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IN DOPING ANALYSIS  
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Observation of Degradation of Some Doping Agents in Plasma

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## **Observation of Degradation of Some Doping Agents in Plasma**

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**Abstract:** Four doping agents and their metabolites were determined in plasma by liquid chromatography/mass spectrometry (LC/MS) using atmospheric pressure chemical ionization (APCI). The metabolic curves of the drugs in plasma were plotted using the MS abundances

**Keywords:** diamorphine, 6-acetylmorphine, acetylcodeine, spironolactone, LC/MS, APCI

### **Introduction**

Some drugs related to doping are degradable in plasma of mice. In our experiment, diamorphine, 6-acetylmorphine, acetylcodeine and spironolactone were added to plasma of mice to observe the process of degradation. The plasma was incubated at 39 °C. The drugs were determined using selected ion monitoring (SIM) of LC/MS/APCI. APCI was suitable for the ionisation of these compounds and had a higher sensitivity compared to HPLC. The process of sample preparation was rather simple. The metabolites of the drugs were detected simultaneously.

### **Experimental**

#### **1. Instruments and Experimental Conditions**

LC/MS: HP 1090 / HP5989 with APCI interface

HPLC column: C-18 reversed-phase column, 5 $\mu$ m, 2.1mm $\times$ 15cm (Zorbax, USA)

Mobile phase: A: acetic acid 1% in H<sub>2</sub>O, B: acetonitrile

Gradient: 0-10 min: B= 0-20%, 10-20 min: B=20-70%

flow rate: 0.4 ml/min.

Mass calibrator: a mixture of valine, tri-valine and hexa-tyrosine (HP company), target ions: m/z 118.08, m/z 508.20, m/z 997.39, settings: CapEx=150 V, EMV 1800V, drying nitrogen flow: 8000 ml/min, drying nitrogen temperature: 350°C nebulizing nitrogen pressure: 80 psi.

## 2. Chemicals and Reagents

Diamorphine, 6-acetylmorphine, acetylcodeine, and spironolactone were purchased from Sigma or from National Laboratory for Narcotics of China. All solvents and reagents used in the experiments were of chromatographic grade (Beijing Chemicals Company).

## 3. Sample preparation

After decapitation of a mouse, the blood with some anticoagulant was centrifuged for 45 min, about 4 mL of plasma were obtained and used in the experiment. To 3 mL of plasma 200  $\mu$ g of the standard were added, the solution was rapidly shaken and kept in a water bath. The temperature of the water was adjusted to 39  $^{\circ}$ C. Aliquots of 100  $\mu$ l were taken at different intervals and mixed with 100  $\mu$ l of perchloric acid. After shaking for 1 min and centrifuging for 15 min (3000 rpm), 20  $\mu$ l of the supernatant were subjected to LC/MS/APCI analysis.

## Results and Discussion:

### 1. APCI mass spectra of the drugs:

APCI mass spectra of the drugs studied in this paper are shown in figs.1-4. The base peaks of the drugs were chosen for monitoring the time course of the concentration of the drugs.

