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Detection of mesocarb metabolites excreted free and conjugated with sulphate in human urine by UPLC-MS/MS

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Introduction

Mesocarb is a substance with a central nervous system stimulant activity included on the list of prohibited substances of the World Anti-Doping Agency. Unchanged mesocarb and ten metabolites were identified in human urine in previous studies [1,2,3,4].

In this work, methods to screen and to confirm for the presence of mesocarb metabolites in human urine have been optimized based on the detection of urinary mesocarb metabolites excreted free and conjugated with sulphate.

Experimental

Chemicals

Reference standards of p-hydroxymesocarb and p-hydroxymesocarb-sulphate were supplied by United Laboratories Ltd. (Helsinki, Finland).

Sample preparation

The sample preparation for screening and confirmation purposes was based on a procedure previously described [1,2,5]. For screening purposes UPLC conditions described in a previous work have been used [5]. For confirmation purposes, a slow gradient elution was used: from 0 to 1 min, 5% B; from 1 to 16 min, to 90% B; during 1.6 min, 90% B; from 17.6 to 18 min, to 5% B; from 18 to 20 min, 5% B, (A: 0.01% formic acid in deionized water; B: 0.01% formic acid in acetonitrile) at a flow rate of 0.4 ml/min. MS/MS conditions for screening and confirmation are shown in **Table 1**.

The procedure was validated for p-hydroxymesocarb and p-hydroxymesocarb sulphate using a procedure previously described [6]. The following parameters were evaluated: selectivity and specificity, limit of detection (LOD), extraction recovery and intra-assay precision.

Excretion study samples

Urine samples obtained after administration of a single dose of 10 mg of mesocarb (Sydnocarb®) to a healthy volunteer were collected for 48h, according to a clinical protocol was approved by the Local Ethical committee (CEIC-IMAS, Institut Municipal d'Assistència Sanitària, Barcelona, Spain).

Results

For screening purposes, multiple reaction monitoring (MRM) of one transition of the main metabolites (p-hydroxymesocarb and p-hydroxymesocarb sulphate) was used. The extraction recoveries were 100.3±0.8% and 105.9±10.8% (n=4) for p-hydroxymesocarb and p-hydroxymesocarb sulphate, respectively. Limits of detection were 50 ng mL^{-1} for both metabolites. The intra-assay precision was estimated at two concentrations (50 and 250 ng mL^{-1}, n=4) and relative standard deviations were lower than 15% in all cases. The compounds were included in the screening method for diuretics and acidic compounds [5].

For confirmation purposes, a slow gradient elution was used. After analysis of excretion study samples ten metabolites were detected including free compounds and conjugates with sulphate. Free metabolites previously described by other authors were detected [1,3,4]. Sulphated metabolites were identified by the characteristic fragmentation profile of their pseudomolecular ion, $[M+H]^+$. The main metabolite was p-hydroxymesocarb conjugated with sulphate. Product ion mass spectra of the main mesocarb metabolites and proposed fragmentation profile are presented in **Figure 1**. Chromatograms of excretion study samples are presented in **Figure 2**.

Conclusions

The screening method allows determination of most abundant metabolites of mesocarb with a simple extraction step and a chromatographic analysis of 5 minutes [5]. The confirmation method allows detection of ten mesocarb metabolites. The suitability of the developed methods to detect mesocarb ingestion was demonstrated by analysis of urines obtained up to 48 h after administration of a single oral dose of mesocarb to healthy volunteers.

References

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Table 1. UPLC-MS/MS data: ionization mode (ESI +/-), retention time (RT), precursor ion (PI), product ion (DI), cone voltage (CV), and collision energy (CE).

Compound	ESI	RT (min)	PI (m/z)	CV (V)	DI (m/z)	CE (eV)
Screening method						
7-Propyltheophylline	+	2.11	223	25	181	15
p-Hydroxymesocarb	+	2.54	339	20	91	15
p-Hydroxymesocarb sulphate	+	2.40	419	15	119	10
Confirmation method						
Mesocarb	+	8.84	323	20	119	10
p-Hydroxymesocarb	+	7.00	339	20	193	10
p-Hydroxymesocarb sulphate	+	6.13	419	15	119	10
Hydroxymesocarb (2 isomers)	+	7.60/7.72	339	20	177	10
Hydroxymesocarb sulphate	+	6.00	419	20	177	10
Dihydroxymesocarb (2 isomers)	+	5.14/5.24	355	20	193	10
Dihydroxymesocarb sulphate (2 isomers)	+	5.46/5.56	435	15	273	10
Trihydroxymesocarb (2 isomers)	+	4.46/4.52	371	20	193	10

Figure 1. Product ion mass spectra of $[M+H]^+$ of p-hydroxymesocarb ($[M+H]^+$ 339) and p-hydroxymesocarb sulphate ($[M+H]^+$ 419).

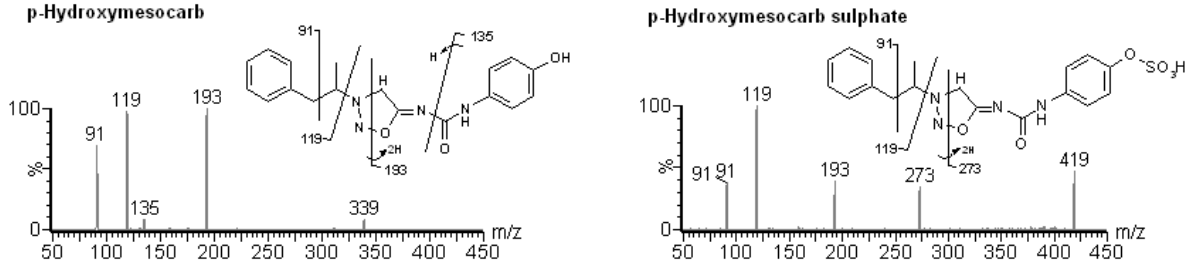

Figure 2. Confirmation method: MRM chromatograms of mesocarb metabolites: blank urine (left); urine obtained 4-8h after administration of mesocarb (middle); urine obtained 24-36h after administration of mesocarb (right).