# Determination of cocaine and benzoylecgonine in guinea pig's hair after a single dose administration by LC-MS/MS

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**Abstract**: A sensitive LC-MS/MS method to determine cocaine and its major metabolite benzoylecgonine in guinea pig's hair has been established. About 20 mg of decontaminated hair sample was hydrolyzed with 0.1 mol · $L^{-1}$ HCl at 50 ℃ overnight, in the presence of cocaine-$d_3$ and benzoylecgonine-$d_8$ used as internal standards, and then extracted with dichlormethane. The analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Positive electrospray ionization (ESI +) and multiple reactions monitoring (MRM) mode were used. The limit of detection (LOD) for cocaine and benzoylecgonine was 1 pg · $mg^{-1}$. The calibration curves of extracted standards were linear over the range from 5 pg · $mg^{-1}$ to 250 pg · $mg^{-1}$ ($r^2 \geq 0.999\ 7$). The method was validated and applied to the analysis of guinea pig's hair after a single dose administration of cocaine hydrochloride. Cocaine and benzoylecgonine were not only detected, but also quantified in guinea pigs hair.

**Key words**: cocaine; benzoylecgonine; LC-MS/MS; single dose; hair

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# LC-MS/MS 法测定豚鼠毛发中可卡因及其代谢物苯甲酰爱康宁

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**摘要**: 本实验旨在建立豚鼠毛发中可卡因及其代谢物苯甲酰爱康宁的液相色谱-串联质谱分析方法,并考察单次给药后豚鼠毛发中可卡因和苯甲酰爱康宁的质量浓度。毛发洗涤、晾干后剪成 1 ~ 2 mm 小段,取 20 mg,加入 0.1 mol · $L^{-1}$ HCl 1 mL 和内标溶液(50 ng · $mL^{-1}$ $d_3$-可卡因和 $d_8$-苯甲酰爱康宁) 20 μL,50 ℃水浴中酸水解过夜;调 pH 为中性,用二氯甲烷液液提取,移取有机相于 60 ℃水浴下挥干,用甲醇 100 μL 复溶,进样 5 μL。目标化合物用 Allure PFP 丙基柱分离,以甲醇-20 mmol · $L^{-1}$ 乙酸铵(0.1% 甲酸)(80∶20)为流动相。质谱采用电喷雾电离-正离子模式(ESI +),多反应监测模式(MRM)。动物实验:豚鼠 8 只分两组,分别以 10 和 0.4 mg · $kg^{-1}$ 的剂量腹腔注射盐酸可卡因水溶液一次,在给药后 d 7 和 d 14 分色剃取毛发进行检测。结果显示,毛发中可卡因和苯甲酰爱康宁的最低检出限均为 1 pg · $mg^{-1}$,在 5 ~ 250 pg · $mg^{-1}$ 线性良好($r^2 \geq 0.999\ 7$)。在给药后 d 7 收集的豚鼠毛发中同时检测到可卡因和苯甲酰爱康宁;d 14 收集的毛发中只检测到可卡因。所建 LC-MS/MS 法灵敏度高,特异性强,适用于豚鼠毛发中低质量浓度的可卡因和苯甲酰爱康宁的分析。

**关键词**: 可卡因; 苯甲酰爱康宁; LC-MS/MS; 单次给药; 毛发分析

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## Introduction

In the scene of forensic toxicology, hair enjoys a growing reputation in drug testing because it provides a larger surveillance window, as well as its easy and no intrusive way of sample collection. In particular, to identify or quantify some specific chemicals in hair after a single dose administration is of great importance in drug-facilitated crimes (DFC), such as sexual assault and robbery[1]. However, hair analysis after a single dose administration is characterized by low drug concentration but significant background noise coming from hair matrix. Yet, with the help of high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS), which is of high sensitivity and specificity in identifying drugs from complex biological matrix, it is possible to achieve such trace analysis in hair.