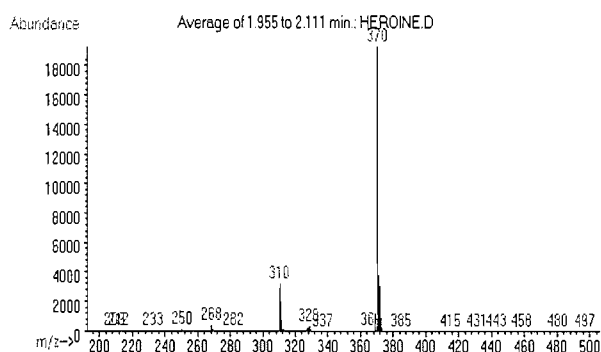


Fig.1 APCI mass spectrum of diamorphine

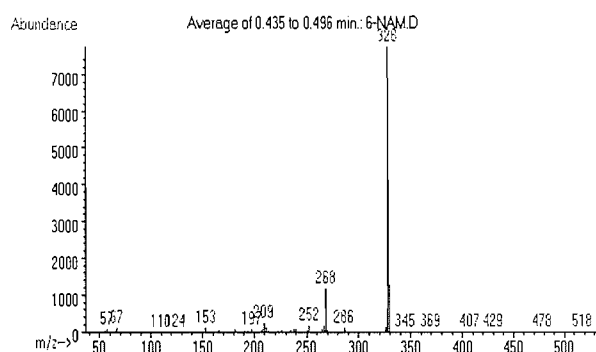


Fig.2 APCI mass spectrum of 6-acetylmorphine

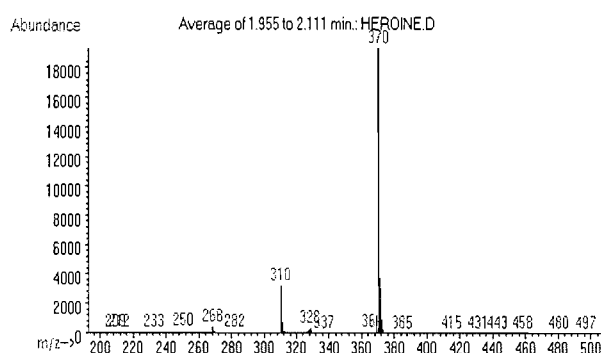


Fig.3 APCI mass spectrum of acetylcodeine  
spironolactone

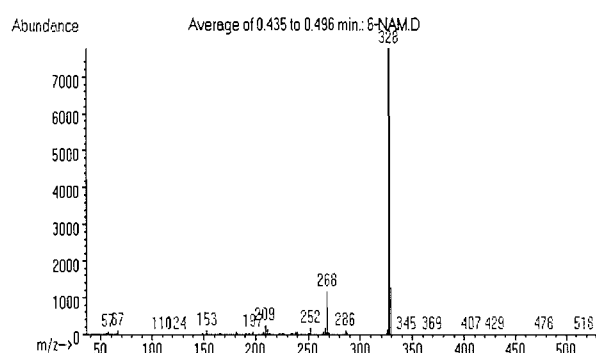
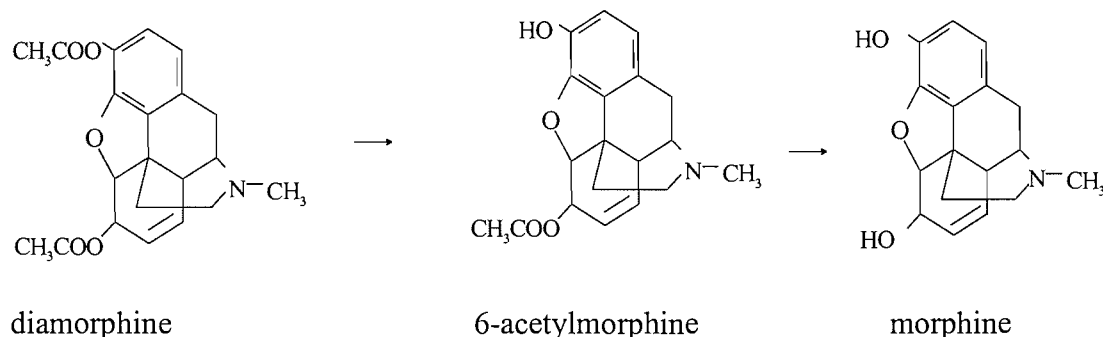


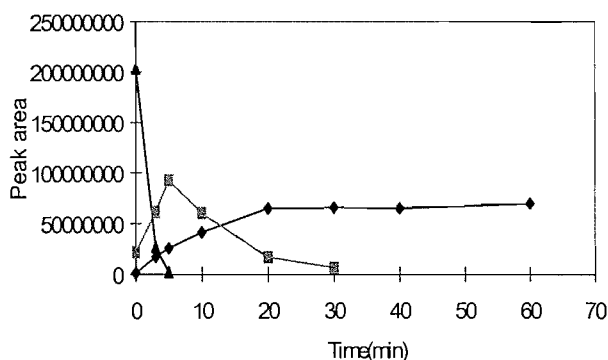
Fig.4 APCI mass spectrum of  
spironolactone

## 2. Diamorphine in plasma

The metabolic pathways of diamorphine are shown in fig.5, and the corresponding curves are plotted in fig.6.



**Fig.5 Metabolic pathways of diamorphine**

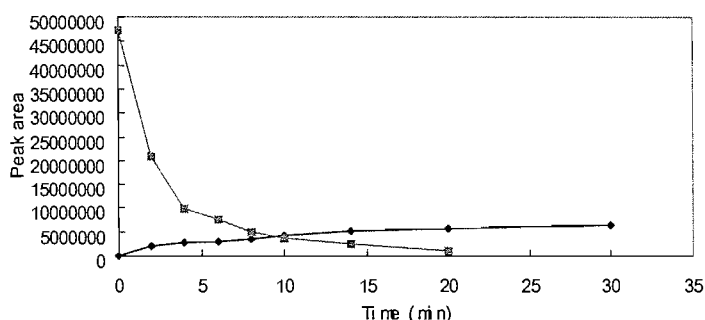


**Fig.6 Variation of the concentration of diamorphine and its metabolites in plasma:  $\square$  diamorphine,  $\circ$  6-acetylmorphine,  $\triangle$  morphine**

Fig.6 demonstrates that diamorphine is degraded rapidly in plasma and nearly completely within 5 min. The intermediate metabolite 6-acetylmorphine is generated simultaneously and further transformed to morphine. Metabolism of morphine was not observed in this experiment.

