# II.2.4 Cocaine and its metabolites

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## Introduction

Cocaine is an alkaloid being contained in the leaves of *Erythroxylon coca* Lam. or *Erythroxylon novogranatense* (Morris) Hieronymus of Erythroxylaceae plants. The compound is being used as a local anaesthetic. It also shows stimulating action on the central nervous system; when it is used continuously, psychic dependence on its use appears, resulting in its chronic toxicosis [1]. Cocaine is being abused worldwide; its abuse is most serious in the north and south American continents. Fortunately, in Japan, its abuse is not so many; but it cannot be overlooked.

In this chapter, the methods of extraction[a] and GC/MS analysis for cocaine and its main metabolites, such as ecgonine methyl ester (EME) and benzoylecgonine (BE), in urine and blood, are presented; a fatal cocaine poisoning case, which the author experienced, is also demonstrated.

## Chemical synthesis of the metabolite standards [2]

- Cocaine hydrochloride (Shionogi & Co., Ltd., Osaka, Japan; Takeda Chem. Ind. Ltd., Osaka, Japan; or Sigma, St. Louis, MO, USA) is dissolved in 0.2 M hydrochloric acid aqueous solution and heated at 90 °C with refluxing (gentle mixing) for 24 h to produce ecgonine. The solution is extracted with ethyl ether to remove benzoic acid. The dried residue of ecgonine hydrochloride is dissolved in 10 % hydrochloric acid methanolic solution for its methylation to synthesize EME [2].
- Aqueous solution of cocaine hydrochloride is carefully adjusted to pH 7 by repeated addition of 0.1 M NaOH solution with refluxing for 3 days; precipitated BE can be obtained. For more details of procedure, see reference [2].

## Reagents and their preparation

- Bond Elut Certify® LRC[b] (Varian, Harbor City, CA, USA)
- Extrelut®[c] (Merck, Darmstadt, Germany)
- 0.1 M Phosphate buffer solution (pH 6):13.6 g of potassium dihydrogenphosphate is dissolved in 900 mL distilled water and its pH is adjusted to 6.0 (± 0.1) by adding 0.1 M potassium hydroxide solution; the final volume is brought to 1,000 mL by adding distilled water.
- 0.1 M Hydrochloric acid solution: an 833-µL aliquot of concentrated hydrochloric acid is added to distilled water to prepare 100 mL solution.
- Eluent-1: dichloromethane/isopropanol/28% ammonia water (80:20:2, v/v) Eluent-2: chloroform/isopropanol (9:1, v/v)

- Standard stock solutions: 11.2 mg of cocaine hydrochloride (10 mg of the free form) is dissolved in dilute hydrochloric acid solution (0.1 mM, pH 4.0) to prepare 100 mL solution; EME and BE, 10 mg each, are dissolved in 100 mL methanol respectively. These stock solutions are appropriately diluted just before use to serve for standard solutions.
- As internal standard (IS)[d], 3 mg of scopolamine hydrobromide (Kanto Chemicals, Tokyo, Japan and any other manufacturer) is dissolved in 100 mL distilled water to prepare 30 μg/mL solution.
- Derivatization reagents[e]: pentafluoropropionic anhydride (PFPA, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan and other manufacturers); trifluoroacetic anhydride (TFAA, manufacturers the same as above); hexafluoroisopropanol (HFIP, Kanto Chemicals, Tokyo, Japan and other manufacturers).

## Storage of specimens[f] [3–5]

Cocaine is rapidly metabolized[g] by enzymes such as cholinesterase and by chemical reaction after its absorption into bodies; these reactions continue postmortem. Therefore, the analysis of cocaine in an unchanged form in biomedical specimens depends on how to stop the metabolism and decomposition of cocaine in a matrix and how to keep its concentrations at the point of analysis. For these purposes, the following treatments should be made as soon as possible after sampling specimens:

- Addition of cholinesterase inhibitors (sodium fluoride, eserine, organophosphorus compounds and others)
- Adjustment of pH to 5.0 for specimens by adding acidic solutions
- Storage in a refrigerator or in a frozen state.

## GC/MS conditions[h]

Gas chromatograph/mass spectrometer: Finnigan Polaris Q (Thermoquest, Austin, TX,USA); column: Rtx-5MS (30 m × 0.25 mm i. d., film thickness 0.25 μm, Restek, Bellefonte, PA, USA); column temperature: 50 °C → 25 °C/min →150 °C(3 min) →10 °C/min → 300 °C; injecor temperature: 250 °C; transfer line temperature: 300 °C; carrier gas: He; its flow rate: 0.9 mL/min; injection mode: splitless; measurement: scan mode.