As a potent central neural system stimulant, cocaine (COC) is a drug of widespread abuse. Benzoylecgonine (BZE) is its major metabolite in body. To identify these two chemicals in biological samples can help to confirm the consumption of COC[2]. So far, published literatures mostly described the determination of COC after repeated administration[3-10]. Although Hubbard[11], Henderson[12] and Jurado[13] had adopted a single dose administration of COC (or COC-$d_5$), they used a gas chromatography/mass spectrometry device to determine the analytes in hair. The sensitivities of these methods (with a LOD around 0.1 ng · $mg^{-1}$) are not adequate for hair analysis involved in a single dose administration, which requires a LOD/LOQ at pg · $mg^{-1}$ level[1].

The aim of this work is to establish a specific and sensitive method to detect COC and its main metabolite BZE in hair using LC-MS/MS. By applying this method to analyze hair samples from guinea pigs who received a single dose of COC, we could verify if it would be possible to detect COC and BZE in animal's hair after a single administration.

## Materials and methods

**Chemicals and reagents** Deionized water was purified using a Milli-Q system (Millipore, Molsheim, France). Solid COC and BZE came from the National Narcotics Lab (Beijing, China). Methanol solutions of COC-$d_3$ (1.0 mg · $mL^{-1}$) and BZE-$d_8$ (1.0 mg · $mL^{-1}$) were obtained from Cerilliant (Texas, USA). Methanol (HPLC gradient grade) was purchased from Fisher (Fairlawn, NJ, USA). Ammonium acetate and formic acid were got from Fluka (Buchs, Switzerland). All the other solvents and inorganic chemicals were of analytical grade. COC hydrochloride used in animal experiment was supported by the National Narcotics Lab.

**Stock solutions and quality control samples** Solid COC and BZE were dissolved in methanol to get 1.0 mg · $mL^{-1}$ solution.

For simultaneous determination of COC and BZE, a mixed 100 ng · $mL^{-1}$ of COC-$d_3$ and BZE-$d_8$ methanol solution was prepared as internal standard (IS). Also a mixed 50 ng · $mL^{-1}$ of COC and BZE in methanol was prepared as standard stock solution.

COC hydrochloride was dissolved in water so that 0.4 mg · $mL^{-1}$ and 10 mg · $mL^{-1}$ of COC injection solutions were obtained.

**Hair sample collection** Hair samples were obtained from a controlled animal treatment experiment. Eight guinea pigs (Shanghai, China) weighing 200 - 450 g were kept in a constant environment at 26 ℃, with an alternating 12 h light-dark cycle. Clean food and water were available. Animals were housed individually in hanging wire cages to prevent urine or saliva contamination from each other. Before the experiment, a 3 cm × 4 cm area of hair stem on the animals' back was shaved to the skin with an electric shaver and drug-free hair was preserved. Four guinea pigs which had multiple colored hairs (white, brown or black) were injected with high dose of COC (10 mg · $kg^{-1}$) and the other four received low dose (0.4 mg · $kg^{-1}$) intraperitoneally. Afterwards, hair samples were collected on the 7th and 14th day. Hair of different color was collected and saved separately. All samples were stored at ambient temperature before analysis.

**Sample preparation** Prior to analysis, decontamination was performed following the procedure reported by Xiang, *et al*[14]. Hair samples were washed with 0.1% sodium dodecyl sulfate (SDS), water and acetone successively. After dried, hair was cut into segments of 1 - 2 millimeter with a scissors. 20 mg of decontaminated hair was incubated in 1 mL of 0.1 mol · $L^{-1}$ HCL, at 50 ℃ overnight, in the presence of IS. The cooled homogenate was neutralized with 2.5 mol · $L^{-1}$ NaOH 0.05 mL and 1 mol · $L^{-1}$ phosphate buffer (pH 6.8) 2 mL. Afterwards, dichloromethane 2 mL was added and the vortex-mixed mixture was centrifuged at 868 × $g$ for 3 min. The organic layer was then transferred into a 5 mL glass tube and evaporated to dryness at 60 ℃. Finally the

residue was reconstituted in 100 μL methanol and 5 μL was injected into the LC-MS/MS system.