## 3. 6-Acetylmorphine in plasma

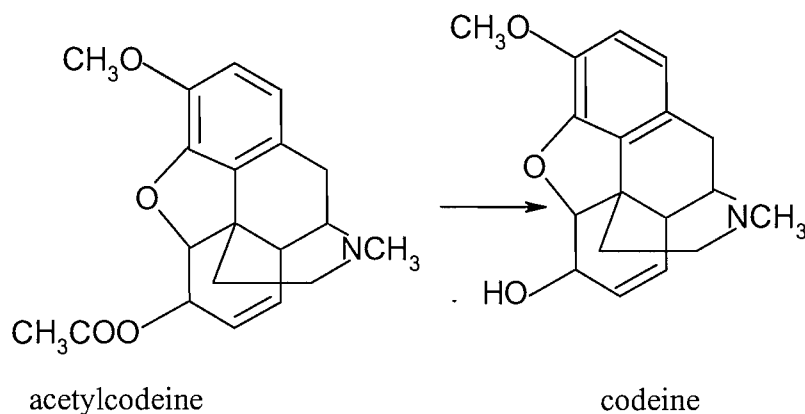
6-Acetylmorphine was utilized as parent compound. The metabolic curves are presented in fig.7. The chart shows that 6-acetylmorphine degrades slower than diamorphine in plasma, and it took more than 20 min to finish the biotransformation.



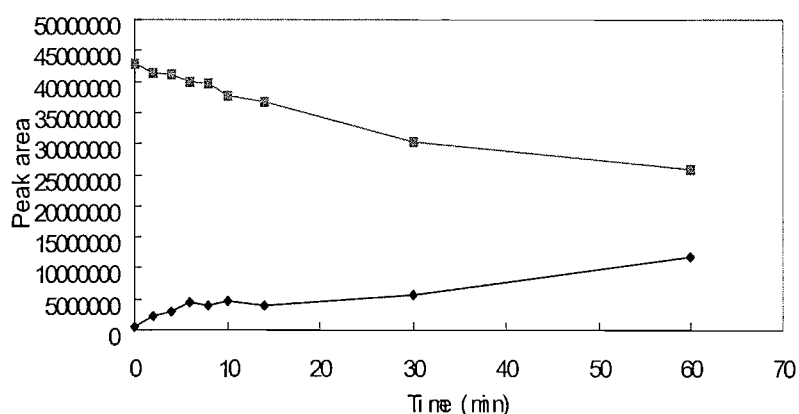
**Fig.7 Variation of the concentration of 6-acetylmorphine and its metabolite morphine in plasma:  $\square$  6-acetylmorphine,  $\circ$  morphine**

#### 4. Acetylcodeine in plasma

The metabolic process of acetylcodeine was relatively slower than that of diamorphine or 6-acetylmorphine. Within 60 min, only 40% of the parent compound were transformed to codeine. The concentration of codeine was thereby raised slowly in the meanwhile.



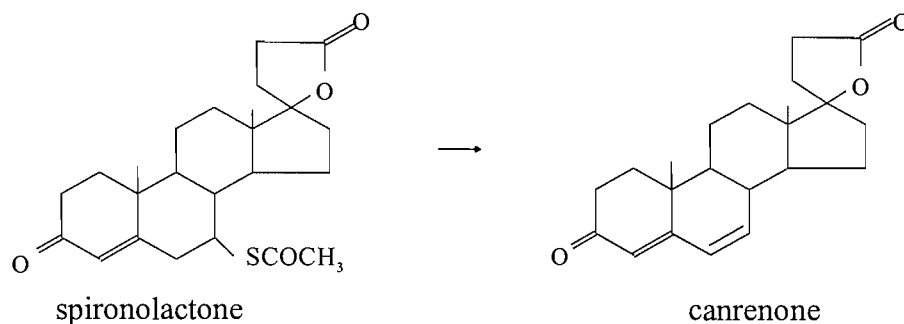
**Fig.8 Metabolic pathways of acetylcodeine**



**Fig 9 Variation of the concentration of acetylcodeine and its metabolite codeine in plasma:**  
 □ acetylcodeine,    ♦ codeine

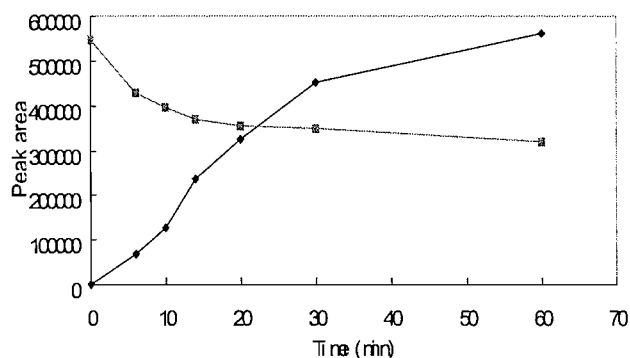
#### 5. Spironolactone in plasma

Spironolactone was biotransformed into canrenone by elimination of thioacetic acid.



**Fig.10 Metabolic pathway of spironolactone**

The variation of the concentration is shown as follows



**Fig.11 Variation of the concentration of spironolactone and its metabolite in plasma:**

**□ spironolactone,    ○ canrenone**

### Conclusion

LC/MS/APCI is very convenient for the analysis of some doping agents without derivatization. The treatment of the sample was rather simple. The experiment was suitable for the monitoring of drugs which easily degrade in plasma, blood and other biologic fluids.

### References:

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2. Xu Youxuan, Peng Shiqi, Recent advances in doping analysis (7), Proceedings of the Manfred Donike Workshop, 17<sup>th</sup> Cologne Workshop on Dope Analysis, 1999, p. 377-81, Sport&Buch Strauß