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Determination of prednisolone and prednisone in urine after therapeutic administration of an ophthalmic solution

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INTRODUCTION

According to WADA 2009 Prohibited List, the administration of glucocorticosteroids as an ophthalmic preparation is not prohibited. Moreover it does not require a Therapeutic Use Exemption or a declaration of use. It would therefore be of interest to ascertain the level of these steroids in the urine when used in this manner, especially whether it is above or below the Minimum Required Performance Limit, MRPL (30 ng/mL).

In this work, the concentration of prednisolone and prednisone in urine was determined after administration of a prednisolone ophthalmic eye drop solution.

EXPERIMENTAL

The subject (a healthy 64 years old male) had undergone a cataract operation to one eye and applied the prednisolone ophthalmic eye drops (Pred Forte[®], Allergan[®], 1% prednisolone acetate suspension) daily for 9 days, as directed by the physician. The medication was administered one drop (25-30 µL) every 2 hours, for a total of 9 times per day. Spot urine samples were collected over 9 days and stored frozen until analyzed.

Sample preparation

Urine samples (2.5 mL) were hydrolyzed by β -glucuronidase (*E. coli*) for 1 hr at 55°C. An extraction at basic condition followed by a second extraction at acidic condition with *t*-butyl methyl ether were performed. The combined extracts were evaporated to dryness under a N₂ gas stream, and then reconstituted with mobile phase (1% acetic acid in water:acetonitrile, 80:20,v/v) for subsequent instrumental analysis by LC+ESI-MS/MS in MRM mode.

Instrumentation

The analyses were performed on an Agilent 1200 Series liquid chromatography coupled to a 6410 Triple Quadrupole mass spectrometer. The LC was equipped with a Hypersil ODS-C18 analytical column (60 mm x 4.6 mm i.d., 3 µm particle size) from Thermo Scientific. The mobile phase consisted of 1% acetic acid in water (A) and acetonitrile (B). The gradient started at 30% B increasing to 65% in 5 min and held for 10 min, then back to 30% B in 1.5 min and held for 2 min, with a flow rate of 0.5 mL/min. The mass spectrometer was operated in positive electrospray ionization mode (ESI+) with a capillary voltage of 5000 V at 325°C. All analytes as well as the internal standard (ISTD, Methyltestosterone) were detected by means of characteristic product ions utilizing the multiple reaction monitoring mode (MRM).

Table 1. MRM parameters for the steroids.

Compound	Precursor ion, m/z	Product ion, m/z
Prednisolone	361.0	343.2, 307.2
Prednisone	359.2	147.1, 267.1
Methyltestosterone (ISTD)	303.2	97.0, 109.1

RESULTS AND DISCUSSION

After the first application of the eye drop solution, prednisolone was detected after 4 hours. Prednisone was also observed due to the *in vivo* interconversion of prednisolone and prednisone ⁽²⁾, and was first excreted after 14 hours. Since the number of urine samples collected varied from 1 to 5 samples per day, only the mean daily concentration of the spot urine samples is shown in Figure 1 and 2. The maximum levels were 45 ng/mL and 90 ng/mL for prednisolone and prednisone, respectively. The ratio of prednisolone:prednisone varied from 0.2 to 1.0, with mean of 0.6 ± 0.2 (SD).

Prednisolone and prednisone could be detected for up to 24 hours after end of administration, *i.e.* after Day 9. The large fluctuation in the levels of the 2 steroids may be due to the variability in the absorption of the colloidal solution.

The limit of detection of prednisolone and prednisone are 0.01 ng/mL and 0.004 ng/mL, and the linear range of the calibration is 0.1 ng/mL to 500 ng/mL, respectively. The precision of the screening procedure is 9.5% RSD.

CONCLUSION

This study showed that prednisolone and prednisone were detected in some spot urine at levels exceeding the current WADA specified reporting MRPL of 30 ng/mL.

The administration of a prednisolone ophthalmic solution can lead to positive urines.