**Chromatography and MS/MS conditions** The LC-MS/MS system consisted of an Agilent (Palo Alto, USA) 1100 HPLC system equipped with a triple quadrupole tandem mass spectrometer API-4000 instrument (Applied Biosystems/MDS SCIEX, Toronto, Canada). Analyst 1.4 software package was used for instrument control and data acquisition.

The analytical column was a Restek (Bellefonte, Pennsylvania, USA) Allure PFP column (100 mm × 2.1 mm ID, 5 μm) preceded with a Phenomenex (Torrance, CA, USA) guard column (12.5 mm × 2.1 mm ID, 5 μm), at room temperature. The mobile phase was a mixture of methanol and 20 mmol · $L^{-1}$ ammonium acetate buffer containing 0.1% formic acid (80∶20, *v/v*), with a flow rate of 0.2 mL · $min^{-1}$.

The mass spectrometer was operated in the positive electrospray ionization mode with ion spray voltage of 5.5 kV and source temperature 450 ℃. Nitrogen was used as nebulizing gas (GS 1, 241 kPa or 35 psi), turbo spray gas (GS 2, 241 kPa or 35 psi) and curtain gas (172 kPa or 25 psi). Declustering potential (DP) was optimized while ion spray voltage, nebulizing and curtain gas conditions were used in default mode. The dwell time was set at 100 ms.

The product ion spectrum (MS/MS) was generated at optimized DPs to identify the prominent product ions of the analytes using nitrogen as the collision gas. Collision energies (CE) optimization for the precursor to product ions transition was obtained by CE ramping by direct infusion. The established MRM operating conditions and retention times for COC, BZE and IS are listed in Table 1. All the quantification of analytes was calculated with the first MRM transition of each.

**Table 1** MS/MS conditions and retention times for COC, BZE and IS

| Analyte | Ion transition | Declustering potential/V | Collision energy/eV | Retention time/min |
|---|---|---|---|---|
| COC | 304.2→182.3 | 60 | 28 | 6.54 |
| | 304.2→150.1 | | 35 | |
| BZE | 290.2→168.3 | 70 | 26 | 2.50 |
| | 290.2→105.1 | | 43 | |
| COC-$d_3$ (IS) | 307.1→185.3 | 60 | 27 | 6.54 |
| | 307.1→153.2 | | 35 | |
| BZE-$d_8$ (IS) | 298.3→171.0 | 60 | 27 | 2.50 |
| | 298.3→110.2 | | 46 | |

## Method validation

**Specificity** Drug-free hair samples from guinea pig were analyzed to ensure no endogenous interference with the mass transitions chosen for COC, BZE and IS at the appropriate retention times.

**Calibration curves** COC and BZE standards of 6 concentration levels at 5, 10, 25, 50, 100, and 250 pg · $mg^{-1}$ (2 replicates) were spiked to blank hair. Then the hair samples were extracted and assayed. The calibration curves ($Y = aX + b$) were constructed by plotting the ratio of analytes' peak area to IS's against the spiked concentration. Linear regression $1/x$ weighting was used.

**LOD and LOQ** The LOD was defined as the spiked drug concentration in hair which corresponded to three times the baseline noise ($S/N \geqslant 3$). The LOQ was defined as the lowest concentration on the calibration curve at which precision and accuracy were within ±20%.

**Accuracy and precision** The intra-day and inter-day precision were determined at three concentration levels (5, 50 and 200 pg · $mg^{-1}$). The intra-day variation was determined by assaying 6 spiked hair samples at each concentration level on the same day and inter-day variation was assayed for 24 replicates on four days. The precision was expressed as the relative standard deviation (% RSD). Calculated from the intra-day data, accuracy was established by comparing the determined concentration with the spiked concentration. Provided an 85% – 115% accuracy, a % RSD no more than 15% in measuring range (20% at LOQ) was demanded.

**Recovery** The recovery was calculated by comparing the peak area ratio of analytes of extracted samples with those of extracted blanks spiked with the same amounts of analytes. This procedure was repeated for samples at three concentration levels.