## Procedures

Since cocaine in biomedical specimens is rapidly hydrolyzed, the specimens[i] should be treated for its preservation immediately after sampling, and processed for extraction and derivatization procedure. Good recoveries of cocaine and its metabolites can be obtained with a mixed type extraction column (Bond Elut Certify®) and a diatomite column (Extrelut®). The extraction with the diatomite column is somewhat inferior in purification ability, but gives good recoveries with simple procedure and thus is useful in forensic toxicology.

## a) Procedure I (Bond Elut Certify®) [6–8]

### i. Extraction from urine and derivatization

i. A 5-mL volume of urine is mixed with 5 mL of 0.1 M phosphate buffer (pH 6) and 100 μL IS solution.
ii. The column is activated by passing 3 mL methanol and then 3 mL of 0.1 M phosphate buffer solution (pH 6) through it[j].
iii. The above urine mixture is poured into the column to let it flow slowly (about 1 mL/min)[k].
iv. The column is washed with 5 mL distilled water, 3 mL of 0.1 M hydrochloric acid solution[l] and 9 mL methanol, and aspirated with reduced pressure for more than 5 min to dry the column[m].
v. Cocaine and its metabolites are eluted with 3 mL of the Eluent-1.
vi. The eluate is evaporated to dryness under a stream of nitrogen[n] with warming at 50 °C. The residue is dissolved in a small amount of methanol[o] and transferred to a reaction vial. The solution is again evaporated to dryness under a stream of nitrogen, mixed with 100 μL PFPA and 50 μL HFIP, capped airtightly, and heated at 70 °C for 30 min for derivatization.
vii. After the reaction, the solvent is removed under a gentle stream of nitrogen; the residue is dissolved in 100 μL ethyl acetate to be subjected to GC/MS analysis.

### ii. Extraction from blood and derivatization

i. A 1-mL volume of blood (plasma) is mixed with 100 μL IS solution and 5 mL methanol, vortex-mixed for 1 min and centrifuged to separate a supernatant fraction. Another 3-mL methanol is added to the sediment in the centrifuge tube, vortex-mixed and centrifuged in the same way.
ii. The supernatant fractions are combined and concentrated (under a stream of nitrogen or in a rotary evaporator) to not larger than 1 mL, mixed with 5 mL of 0.1 M phosphate buffer solution (pH 6) and centrifuged. The supernatant fraction is poured into the Bond Elut Certify column.
iii. The following procedure is exactly the same as described above for the urine specimen.

## b) Procedure II (Extrelut®) [5]

i. A 1-mL volume of blood (plasma) is mixed with 4 mL distilled water; in the case of urine, 5 mL is used without any dilution. The 5 mL specimen is mixed with 100 μL IS solution and its pH is adjusted to 8–9[p] with 10 % ammonia water.
ii. The above solution is poured into the Extrelut® column and left for 20 min.
iii. Cocaine and its metabolites are eluted from the column with 30 mL of the Eluent-2.
iv. The eluate is evaporated to dryness under reduced pressure with warming at 50 °C. The residue is dissolved in 100 μL PFPA and 50 μL HFIP, capped airtightly and heated at 70 °C for 30 min for derivatization. A 1–2 μL aliquot of it is subjected to GC/MS analysis.

### c) Construction of calibration curves[q]

i. To each blank blood (1 mL) and urine (5 mL), are added 100 μL IS solution, cocaine (100–1,000 ng/mL), EME (100–1,000 ng/mL) and BE (200–1,000 ng/mL).
ii. The specimens are subjected to either Procedure I or Procedure II.
iii. For each calibration curve, peak area ratio of a compound to IS is used for the vertical axis. Monitor ions used are ; EME-PFP: *m/z* 82; BE-HFIP: *m/z* 318; cocaine: *m/z* 82; scopolamine-PFP (IS): *m/z* 138.

## Assessment and some comments on the methods

It is not easy to simultaneously extract cocaine, EME and BE by liquid-liquid extraction due to the high polarity of BE. However, the three compounds can be simultaneously extracted with the Bond Elut Certify® and Extrelut® columns with high efficiency. The recovery rates were not lower than 86 % for the three compounds with the Bond Elut Certify® column [7,8] and not lower than 81 % with the Extrelut® column [5].

*Figure 4.1* shows a TIC and mass chromatograms (MCs) of cocaine (100 ng), EME (100 ng), BE (200 ng) and IS, which had been spiked into 1 mL blood before extraction with a Bond Elut Certify® column and PFP-HFIP derivatization. In the TIC, many big impurity peaks appeared; the test peaks were small. Each mass spectrum contained some impurity peaks, but by subtracting background impurity peaks from the test mass spectrum, a clean spectrum can be obtained, which is almost identical to that of the authentic standard. By mass chromatography, quantitative analysis can be achieved without any interference by impurities. *Figure 4.2* shows mass spectra of the authentic cocaine, EME and BE after derivatization.

