphetamine	15	9
Morphine	12	8
Methamphetamina + morphine	6	1
Nonspecified	6	0

*Morphine was detected.

Fig. 1. EMIT response for methamphetamine and morphine.

Fig. 2. Methamphetamine detection by 2-HPTLC.

Fig. 3. Chromatogram of methamphetamine detection by GC-NPD.
CYHA – cyclohexylamine, internal standard.
MAMPH – methamphetamine.
Papaverin(e) – external standard for GC/NPD.

Fig. 4. Chromatogram of methamphetamine detection by GC-ECD.
MAMPH – methamphetamine.
EF – ephedrine.
Griseofulvin(e) – external standard for GC/ECD.

TABLE III. COMPARISON OF POSITIVE FINDINGS IN MECONIUM AND URINE

Substance		Methamphetamine	Morphine
Number of cases with both specimens		6	8
Positive:	Meconium + urine	4	6
Meconium		–	2
	Urine	2	–

In all real cases with enough meconium available at least two isolation procedures were employed and two analytical methods were used. In this way we were able to evaluate the extraction efficiency and the reliability of the screening at the low cut-off values better than for the spiked samples. The Qual values of Emit in urine were about 10 times higher than in meconium, but according to the semiquantitative estimation of the chromatograms the differences in the concentration between both specimens were not so high. The explanation is probably the colour of the meconium eluate.

In one case the Qual value for methamphetamine in the urine of the newborn was extremely high, appearing rarely even in the urine of drug dependent patients, and methamphetamine, amphetamine and ephedrine were identified in urine. Meconium

was negative. The probable interpretation was, that the mother has applied a high dose of methamphetamine not long before the delivery.

CONCLUSION

1. The isolation procedure is the crucial step for the analysis of meconium and must be tested for each drug and analytical method employed.
2. 2-HPTLC proved to be sufficiently sensitive and can be used as a method of choice for the analysis of meconium.
3. The last drug intake is difficult to determine, if urine and meconium are positive and the analysis of mother's urine is not available.

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