**Evaluation of matrix effect** Matrix effect (co-eluting, undetected endogenous matrix compounds that may influence objects ionization) was investigated by extracting blank hair. The extract was spiked with controlled amount of COC and BZE at three concentration levels, and evaporated to dryness at 60 ℃. The residue was reconstituted in 100 μL methanol. These samples were analyzed and the peak areas of analytes ($A$) were compared with those in pure neat solutions ($B$) with the same amounts of analytes. The matrix effect was expressed as $A/B \times 100\%$.

**Autosampler stability** Autosampler stability was measured by analyzing samples immediately after workup, and repeating the analysis 24 hours later. Concentrations were calculated and the corresponding means were compared. The results within 10% deviation would be accepted.

## Results and discussion

### 1 ESI mass spectra and MS-MS conditions

The analytes gave high MS responses in positive ionization mode. When tuned with flow injection analysis using single standard solution, obvious protonated molecules $[M+H]^+$ were observed in Q1 full-scan. Then fragments of protonated molecules were obtained in product ion scan at collision cell. Figure 1 presents the mass spectrum and product ion spectrum of protonated COC, BZE and IS. The MS/MS analysis showed the predominant fragment ions for COC were at *m/z* 150.1 and 182.3 while those for BZE were at *m/z* 105.1 and 168.3.

As Figure 2 shows, the similar structure determines that COC and BZE have the same loss of benzoic acid (*m/z* 122).

### 2 Validation results

**2.1 Specificity** High specificity was found in MRM mode for the determination of COC and BZE in hair samples, for no interfering compounds from endogenous substances observed at the retention times of COC and BZE, as shown in Figure 3.

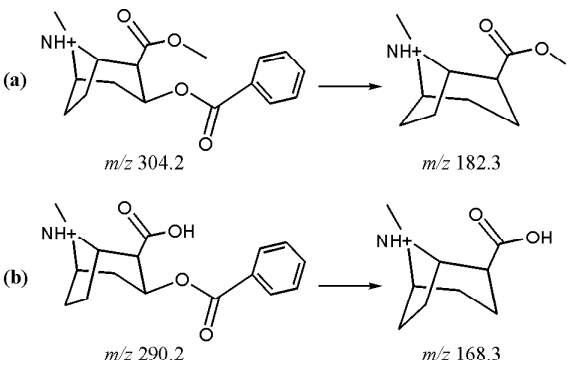


**Figure 2** The fission procedure of COC (a), and BZE (b)

**Table 2** Calibration curves and LOD for COC and BZE in hair

| Analyte | Range /pg · mg$^{-1}$ | Calibration curve/$n=6$ | $r^2$ | LOD /pg · mg$^{-1}$ |
|---|---|---|---|---|
| COC | 5 - 250 | $Y=0.011\,9X+0.002\,3$ | 0.999 9 | 1 |
| BZE | 5 - 250 | $Y=0.029\,6X-0.001\,1$ | 0.999 7 | 1 |

**2.2 Calibration and sample quantification** Over concentration range from 5 to 250 pg · mg$^{-1}$, regression analysis indicated that there was a good linearity between peak area ratio and analytes' concentration ($r^2 \geqslant 0.999\,7$). Calibration curves for COC and BZE are shown in Table 2.

**2.3 LOD and LOQ** The LOD value in hair for both analytes was 1 pg · mg$^{-1}$, with a signal-to-noise ratio of 3. The LOQ was defined as the lowest