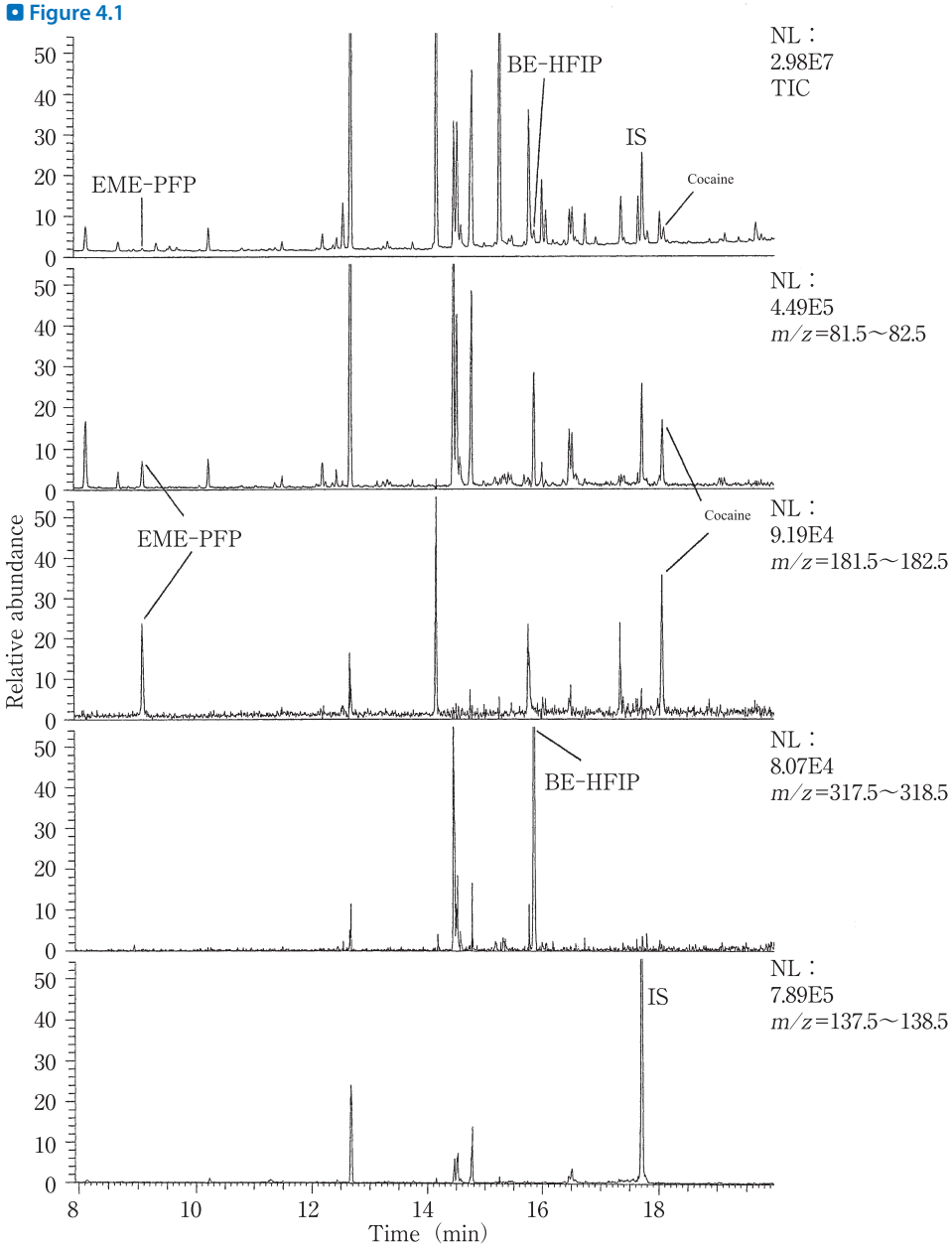
Setting up of cutoff values are very common in USA and Europe as a part of QC (quality control) and QA (quality assurance) system; the idea is useful to suppress the discrepancies of results among testing institutions and to secure the accuracy of each analysis. The cutoff values [9] of BE in urine for cocaine abuse being established in U.S.A. are 300 ng/mL for the primary judgement by immunoassays, and 150 ng/mL for the secondary judgement by GC/MS. However, in Japan, such criteria set by official institutions are not available for drugs of abuse. Upon judgements of analytical data, the idea of cutoff values should be adopted with reference to the values established in USA or Europe. When BE in urine is analyzed by GC/MS in the full scan mode for both mass spectral measurements and quantitation by mass chromatography, the most suitable cutoff value seems to be 300 ng/mL.

## Poisoning case

Numerous fatal cocaine poisoning cases were reported in USA, but in Japan the number of such cases is limited. The author et al. experienced a fatal cocaine poisoning case and measured concentrations of cocaine and its metabolites in body fluids and various organs.