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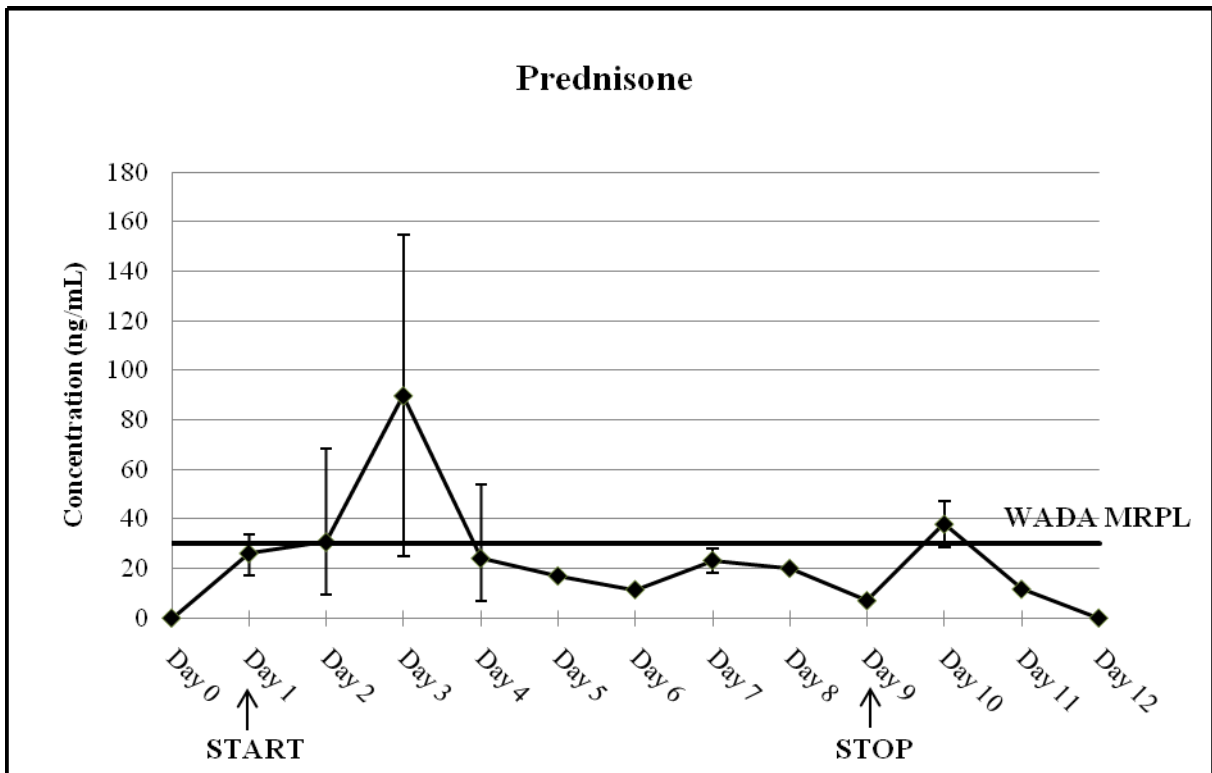
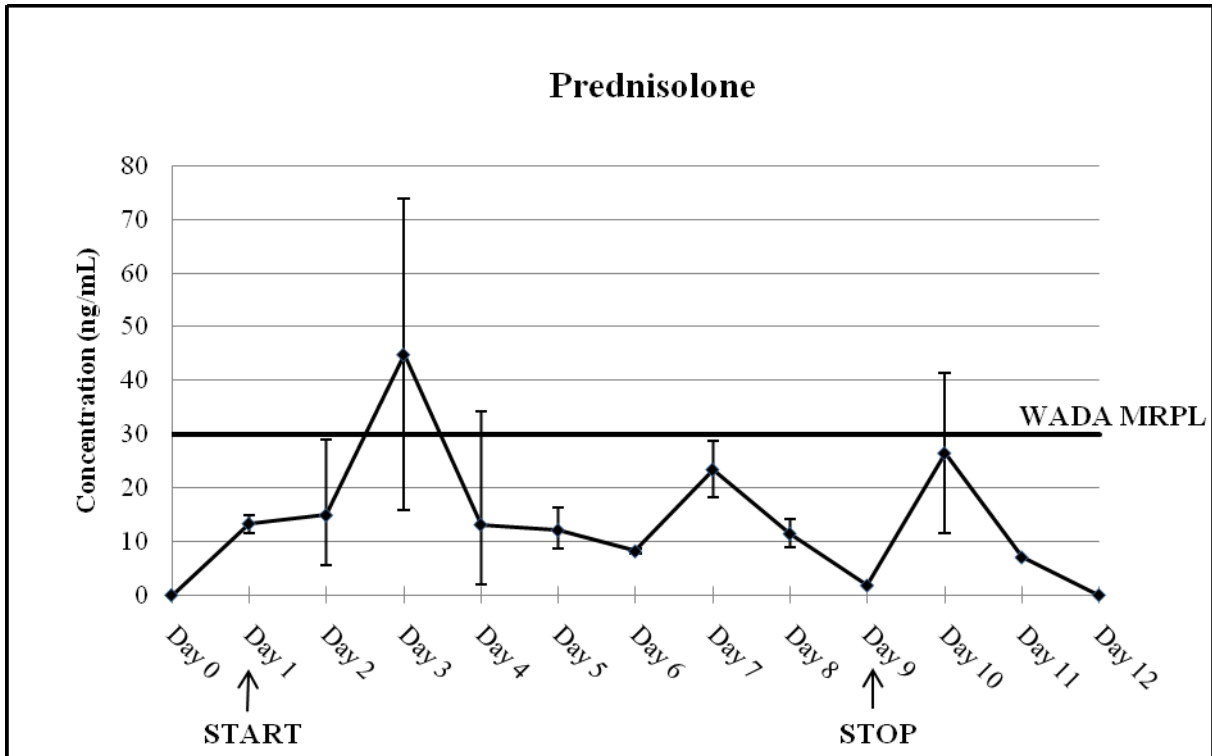


Figure 1. The daily mean concentration of prednisolone excretion. (upper)

Figure 2. The daily mean concentration of prednisone excretion. (lower)

The error bar indicates the range of concentration of the urine samples.