

Determination of morphine in urine after the therapeutic administration of a common cough mixture, “Brown Mixture”, containing camphorated opium tincture

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Introduction

Morphine and codeine are naturally occurring alkaloids that are found in opium, a substance exuded from the unripe seed pod of the opium poppy *Papaver somniferum*. Opium contains morphine at 4-21 wt% and codeine at 0.7-3 wt% [1]. Morphine and codeine are potent narcotic analgesic substances and are found in some medication. Because of its severe side effects and drug addiction effect, morphine is a controlled substance and, for athletes, its use is prohibited by the World Anti-Doping Agency (WADA) code. A morphine urinary concentration (free and glucuronide conjugate) greater than 1 µg/ml constitutes an adverse analytical finding unless it may have resulted from the administration of a permitted substance such as codeine [2]. In this work, the concentration of morphine and codeine in urine was determined after administration of a common cough mixture containing camphorated opium tincture known in Thailand as Brown Mixture (BM) (Fig. 1). These medications are sold without prescription.



Figure 1: Pictures of Brown Mixture products A. MIST SCILL AMMON from GPO B. LEOPARD MEDICAL BRAND and C. GPO (Government Pharmaceutical Organization)

Excretion study

The aim of the first study was to determine the concentration of morphine and codeine in human urine after therapeutic use of BM from three different manufacturers. Three healthy

male volunteers took the medication as recommended on the bottle label, i.e. 10 ml, 3-4 times per day, for 3 days. Spot urine samples were collected over 5 days and kept frozen until analyzed. The second study took place 7 days after the first study. This involves an increased dose of BM from the same three manufacturers. Three healthy male volunteers took the medication at dose of 20 ml, 3-4 times a day. Spot urine samples were collected during 3 days and frozen until analyzed.

Sample Preparation

The urine samples were analyzed for morphine and codeine according to our standard operating procedure for the screening of narcotics and β -blockers (Screening Procedure VI) [3]. The morphine glucuronides were enzymatically hydrolyzed by β -glucuronidase from Helix Pomatia, at pH 5.2 and 55 °C, for 3h. The free morphine was extracted from urine at pH 9.6 with TBME. After centrifugation, the organic layer was transferred into a glass tube and evaporated to dryness. The residue was derivatized with 100 μ L of MSTFA, with heating at 80 °C for 10 min and then 30 μ L MBTFA added, with heating at 80 °C for 10 min. However it has been reported [4] that acid hydrolysis is more efficient than enzyme hydrolysis.

GC-MS analysis

GC-MS analysis was carried out on an Agilent 6890 gas chromatograph directly coupled to an Agilent 5973 mass selective detector (Agilent, Palo Alto, USA). The GC column was an Ultra 1 (J&W, Folsom, USA), 100% methylsilicone with a length of 25 m, internal diameter of 0.2 mm and film thickness of 0.11 μ m. The oven temperature program was as follows: 120 °C (0 min) - 25 °C/min - 320 °C (3 min). Injection (2 μ l) was by split mode (10:1) at 280 °C. The mass spectrometer was operated in the SIM mode.

Results

The retention times (RT) and characteristic ions of codeine, morphine and ISTD (Nalorphine) as the TMS derivatives are listed in Table 1. The urinary excretion profiles of morphine for the first study after the recommended dose of BM from three different manufacturers are shown in Figs. 2, 3 and 4, respectively. The urinary excretion profiles of morphine in the second study are shown in Fig. 5. Morphine and codeine in urine were quantified using a sample of blank urine spiked with standard morphine and codeine at 1.0 μ g/ml.

Table 1. Retention time (RT) of Codeine, Morphine and Nalorphine (ISTD) and characteristic ions of TMS derivatives

Compound	RT (min)	Ions (m/z)
Codeine	6.31	371, 178, 234
Morphine	6.54	429, 401, 414
Nalorphine (ISTD)	6.92	455, 414

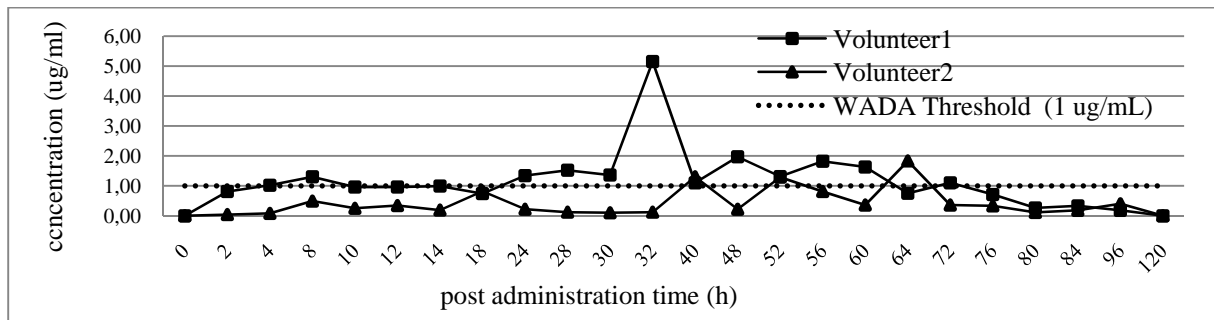


Fig. 2. Urinary morphine concentration profiles after the administration of MIST SCILL AMMON from GPO. Volunteer 3 was not available during this study.