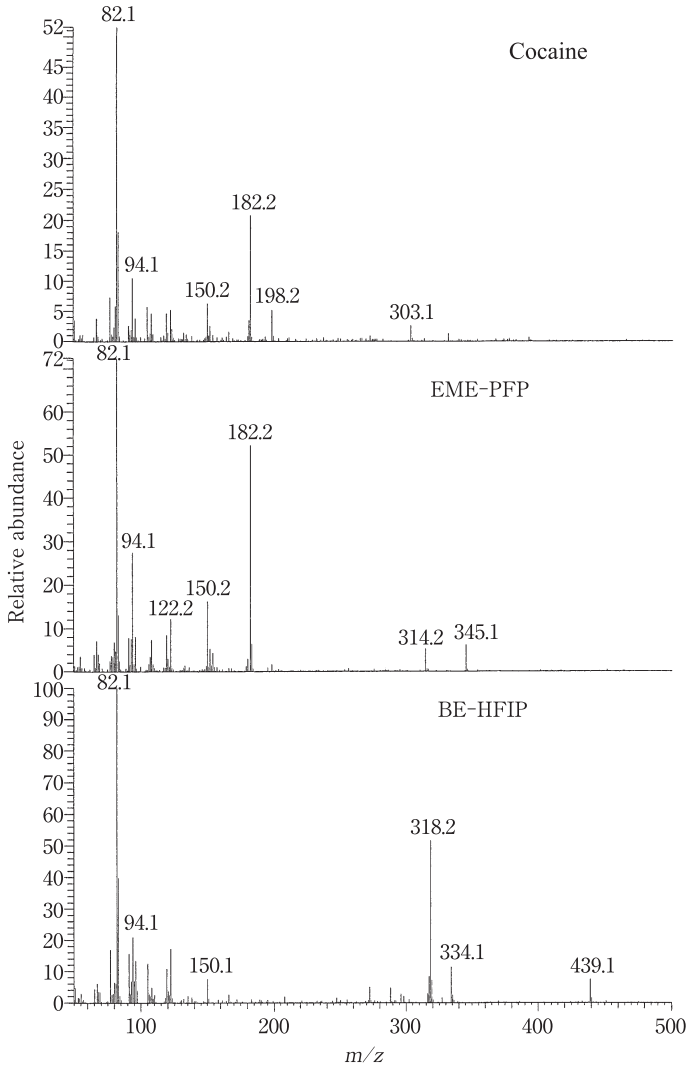
A 41-year-old male was found dead in a hotel room. The clothes on the corpse were not disordered; no external wounds could not be found. However, the skin around both nostrils was reddened erosively. In his room, nine small empty polyethylene bags, to which small amounts of cocaine powder adhered were found; methamphetamine powder, cannabis resin

**Figure 4.1**




**Total ion chromatogram (TIC) and mass chromatograms for the PFP-HFIP derivatives of the extract of 1 mL blood, to which 100 ng cocaine, 100 ng EME and 200 ng BE had been added.**

**Figure 4.2**




**Mass spectra of the authentic cocaine and its metabolites.**

and tablets of 3,4-methylenedioxymethamphetamine (MDMA) were also discovered. The cadaver was subjected to forensic autopsy with suspicion of drug poisoning.

The analytical results are summarized in *Table 4.1*; the TIC and mass spectra for heart blood of this victim are shown in *Fig. 4.3*. The level of cocaine found in heart blood (19.4 μg/mL) was considered to be in the fatal range, because those reported in the previous fatal cases were largely not higher than 20 μg/mL [10–13]. Because of the presence of red skin around the nostrils, inhalation of cocaine was suspected; but its level in the lung was not higher than those in other organs. The cocaine levels in stomach contents and the stomach wall were especially high, while the levels of its metabolites EME and BE were low in both speci-

