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## **Investigation of possible interference of a metabolite of etoricoxib (Arcoxia®) in the detection of 16a-hydroxyprednisolone by LC-MS/MS**

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### **Abstract**

Etoricoxib (Arcoxia®) is a recent drug for the effective treatment of pain, inflammation and fever. It is available without prescription in Thailand but may require a physician's prescription in other countries. This investigation was initiated by the presence of a large peak in about 10% of the MRM chromatograms of our screening procedure for corticosteroids by LC-MS/MS. This peak was in the time window of the MRM transition for 16a-hydroxyprednisolone. Fortunately for some of these samples the data sheet for previous use of medication stated the use of Arcoxia®.

To confirm that the interfering peak was due to a metabolite of the drug etoricoxib (Arcoxia®) an excretion study of a male volunteer who had taken a 30-mg single oral dose of etoricoxib was performed. Metabolites of the drug were identified by comparison of the observed mass spectra (both full scan and product ion scan) with that reported in the literature. It was shown that the interference peak was 6'-hydroxymethyl-etoricoxib. The mass spectrum information of this metabolite was then used to modify the MRM transition for 16a-hydroxyprednisolone in the screening method.

### **Introduction**

Analgesic drugs are widely available without prescription in Thailand and may be used by athletes. Etoricoxib (Arcoxia®) is a common analgesic drug sold in drug stores. We noted an interference peak in the LC-MS/MS screening method for corticosteroids from athletes who declared the use of this drug. An excretion study of this drug was performed to confirm the source of this peak and subsequent modification of the screening parameters.

### **Experimental**

An excretion study of a male volunteer who had taken a single 30 mg oral dose of etoricoxib was carried out. The urine was collected over 6 hours post administration. The pooled urine was analyzed by LC-MS/MS in both full scan and product ion scan modes. Excretion urine and samples that had declaration for use of Arcoxia® were analyzed together using the standard LC-MS/MS screening procedure [1]. The sample preparation consisted of enzymatic hydrolysis (glucuronidase, E.coli) and liquid-liquid extraction with *tert*-butylmethylether in basic and acidic condition [2]. Chromatography was carried out on an Agilent 1090 triple-quadrupole system using a Phenomenex Luna C18(2) column (100 x 2.0 mm, 3 µm). The mobile phase was 0.1% formic acid in ammonium acetate (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a constant flow rate of 0.3 mL/min. The gradient program was as follows: 10% B to 40% B in 13.5 min and then to 80% B in 7.5 min, returning to 10% B over 4 min. Total run time was 25 minutes.

Metabolites of the drug in the excretion urine sample were identified by comparison of the observed mass spectra with that reported in the literature [3].

## Results and Discussion

The parent compound and three metabolites of etoricoxib (6'carboxy-etoricoxib, 6'hydroxymethyl-etoricoxib glucuronide and 6'hydroxymethyl-etoricoxib-1'-N-oxide) were identified in the excretion urine [3]. From the chromatogram, etoricoxib PC was found at 11.651 min ( $[M+H]^+$ , m/z 359), 6'carboxy-etoricoxib at 12.017 min ( $[M+H]^+$ , m/z 389), 6'hydroxymethyl-etoricoxib at 11.760 min ( $[M+H]^+$ , m/z 375) and 6'hydroxymethyl-etoricoxib-1'-N-oxide at 12.017 min ( $[M+H]^+$ , m/z 391) (Figure 1). The interference peak in an athlete's sample was found at 11.787 min (Figure 2) with an intensity 2 times higher than that of 16 $\alpha$ -hydroxyprednisolone (at MRPL level) which has a retention time of 10.261 min.

