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Short communication

Detection of methadone in diaper matrix: A downscaled protocol for pediatric diagnostics

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Introduction

The utilization of methadone within the pediatric population has gained significant momentum over the last decade. Primarily administered in controlled clinical settings under medical supervision, it is indicated for pain management, palliative care, and the treatment of neonatal abstinence syndrome following intrauterine exposure (Mott et al., 2018). Regarding lactation, methadone is not contraindicated, as it exhibits low excretion into breast milk, provided the maternal dosage remains stable and monitored (NICHD, 2026).

Despite its clinical justification, there is a rising incidence of pediatric methadone intoxication (Jelodarzadeh et al., 2021). Such toxicity also may result from accidental ingestion or, in distressing instances, intentional harm, such as child abuse.

In pediatric diagnostics, particularly for children under the age of three, biological sample collection presents significant physiological and volume constraint. When conventional matrices, as blood and urine, are unavailable or insufficient, there is a crucial need for a substantiated extraction procedure for the reliable detection of methadone in alternative samples, such as diapers. The objective of this study is to establish a standardized sample preparation protocol for the qualitative analysis of methadone in diaper material.

Materials and methods

Stock standard solutions of methadone and methadone-d9 were supplied by Sigma Aldrich (Germany) and Lipomed (Switzerland), respectively. Working solutions were prepared by diluting the stock solutions with methanol HPLC grade obtained from Fisher Chemical (Belgium). Aqueous calcium chloride solution (150 g/L) was prepared using $CaCl_2$ purchased from Merck (Germany). Commercially available disposable diapers (Sleepy) were used for the analyses.

Sample preparation was adapted from the method described by Liu et al. (2012), with significant modifications optimized for methadone detection. A urine-saturated section of the diaper was cut into a 3 x 3 cm specimen and placed in a plastic tube. To this, 16 mL of aqueous $CaCl_2$ solution was added. The mixture was vortexed for 1 min, subjected to ultrasonic pre-treatment for 15 min, and the resulting extract was filtered through Whatman Grade 42 filter paper. Methadone-d9 was added as an internal standard achieving a final concentration of 200 ng/mL, and the filtrate was centrifuged for 15 min at 3000 rpm. Following these pre-treatment steps, the

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sample underwent solid-phase extraction using Oasis MCX cartridges (Waters Co., USA) according to the manufacturer's protocol. The eluates were evaporated to dryness and reconstituted in 0.2 mL of methanol.

Chromatographic analysis was performed using a GC-MS 2010 Ultra System (Shimadzu, Japan) equipped with a SPB-5 30 m x 0.25 mm x 0.25 μm capillary column (Supelco, USA) and helium carrier gas. SIM mode was utilized for analyte identification. Methadone was monitored using *m/z* 72 as the target ion, and *m/z* 223, 165, and 294 as reference ions. For methadone-d9, *m/z* 78 and 303 were monitored as the target and reference ions, respectively.

Results and discussion

Method selectivity was confirmed by the absence of interferences at the retention time of methadone and the IS. The relative abundances of the reference to target ions were 0.96% for *m/z* 223 and 0.72% for *m/z* 165. The stability of these ion ratios was successfully verified, yielding acceptable coefficients of variation (CV) of 3.78% and 5.76%, respectively. Although the abundance of the *m/z* 294 ion ratio fell below the reliable threshold due to low signal intensity, its consistent detection provides critical qualitative confirmation for methadone, offering structural specificity. The limit of detection was established at 200 ng/mL, aligning with standard regulatory decision points for methadone confirmation in urine screenings (EWDTS, 2022). Furthermore, the extraction efficiency was satisfactory, achieving 53.03% recovery.

Compared to existing literature protocols that demand substantially larger sample sizes and consequently elevated reagent volumes (Lucarini et al., 2021), this optimized method offers distinct advantages in scaling down sample consumption and minimizing chemical waste.

To bypass the challenge of dealing with an irregular and absorbing matrix, a gravimetric approach was introduced to estimate the volume of absorbed urine based on the mass of the saturated specimen. Standardizing the diaper matrix dimensions to 3 x 3 cm yielded a reproducible dry weight of 0.67 g. A linear correlation was observed between fluid absorption and mass, with every 1 mL of introduced urine consistently contributing an additional 1.01 g to the total mass. The same linear correlation was observed up to 11 mL of urine added on the declared sample size. This predictable gravimetric relationship enables reliable estimation of unknown urine volumes during routine qualitative forensic or clinical screenings.

Conclusion

Despite the challenges of the sample matrix, the proposed method has shown accurate and reliable results. Additionally, minimal sample size and chemicals are required for analysis of such a complex sample material. Beside the favorable findings, there are variables that should be further tested to prove the applicability of the method for broader spectrum of compounds and different sample conditions.

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