**Table 4.1**

**Concentrations of cocaine, EME and BE in the autopsy specimens***

| Specimen | Cocaine | EME | BE | Total cocaine** |
|---|---|---|---|---|
| Heart blood | 19.4<br>(0.06) | 30.9<br>(0.16) | 28.5<br>(0.01) | 96.5<br>(0.32) |
| Urine | 81.5<br>(0.27) | 242<br>(1.22) | 487<br>(1.69) | 962<br>(3.17) |
| Stomach contents | 764<br>(2.52) | 66<br>(0.33) | 155<br>(0.54) | 1,027<br>(3.39) |
| Bile | 82.4<br>(0.27) | 47<br>(0.24) | 20.3<br>(0.07) | 175<br>(0.58) |
| Brain | 41.1<br>(0.14) | 2.9<br>(0.02) | 5.8<br>(0.02) | 51.6<br>(0.17) |
| Lung | 103<br>(0.34) | 28.5<br>(0.14) | 41.5<br>(0.14) | 190<br>(0.63) |
| Heart | 44<br>(0.15) | 6.1<br>(0.03) | 5.1<br>(0.02) | 58.9<br>(0.19) |
| Pancreas | 148<br>(0.49) | 32.4<br>(0.16) | 23.0<br>(0.08) | 222<br>(0.73) |
| Liver | 171<br>(0.56) | 41.5<br>(0.21) | 160<br>(0.55) | 403<br>(1.33) |
| Spleen | 109<br>(0.36) | 51.1<br>(0.26) | 46.3<br>(0.16) | 235<br>(0.78) |
| Kidney | 71.3<br>(0.24) | 25.9<br>(0.13) | 15.8<br>(0.06) | 127<br>(0.42) |
| Stomach wall | 655<br>(2.16) | 30.7<br>(0.15) | 27.4<br>(0.10) | 730<br>(2.41) |

* Unit: μg/mL or g and μmol/mL or g in parentheses

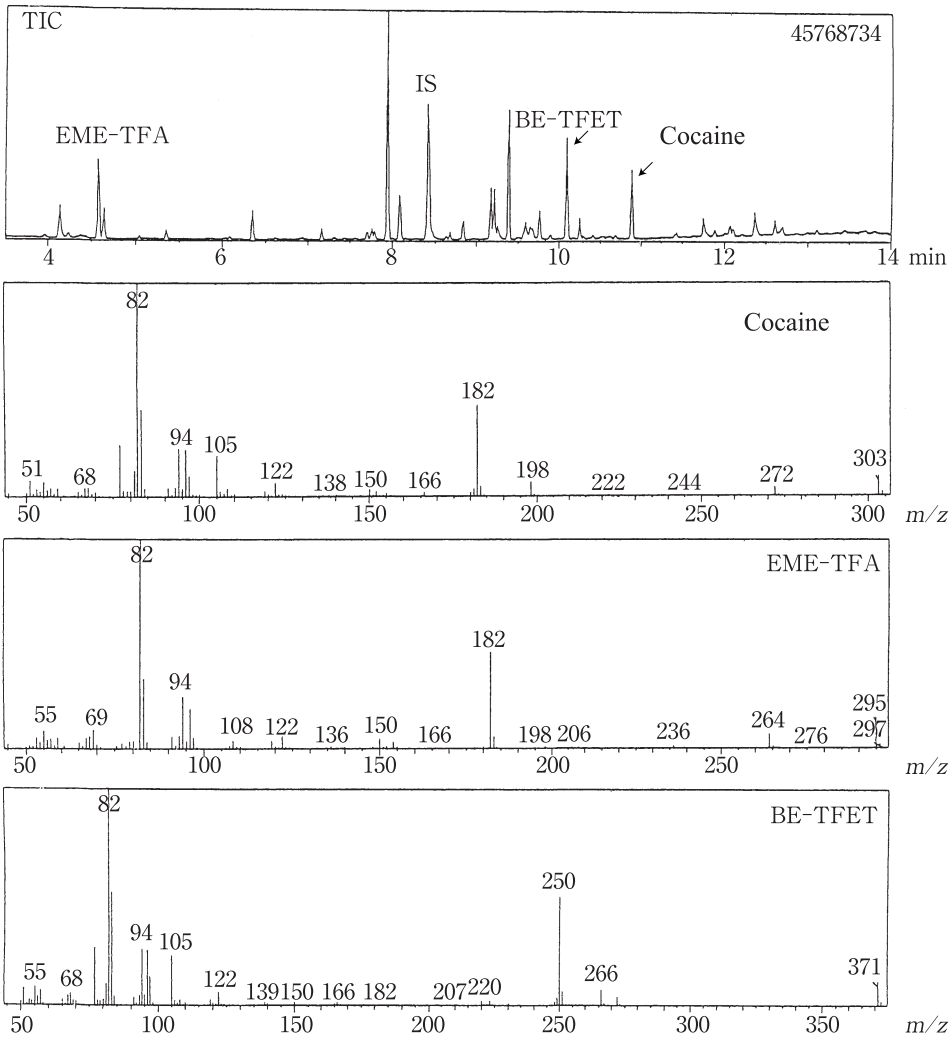
** The total cocaine concentration was calculated after conversion of amounts of EME and BE into those of cocaine

mens. This means that the victim had died relatively shortly after ingestion of cocaine; this idea seems to be also supported by the result that the ratio of unchanged cocaine in urine was as much as 8.5 % *(Table 4.1)*.