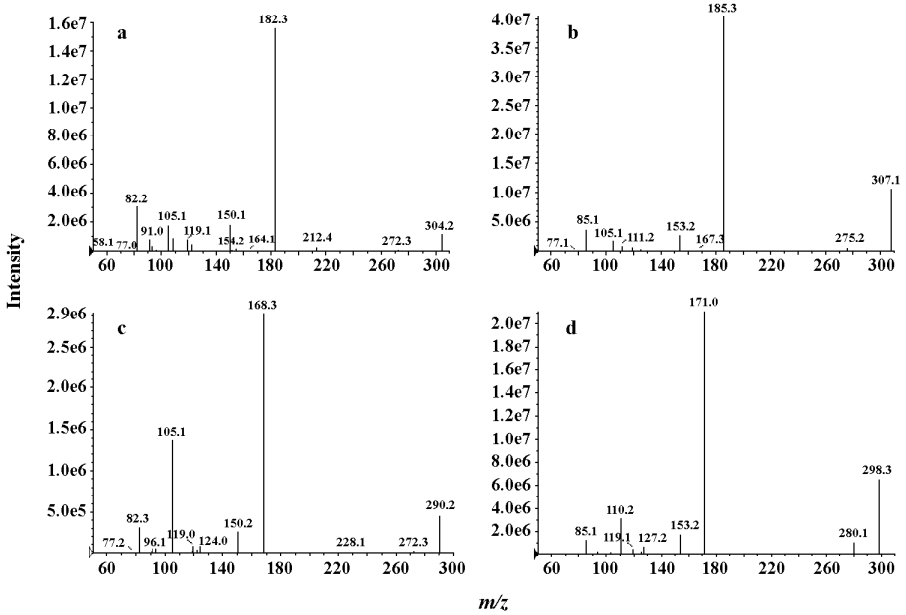


**Figure 1** MS/MS spectrum of COC (a), COC-$d_3$ (b), BZE (c), and BZE-$d_8$ (d)

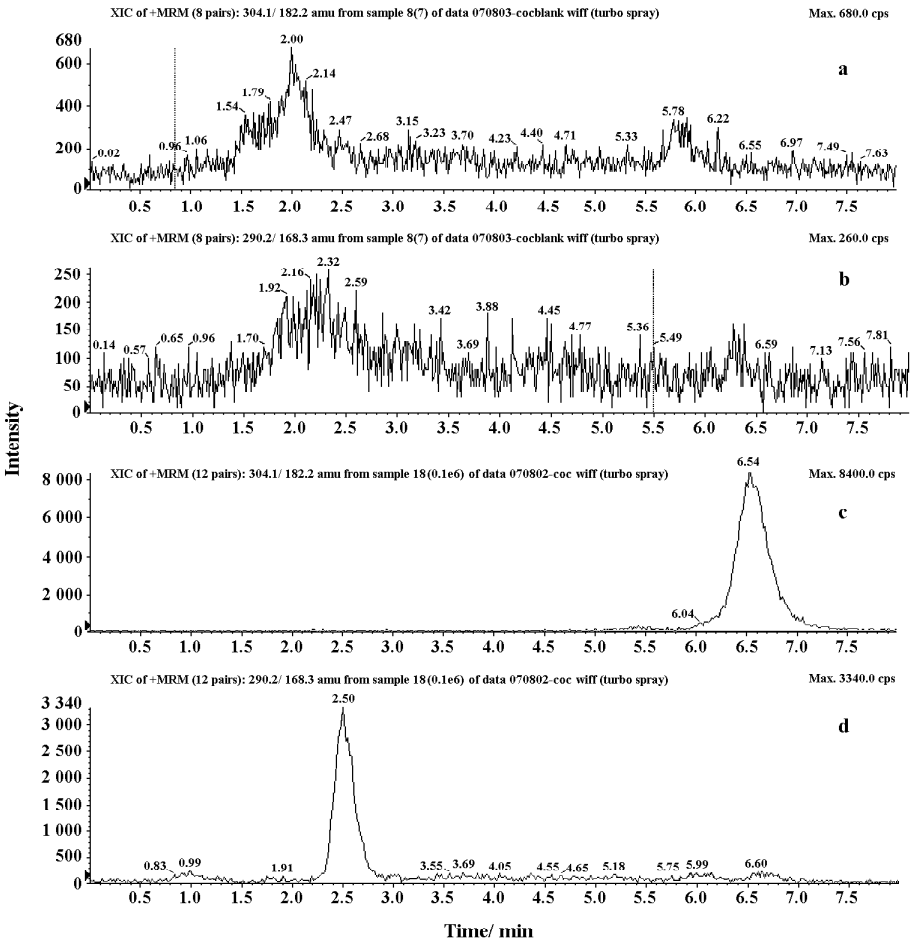


**Figure 3** Representative MRM chromatograms of COC in hair: COC (a) and BZE (b)-free hair; COC (c) and BZE (d) standards spiked hair samples (5 pg · $mg^{-1}$)

