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Determination of oxycodone and its major metabolites in haematic and urinary matrices: Comparison of traditional and miniaturised sampling approaches

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ABSTRACT

Oxycodone is a widely prescribed, full agonist opioid analgesic. As such, it is used clinically to treat different kinds of painful conditions, with a relatively high potential for doping practices in athletes.

In this paper, different classic and innovative miniaturised matrices from blood and urine have been studied and compared, to evaluate their relative merits and drawbacks within therapeutic drug monitoring (TDM) and to implement new protocols for anti-doping analysis. Plasma, dried blood spots (DBS) and dried plasma spots (DPS) have been studied for TDM purposes, while urine, dried urine spots (DUS) and volumetric absorptive microsamples (VAMS) from urine for anti-doping.

These sampling techniques were coupled to an original bioanalytical method based on liquid chromatography-tandem mass spectrometry (LC–MS/MS) for the evaluation and monitoring of the levels of oxycodone and its major metabolites (noroxycodone and oxymorphone) in patients under pain management and in athletes. The method was validated according to international guidelines, with good results in terms of precision, extraction yield and accuracy for all considered micromatrices. Thus, the proposed sampling, pre-treatment and analysis are attractive strategies for oxycodone determination in human blood and urine, with advanced options for application to derived micromatrices. Microsampling procedures have significant advantages over classic biological matrices like simplified sampling, storage and processing, but also in terms of precision (<9.0% for DBS, <7.7% for DPS, <7.1% for DUS, <5.3% for VAMS) and accuracy (>73% for DBS, >78% for DPS, >74% for DUS, >78% for VAMS). As regards extraction yield, traditional and miniaturised sampling approaches are comparable (>67% for DBS, >74% for DPS, >75% for DUS, >75% for VAMS). All dried matrices have very low volumes, leading to a significant advantage in terms of analysis feasibility. On the other hand, this also leads to a corresponding decrease in the overall sensitivity.

1. Introduction

Opioids represent a highly effective class of drugs widely prescribed for the treatment of moderate to severe pain [1]. They include natural (e.g. morphine), semi-synthetic (e.g. oxycodone, OXC) and synthetic (e.g. fentanyl) compounds. Regarding semi-synthetic opioids, one of the most important representatives is certainly OXC ((5*R*,9*R*,13*S*,14*S*)-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one, Fig. 1a), which is synthesised from thebaine [2]. The first clinical use of OXC dates back to the early 20th century, but in the last few years it has become the most prescribed narcotic medication for treating moderate to severe pain [3]. OXC is *N*-demethylated to noroxycodone ((1S,5*R*,13*R*,17S)-17-hydroxy-

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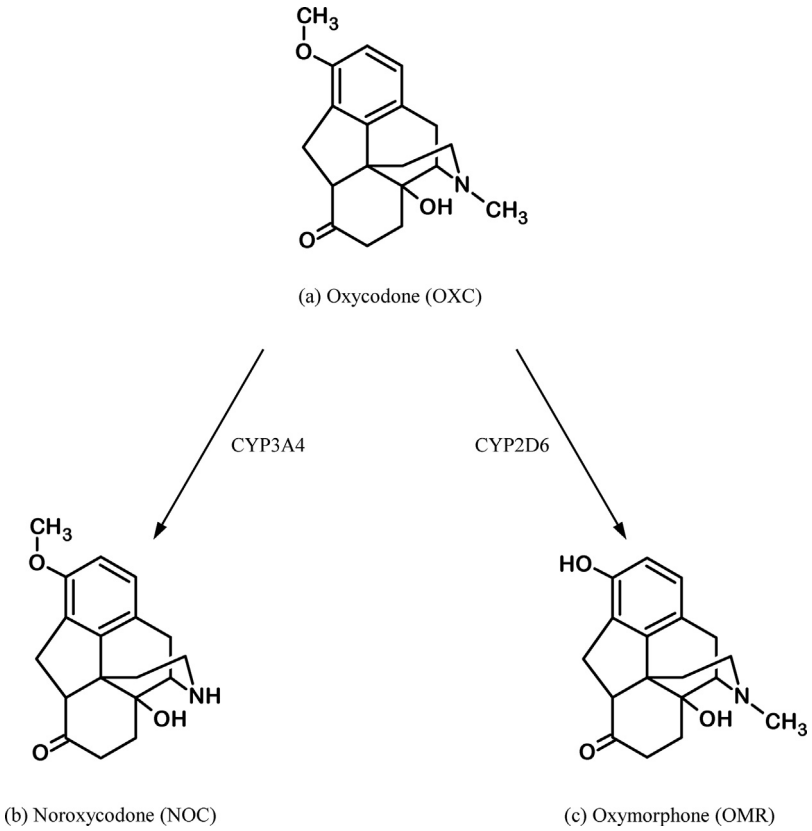