According to human experiments, the urinary ratio of BE to cocaine was reported to be 5.9 on average for urine sampled 0–1 h after cocaine inhalation; the ratio was 10.6 on average for urine sampled 0–8 h after the inhalation. The ratios increased according to the increase in time interval between the inhalation and samplings [13–15]. In the present case, the ratio counted 6.3, suggesting a relatively short period between the times of his ingestion and death. In addition, the ratios of cocaine to BE in blood and in the brain, and the brain-to-blood ratios of cocaine and BE were compared with those described in literature [11]; such comparison also suggested his death to have taken place 1–3 h after cocaine ingestion[r].

Neither amphetamines, cannabis metabolite, MDMA nor ethanol were detected from the present specimens; only cocaine and its metabolites could be detected. Since neither injuries nor diseases could be found, the cause of his death was diagnosed to be cocaine poisoning. Considering the size of nine empty polyethylene bags attached by cocaine powder, the whole

**Figure 4.3**




**TIC and mass spectra for the extract of heart blood in an actual poisoning case. The extraction was made with an Extrelut® column. The derivatization was made with the combination of trifluoroacetic anhydride and trifluoroethanol.**

content of cocaine hydrochloride was estimated to be about 1.5 g, which corresponds to the oral fatal dose of cocaine hydrochloride.

## Toxic and fatal concentrations [16]

Since cocaine in human specimens is rapidly hydrolyzed into EME by enzymatic reaction, and into BE by chemical reaction, the cocaine concentrations detected neither reflect those at the

time of death nor those at the time of samplings. Therefore, there are great variation in the fatal levels of cocaine in literature probably due to the above factors. The fatal doses are, of course, different according to administration routes. Usually, the oral fatal dose for humans is said to be about 1.2 g; but there was a fatal case, in which only 30 mg of cocaine had been applied to mucous membrane of a hypersensitive subject. Among habitual abusers of cocaine, there are cases in which as much as 5 g is used daily. Toxic blood concentrations of cocaine is 0.25–5 μg/mL; there were fatal cases, in which blood cocaine concentration was 1 μg/mL or more.

## Notes

a) Cocaine is a basic compound (p*K*a 8.6) and thus extractable with chloroform under weakly alkaline conditions. BE, however, is highly polar and thus difficult to be extracted with the solvent; to extract BE by liquid-liquid extraction, a polar solvent mixture of chloroform and isopropanol or ethanol should be used. The simultaneous analysis can be made using chloroform/isopropanol (9:1) or (3:1), and using columns of Bond Elut Certify® and Extrelut®.

b) The Bond Elut Certify® column is composed of a mixture of non-polar phase and a cation exchanger phase; the mechanisms are reversed-phase extraction plus cation-exchanging action. The LRC reservoir is advantageous, because as much as 10 mL of sample solution can be applied to it. As extraction columns of a similar type, Oasis® MCX and a disc-type SPEC • PLUS™ • DAU are commercially available.

c) The extraction mode for the Extrelut® column is sometimes classified as solid-phase extraction; but this is not true. It is essentially liquid-liquid extraction; it is not based on molecular interaction between the stationary phase and the mobile phase, which is just the principle of the solid-phase extraction. The essential nature of the Extrelut® column is the partition of a molecule between the water phase adsorbed to the support material and an organic solvent phase, which can be classified as liquid-liquid extraction. The Extrelut® is being sold as packed mini-columns or powder for repacking. The powder is convenient in that the volume of the packing material can be changed according to the volume of a sample solution to be poured. However, the Extrelut® column is inferior to solid-phase extraction columns in view of cleanup ability. When a trace compound is extracted with the Extrelut® column to identify the compound by mass spectral measurements, there are sometimes cases in which a satisfactory mass spectrum cannot be obtained because of contamination by impurity peaks. In the SIM, the possibility of such contamination is much lower and highly sensitive analysis can probably be achieved even with the use of the Extrelut® column.

d) As an IS, the use of a deuterated compound is ideal, because its physicochemical behavior is almost identical to that of a target compound. In USA, many deuterium-labeled compounds are being commercially available. In Japan, the situation is more inconvenient; therefore, a non-labeled IS was used in these experiments. In forensic judgement, the confirmation of a target compound is usually achieved by agreement of its mass spectrum with that of the authentic standard. Since, with simultaneous use of a deuterated IS, a mass spectrum of a target compound is interfered with by the IS, another mass spectral measurement is required without IS. Scopolamine tends to yield a peak due to dehydration of the compound in its mass spectrum, but can be used as IS. The author tried ketamine and an-

tipyrine as ISs for cocaine; ketamine suffered from variation of its peak height and antipyrine showed tailing. Both compounds showed similar problems in reproducibility.

e) The hydroxyl group of EME is acylated and the carboxyl group of BE is esterified for derivatizations simultaneously. As an acylating reagent, pentafluoropropionic anhydride, trifluoroacetic anhydride or heptafluorobutyric anhydride can be used. For estrification, hexafluoroisopropanol (HFIP) or trifluoroethanol (TFET) can be used. The combination of TFAA with TFET can also give good results for derivatizations.