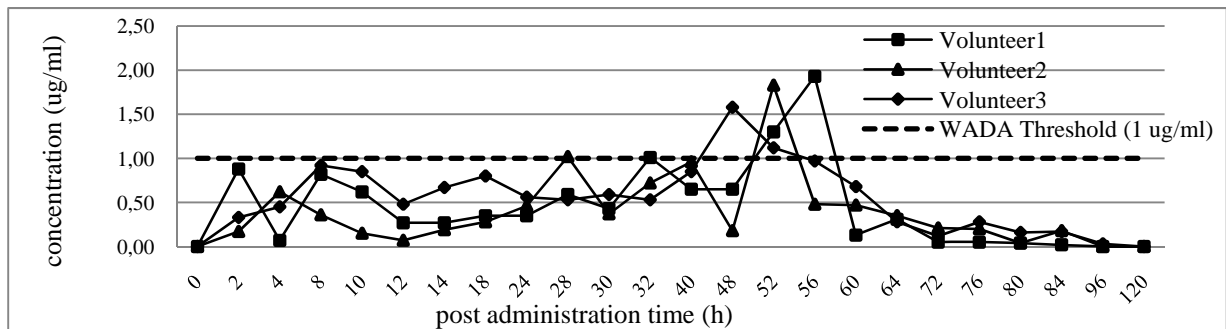


Fig. 3. Urinary morphine concentration profiles after the administration of Brown Mixture from LEOPARD MEDICAL BRAND

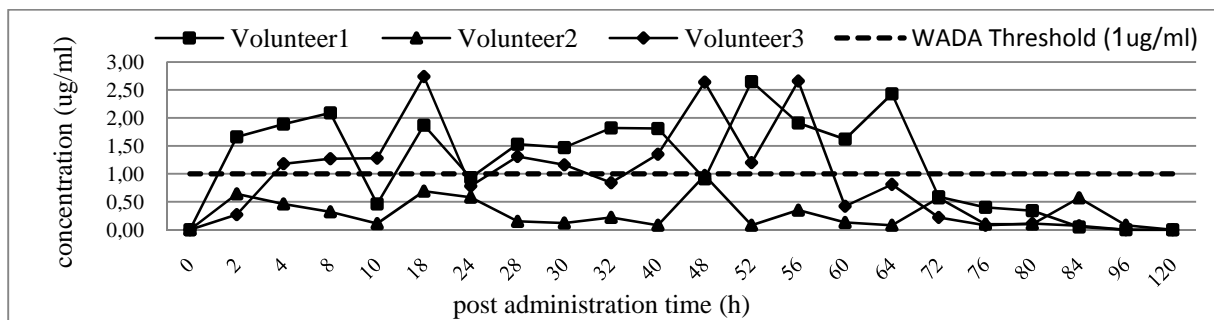


Fig. 4. Urinary morphine concentration profiles after the administration of Brown Mixture from GPO

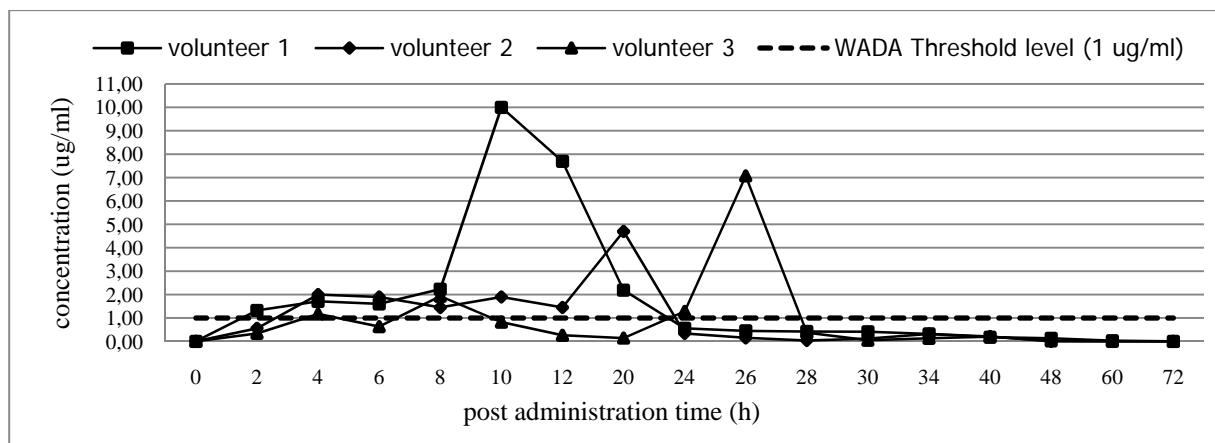


Fig. 5. Urinary morphine concentration profiles after the administration of increased dose of Brown Mixture (BM) from the three manufacturers. Volunteer 1 was administered MIST SCILL AMMON, Volunteer 2 LEOPARD MEDICAL BRAND and Volunteer 3 GPO cough mixture, respectively.

Conclusion

The morphine concentrations in the urine ranged between 0.97 to 5.1 $\mu\text{g/ml}$. Codeine was also found at level between 0.01 to 0.14 $\mu\text{g/ml}$. These findings demonstrate that the administration of a common cough mixture containing camphorated opium tincture, known in Thailand as Brown Mixture (BM), which is sold without prescription, can lead to an adverse analytical finding for morphine.

Reference

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