Fig. 1. Chemical structures of (a) OXC, (b) NOC, (c) OMR.

12-oxa-4-azapentacycloocatadeca-7(18),8,10-trien-14-one, NOC, Fig. 1b) and *O*-demethylated to oxymorphone (4,5α-epoxy-3,14-dihroxy-17methylmorphinan-6-one, OMR, Fig. 1c) [4]. *N*-demethylation is carried out by cytochrome P450 subtype 3A4 (CYP3A4), while CYP2D6 catalyses the *O*-demethylation pathway [5] (Fig. 1). Both NOC and OMR have antinociceptive properties, but NOC activity is considerably lower than that of OXC. OMR potency is similar to that of OXC; however, its plasma levels are usually much lower and its contribution to the overall analgesic effects is still debated [4]. Due to its full opioid agonist activity, OXC can potentially lead to severe side effects, addiction, misuse and withdrawal upon discontinuation [6]. For these reasons, two main areas of OXC use monitoring and control can be individuated. Firstly, chronic pain patients treated with OXC can greatly benefit from Therapeutic Drug Monitoring (TDM), with the determination of blood or plasma levels and their correlation to side, toxic and therapeutic effects [7,8]. Unfortunately, in these last few years several public health problems occurred, particularly in the USA: skyrocketing numbers of side effects, with many fatal cases; correspondingly increasing numbers of misuse cases; thousands of patients in need of addiction treatment [9]. Monitoring the use and potential abuse of OXC during therapy is one of the main actions taken to minimise the risk of addiction and is now becoming a pressing necessity [10], which has often to be carried out in less than optimal settings and conditions.

Secondly, reliable methods to detect this drug use are very important in the field of anti-doping control, in order to reveal possible frauds carried out by professional or amateur athletes.

Since both these two application fields are currently dominated by traditional sampling methods and procedures, aim of this study was the development and application of innovative collection methods for OXC, NOC and OMR determination in biological microsamples, to be compared to classic ones. In particular, TDM is normally carried out on blood samples drawn from patients, from which plasma or serum is then obtained and finally analysed [11]. We have implemented two original sampling procedures based on dried blood spots (DBS) and dried plasma spots (DPS): they sample microamounts of hematic matrix and facilitate pre-treatment, also allowing the easy handling, storage and transportation of many samples with reduced requirements for safety, refrigeration and space [12].

Regarding illicit uses of OXC, doping is one of the areas of concern, where the large majority of assays are carried out in urine. Thus, two different microsampling strategies have been devised and tested for this purpose: dried urine spots (DUS) and volumetric absorptive microsamples (VAMS). DUS provide advantages over classic urine samples that are similar to those of DBS or DPS in comparison to plasma [13]. On the other hand, VAMS are obtained by means of innovative samplers that can produce highly-reproducible dried specimens, overcoming some specific disadvantages of dried sample spots, such as area bias and homogeneity issues [14,15]. Although the VAMS approach has been proposed for blood, information on its application to other biological matrices is rather sparse [16]. This study, in fact, represents one of the first attempts to explore VAMS suitability as an approach to urine sampling for anti-doping purposes.

Both blood-based and urine-based samples were analysed using an original, fully validated LC–MS/MS method, providing high sensitivity and selectivity.

Numerous analytical methods have been published for the determination of OXC in biological fluids (among the most recent ones: [17–24]). However, methods suitable for both human blood- and urine-based matrices are very few [18], as are those dealing with micromatrices [17]. None of them includes the comparison of different, original microsampling procedures with significant