f) For body fluid specimens, such as blood, urine, stomach content fluid and bile, sodium fluoride is added to each fluid at 10 mg/mL and the pH of the fluid is adjusted to 5, before storage in a refrigerator or in a frozen state. For solid specimens such as organs, the tissue are homogenized with a buffer solution (pH 5) containing sodium fluoride at 10 mg/mL, and centrifuged. The supernatant fraction is decanted and stored in a refrigerator or in a frozen state. Care should be taken to shorten the time between the sampling and the above pretreatments for storage.

g) Cocaine is largely metabolized into EME by cholinesterase present in blood and the liver after its rapid absorption into the body, and is also chemically hydrolyzed into BE; they are excreted into urine [17] (*Fig. 4.4*). With human experiments, it was reported that cocaine, EME and BE disappeared from urine in 8–16 h, 24–48 h and 48–72 h, respectively [15]. Although the amounts of the compounds excreted into urine is dependent on the methods of administration, the relative amounts of unchanged cocaine, EME and BE excreted into urine are 1–14 %, 12–60 % and 14–55 % of total amount of cocaine administered, respectively [16, 18, 19]. Because of the basicity of cocaine, its amounts to be excreted into urine are dependent upon pH of urine; its amount for excretion increases for acidic urine [16]. However, it is difficult to detect unchanged form of cocaine from urine more than 24 h after its use. In such cases, EME and BE should be analyzed as target compounds.

h) The optimal conditions are different according to each analytical instrument and separation column to be used. Various conditions including column temperature should be reexamined according to situations. Both recording of full mass spectra and quantitation can be simultaneously made in the scan mode.

i) The concentration of cocaine in blood is very low, and it is rapidly metabolized by cholinesterase; therefore, urine is a better specimen for analysis of cocaine and its metabolites.

j) When the column is dried, it causes the decrease of recovery rate; the column should be kept in a wet state with the buffer solution.

k) When the flow trough the column is rapid, the target compounds are not held in the column sufficiently; the flow rate should be as slow as 1 mL/min.

l) A majority of basic drugs is adsorbed to the cation exchanger phase, and a minor part is adsorbed to the non-polar phase. The latter part is desorbed and adsorbed again to the cation exchanger by washing the column with acidic solution. In place of the 0.1 M hydrochloric acid solution, 0.1 M acetic acid solution can be used.

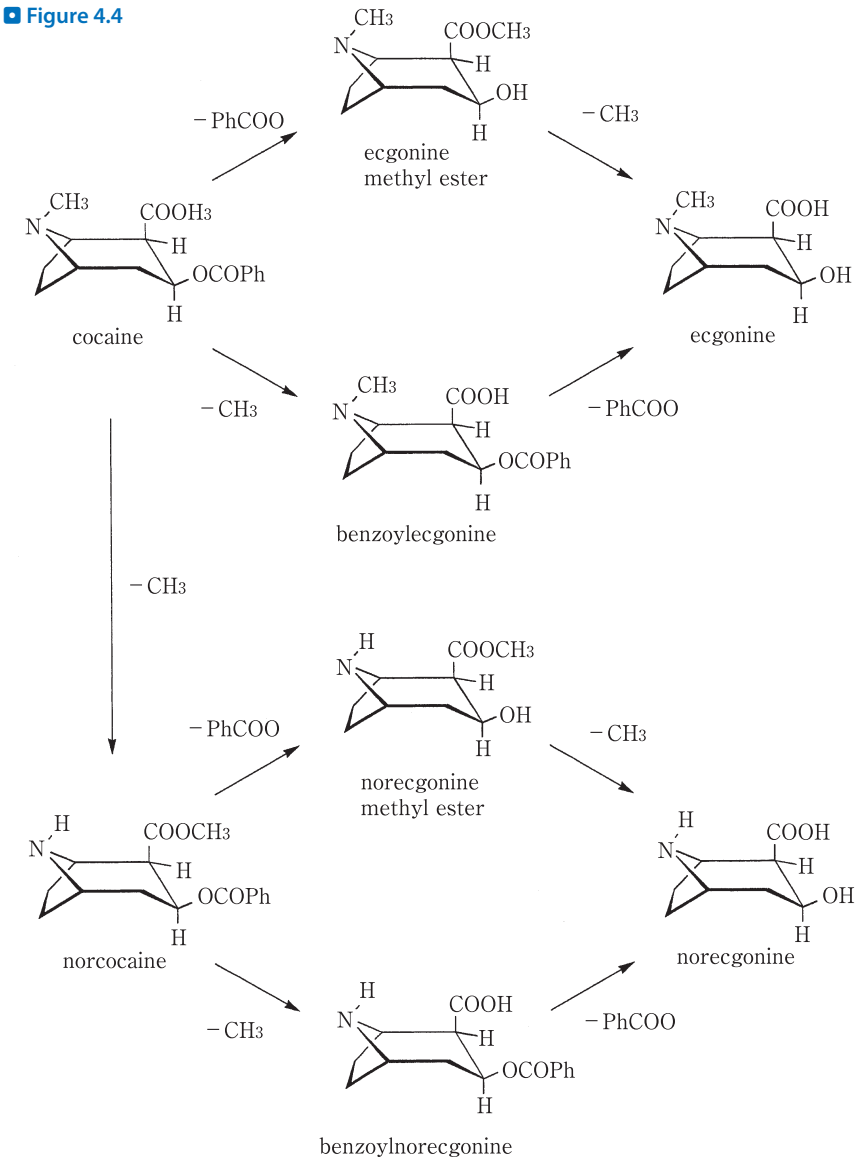
m) It is important to dry the column to secure a high recovery rate and efficient derivatization.