**Table 3** Summary of the precision, accuracy, recovery, matrix effect and autosampler stability of the method ($n=6$)

| Analyte | Concentration /pg · $mg^{-1}$ | RSD /% | | Recovery /% | Accuracy /% | Matrix effect /% | Autosampler stability/% |
|---|---|---|---|---|---|---|---|
| | | Intra | Inter($n=24$) | | | | |
| COC | 5 | 4.2 | 3.3 | 98.0 | 96.6 | 91.9 | 5.1 |
| | 50 | 1.0 | 1.7 | 95.0 | 100 | 91.2 | 3.2 |
| | 200 | 1.3 | 1.1 | 100 | 99.9 | 94.8 | 3.3 |
| BZE | 5 | 5.6 | 4.1 | 99.0 | 97.4 | 71.4 | 6.7 |
| | 50 | 3.0 | 2.5 | 106 | 99.9 | 66.2 | 4.2 |
| | 200 | 1.6 | 4.0 | 101 | 100 | 75.0 | 3.1 |

concentration on the standard curve that can be measured with acceptable accuracy and precision, which was found to be 5 pg · $mg^{-1}$ in hair for COC and BZE.

**2.4 Precision and accuracy** Data from spiked samples was calculated to estimate the precision and accuracy of the method. The deviation of intra-day precision was no more than 6% for both analytes and the accuracy was within (100 ± 5)%. Table 3 presents the results. The inter-day precision deviations were less than 5%. As the deuterated COC and BZE were used as internal standards, the % RSD of precisions was much less than demanded.

**2.5 Recovery** The extraction recoveries of COC under the liquid-liquid extraction condition are displayed in Table 3. The recoveries for COC and BZE

were about 100% at all three concentration levels.

**2.6 Matrix effect** The results listed in Table 3 demonstrates that hair matrix contained little co-eluting and undetected compound which would influence the ionization of COC, but had a significant ion-suppression to BZE.

**2.7 Autosampler stability** Table 3 presents acceptable autosampler stability for COC and BZE.

## 3 Application to controlled animal experiment

After a single dose of COC (0.4 or 10 mg · $kg^{-1}$) administration, the concentrations of COC and BZE were measured. Some of them were out of the range of calibration curve. So, another piece of the same hair sample was incubated in 10 mL of 0.1 mol · $L^{-1}$ HCl and 1 mL of the homogenate was prepared following Section 2.4. Thus, hair samples contained high concentration of COC and BZE were diluted and determined within the range of present calibration curves. The results are shown in Table 4.

**Table 4** The concentrations of COC and BZE in guinea pig's hair following a single dose administration of COC

| Sample | COC/pg · $mg^{-1}$ | | BZE/pg · $mg^{-1}$ | | Ratio:[COC]/[BZE](d 7) |
|---|---|---|---|---|---|
| | d 7 | d 14 | d 7 | d 14 | |
| L1-black hair | 41.2 | 12.8 | -[a] | - | - |
| L2-black hair | 37.6 | 7.4 | 7.7 | - | 0.20 |
| L3-black hair | 43.6 | 4.7 | +[b] | - | - |
| L4-black hair | 169.9 | 8.9 | 6.1 | - | 0.04 |
| H1-white hair | 629.7 | 76.5 | 76.4 | - | 0.12 |
| H1-black hair | 874.6 | 156.2 | 101.6 | - | 0.12 |
| H2-brownhair | 108.7 | 99.6 | 8.9 | - | 0.08 |
| H2-black hair | 1 011 | 136.8 | 104.3 | - | 0.10 |
| H3-white hair | 385.3 | - | 26.5 | - | 0.07 |
| H3-black hair | 2 071.5 | 191.8 | 176.3 | - | 0.09 |
| H4-brown hair | 346.9 | 28.5 | 54.2 | - | 0.16 |
| H4-black hair | 1 365.9 | 113 | 309.7 | - | 0.23 |

[a]Not detected; [b]Detected, not quantified; L: Low dose (0.4 mg · $kg^{-1}$) group; H: High dose (10 mg · $kg^{-1}$) group

Although there was only one administration, COC and BZE could be detected in almost all samples collected in the first week, except L1. And compared with the second week's results, the concentrations were much higher, which indicated that the majority of COC and BZE incorporated into hair in the first week. When it came to the second week, BZE could no longer be detected, while COC still had a lower concentration.