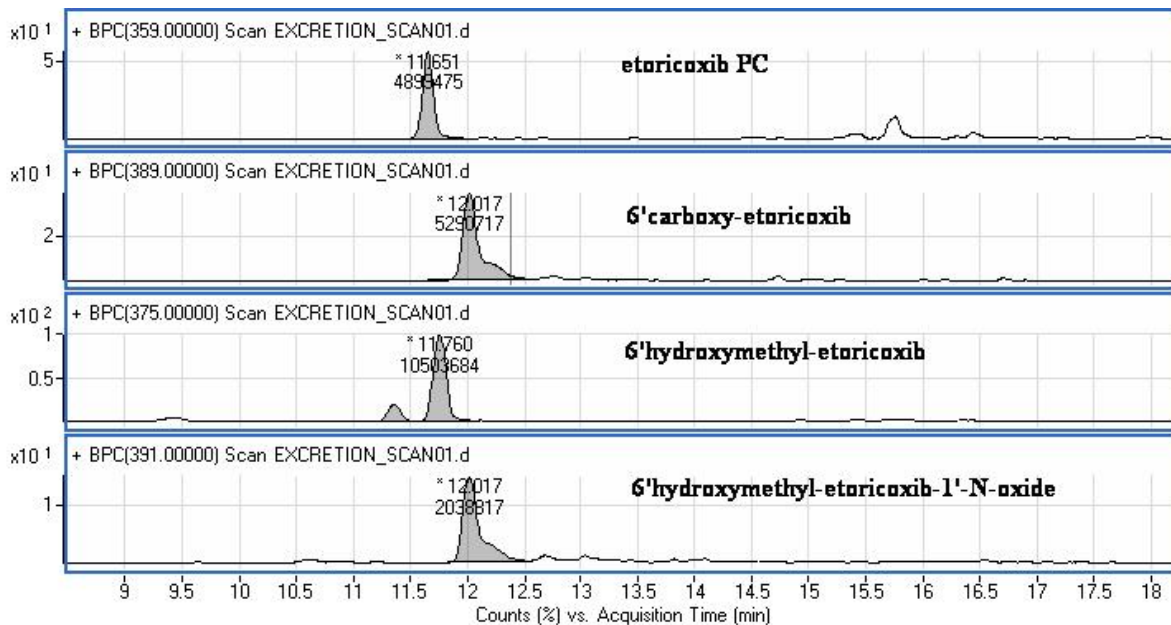


Figure 1: Extracted ion chromatogram from the full scan mass spectrum of the excretion urine.

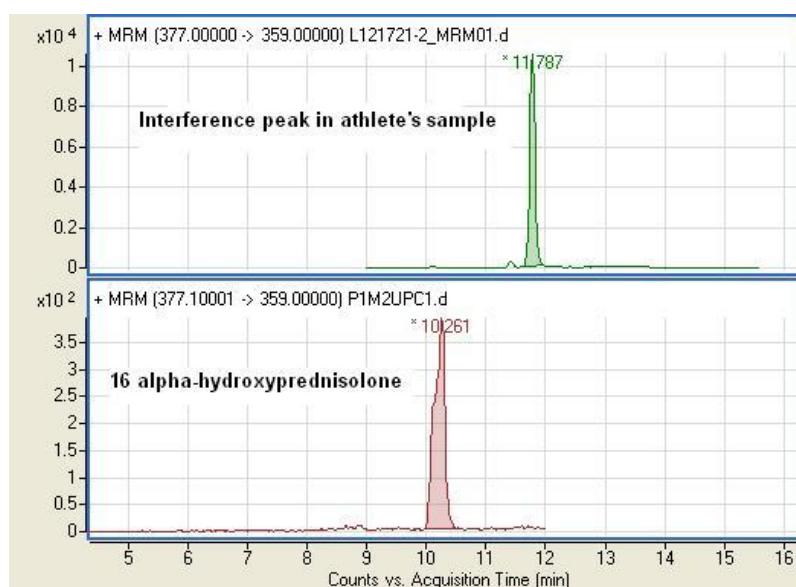


Figure 2: Chromatograms showing the interference peak from an athlete's sample at 11.787 min compared to 16 $\alpha$ -hydroxyprednisolone at 10.261 min. The ion transition is 377 → 359.

The full scan mass spectrum of 6'-hydroxymethyl-etoricoxib ( $M = 374$ ) shows peaks at  $m/z$  375 ( $[M+H]^+$ ),  $m/z$  376 and  $m/z$  377 (Figure 3). The  $m/z$  377 ion  $[M+2+H]^+$  is the isotopic peak of the  $^{37}\text{Cl}$  isotope, with its characteristic isotope ratio of ca. 30%.