Apart from L4-black, all [COC]/[BZE] values were higher than 0.05, ranging from 0.07 to 0.23. According to the criteria proposed by the Society of Hair Testing[15], the analytes detected could be regarded as being incorporated into guinea pigs' hair rather than external contamination.

Comparing the black hair samples of two dose groups, it shows the higher dose of COC administrated the higher concentration of COC and BZE occurred in guinea pig's hair.

In our study, concentrations of COC in hair were much higher than those of BZE. That result was consistent with those reported in earlier publications. Nakahara *et al.*[16,17] explained that is because COC is more lipophilic to penetrate the cell membrane than BZE. And Jurado, *et al*[13]. added that with a higher alkalinity, COC is easier to shift from alkalic blood matrix to acid hair matrix.

Some published literatures indicated that there was a close relationship between the drug concentration and the melanin in hair analysis[13,18,19]. In present work, our data also showed that higher concentration of COC and BZE occurred in black hair than those in white and brown ones. Figure 4 displays COC and BZE determined in different hair samples collected from high dose group.

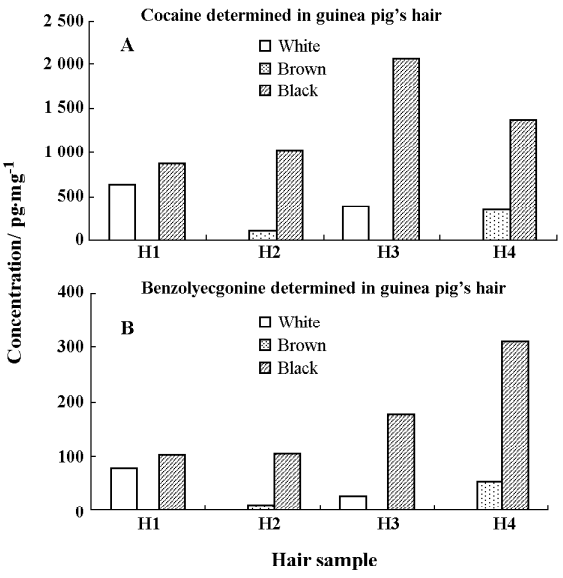


**Figure 4** COC (A) and BZE (B) determined in hair samples from guinea pigs which received high dose (10 mg · $kg^{-1}$) of COC

One suggested explanation is that analytes incorporated with eumelanin, one major subtype of melanins, in hair by electrostatic force. And black hair

has more eumelanin than other color hair[11,19].

On the other hand, in white hair which contains no melanin, COC and BZE were found out, too. This result supported that melanin is not the only predominant factor in the mechanism of analytes' incorporation into hair[20,21].

## Conclusion

A rapid LC-MS/MS method has been established, which is of high specificity and sensibility to analyze COC and its major metabolite BZE in hair after a single dose administration. And the validated method was successfully applied to guinea pigs who received one single dose of COC. Both COC and BZE could be detected in the first week and [COC]/[BZE] indicated few external contamination. Additionally, there was a higher concentration of COC than that of BZE occurred in hair because of their different physicochemical properties; and incorporation of COC and BZE into hair is highly related with given dose and hair color.

## References

[1] Kintz P, Villain M, Ludes B. Testing for the undetectable in drug-facilitated sexual assault using hair analyzed by tandem mass spectrometry as evidence [J]. Ther Drug Monit, 2004,26:211 -214.

[2] Shen M. Analysis for Drugs of Abuse in Body (体内滥用药物分析) [M]. Beijing: Law Publications, 2003: 161 -163.