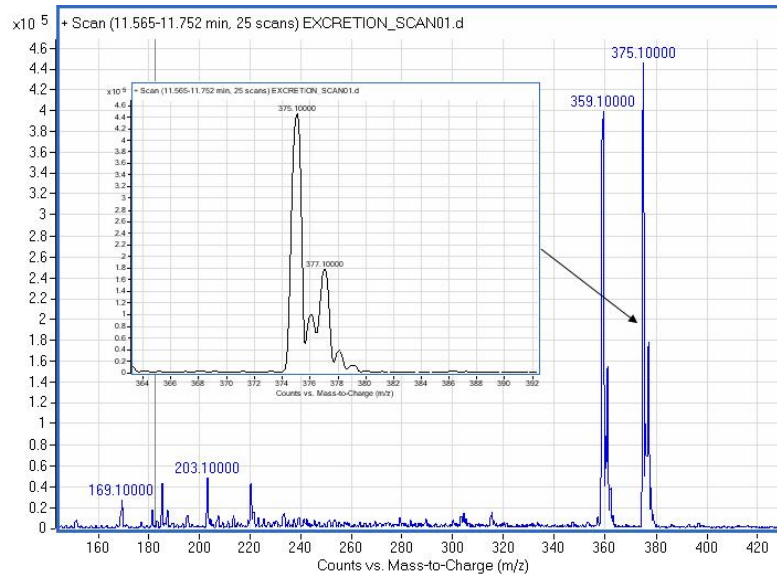


Figure 3: Mass spectrum of 6'-hydroxymethyl-etoricoxib showing the presence of  $[M+2+H]^+$  from  $^{37}\text{Cl}$  isotope.

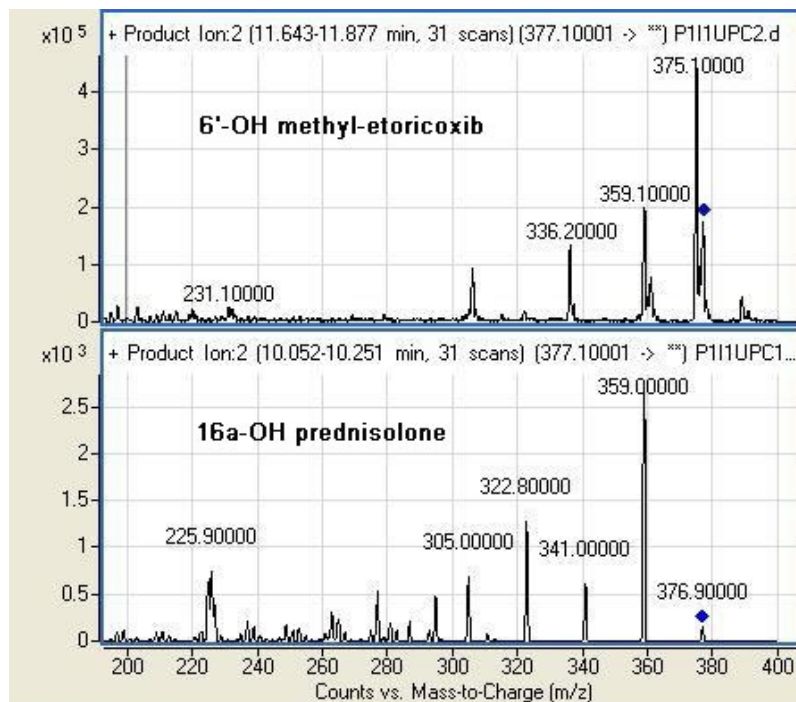


Figure 4: Product ion scans of 6'-hydroxymethyl-etoricoxib and 16a-hydroxyprednisolone. The precursor ion is  $m/z$  377.

The interference peak was found for the MRM transition of 16 $\alpha$ -hydroxyprednisolone, ion transition  $m/z$  377  $\rightarrow$  359. Product ion scan of the isotope peak of 6'-hydroxymethyl-etoricoxib also showed this ion transition (Figure 4). Re-analysis of excretion urine and athletes' samples using the screening procedure showed the interference peak at the same retention time in all the samples, confirming the source of the peak. This MRM transition was edited to avoid this peak.

## Conclusions

The availability of many drugs without prescription in Thailand may lead to possible interfering peaks in chromatograms of screening procedures. In this study, the use of etoricoxib (Arcoxia<sup>®</sup>) led to the excretion of a metabolite that exhibited a large peak in the screening procedure by LC-MS/MS. Analysis of the excretion urine sample found that the **isotopic peak**  $[M+2+H]^+$  from <sup>37</sup>Cl of 6'-hydroxymethyl-etoricoxib ( $M = 374$ ), a metabolite, had the same MRM transition ( $m/z$  377  $\rightarrow$  359) as for 16 $\alpha$ -hydroxyprednisolone. The metabolite also had retention time in the same time window of 16 $\alpha$ -hydroxyprednisolone. The information from this investigation was used to modify the MRM procedure. The retention times and mass spectra of etoricoxib and its metabolites may be of use to other laboratories.

## References

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