[3] Gheorghe A, Van Nuijs A, Pecceu B, et al. Analysis of cocaine and its metabolites in waste and surface water using solid phase extraction and liquid chromatography-ion trap tandem mass spectrometry [J]. Anal Bioanal Chem, 2008,391:1309 -1319.

[4] Cognard E, Rudaz S, Bouchonnet S, et al. Analysis of cocaine and three of its metabolites in hair by gas chromatography-mass spectrometry using ion-trap detection for CI/MS/MS [J]. J Chromatogr B, 2005, 826:17 -25.

[5] Cairns T, Hill V, Schaffer M, et al. Levels of cocaine and its metabolites in washed hair of demonstrated cocaine users and workplace subjects [J]. Forensic Sci Int, 2004,145:175 -181.

[6] Concheiro M, Castro AD, Quintela O, et al. Determination of drugs of abuse and their metabolites in human plasma by liquid chromatography-mass spectrometry: an application to 156 road fatalities [J]. J Chromatogr B, 2006,832:81 -89.

[7] Johansen SS, Bhatia HM. Quantitative analysis of cocaine and its metabolites in whole blood and urine by high-performance liquid chromatography coupled with tandem mass spectrometry [J]. J Chromatogr B, 2007, 852: 338 -345.

[8] Damsa R, Chooa RE, Lambert WE, et al. Oral fluid as an alternative matrix to monitor opiate and cocaine use in substance-abuse treatment patients [J]. Drug Alcohol Depend, 2007,87:258 -267.

[9] Lachenmeier K, Musshoff F, Madea B. Determination of opiates and cocaine in hair using automated enzyme immuno MS/MS analysis screening methodologies followed by gas chromatographic-mass spectrometric (GC-MS) confirmation [J]. Forensic Sci Int, 2006, 159: 189 -199.

[10] Cordero R, Paterson S. Simultaneous quantification of opiates, amphetamines, cocaine and metabolites and diazepam and metabolite in a single hair sample using GC-MS [J]. J Chromatogr B, 2007,850:423 -432.

[11] Hubbard DL, Wilkins DG, Rollins DE. The incorporation of cocaine and metabolites into hair: effects of dose and hair pigmentation [J]. Drug Metab Dispos, 2000,28:1464 -1469.

[12] Henderson GL, Harkey MR, Zhou C, et al. Incorporation of isotopically labeled cocaine and metabolites into human hair: 1. dose-response relationships [J]. J Anal Toxicol, 1996,20:1 -12.

[13] Jurado C, Rodriguez-Vicente C, Menéndez M, et al. Time course of cocaine in rabbit hair [J]. Forensic Sci Int, 1997,84:61 -66.

[14] Xiang P, Shen M, Zhuo XY. Hair analysis for ketamine and its metabolites [J]. Forensic Sci Int, 2006, 162: 131 -134.

[15] Wenning R. Potential problems with the interpretation of hair analysis results [J]. Forensic Sci Int, 2000, 107: 5 -12.

[16] Nakahara Y, Oehiai T, Kikura R. Hair analysis for drugs of abuse V. The facility in incorporation of cocaine into hair over its major metabolites [J]. Arch Toxicol, 1992, 66:446 -449.

[17] Nakahara Y, Takahashi K, Kikura R. Hair analysis for drugs of abuse. X. Effect of physicochemical properties of drugs on the incorporation rates into hair [J]. Biol Pharm Bull, 1995,18:1223 -1227.

[18] Nakahara Y, Kikura R. Hair analysis for drugs of abuse. VII. The incorporation rates of cocaine, benzoylecgonine and ecgonine methyl ester into rat hair and hydrolysis of cocaine in rat hair [J]. Arch Toxicol, 1994,681:54 -59.

[19] Rollins DE, Wilkins DG, Krueger GG, et al. The effect of hair color on the incorporation of codeine into human hair [J]. J Anal Toxicol, 2003,27:125 -134.

[20] Kintz P. Value of hair analysis in postmortem toxicology [J]. Forensic Sci Int, 2004,142:127 -134.

[21] DeLauder SF, Kidwell DA. The incorporation of dyes into hair as a model for drug binding [J]. Forensic Sci Int, 2000,107:93 -104.