n) In place of nitrogen, air can be used for evaporation using an air pump.

o) After evaporation to dryness, the residue is dissolved in methanol; this step is useful for removal of insoluble components and thus contributes to enhance the cleanup.

p) Since cocaine is easily hydrolyzed under the alkaline conditions above pH 9, care should be taken to keep the pH of the solution in the range of pH 8–9.

**Figure 4.4**




**Metabolic pathways of cocaine.**

q) Cocaine, EME and BE generally showed linear calibration curves in the range of 100–1,000 ng/mL. EME-PFP showed variation in a low range (around 100 ng/mL) and in a high range (around 1,000 ng/mL) to some extent. For the extract from blood containing 100 ng/mL of BE, characteristic peaks of the BE-HFIP derivative can be observed in the mass spectrum. However, the mass spectrum obtained from the extract is sometimes interfered with by background impurity peaks, resulting in the difficulty to obtain a clean mass spectrum; while at 200 ng/mL of BE in blood, a satisfactory mass spectrum can be obtained. For BE,

therefore, the calibration curve should be constructed in the range of 200–1,000 ng/mL. When very high concentrations of a target compound can be expected in advance, the mode of injection can be changed from the splitless mode to the split one to obtain good linearity.

r) For autopsy specimens, the postmortem interval (period between death and autopsy) and the *in vitro* interval (period between sampling and analysis) are variable; the hydrolytic rate of cocaine shows great variation. Usually, the metabolism, metabolite accumulation and excretion for cocaine habituals are quite different from those for non-habituals. Therefore, it is difficult to accurately estimate the cause of death, time of death and time after drug use, using the ratio of cocaine to BE and the brain-to-blood ratios of cocaine and BE. The ratios only give rough estimation of them.

## References


1) Editorial Committee of the Manual of the Japanese Pharmacopoeia (ed) (1996) Manual of Japanese Pharmacopoeia, 13th edn. Hirokawa Publishing Co., Tokyo, pp C556–C559 (in Japanese)
2) Hawks RL, Kopin IJ, Colburn RW et al. (1974) A pharmacologically active metabolite of cocaine found in brain. Life Sci 15:2189–2195
3) Baselt RC (1983) Stability of cocaine in biological fluids. J Chromatogr 268:502–505
4) Isenschmid DS, Levine BS, Caplan YH (1989) A comprehensive study of the stability of cocaine and its metabolites. J Anal Toxicol 13:250–256
5) Matsubara K, Maseda C, Fukui Y (1984) Quantitation of cocaine, benzoylecgonine and ecgonine methyl ester by GC-CI-MS after Extrelut extraction. Forensic Sci Int 26:181–192
6) Varian's Bond Elut Certify Instruction Manual. Harbor City, CA, USA
7) Abusada GM, Abukhalaf IK, Alford DD et al. (1993) Solid-phase extraction and GC/MS quantitation of cocaine, ecgonine methyl ester, benzoyl-ecgonine, and cocaethylene from meconium, whole blood, and plasma. J Anal Toxicol 17:353–358
8) Crouch DJ, Alburges ME, Spanbauer AC et al. (1995) Analysis of cocaine and its metabolites from biological specimens using solid-phase extraction and positive ion chemical ionization mass spectrometry. J Anal Toxicol 19:352–358
9) Ueki M (1999) Significance and problems of doping tests and toxin analysis. Nipponrinsho 57:361–363 (in Japanese)
10) Sander R, Ryser MA, Lamoreaux TC et al. (1985) An epidemic of cocaine associated deaths in Utah. J Forensic Sci 30:478–484
11) Spiehler VR, Reed D (1985) Brain concentrations of cocaine and benzoylecgonine in fatal cases. J Forensic Sci 30:1003–1011
12) Karch SB, Stephens B, Ho C-H (1998) Relating cocaine blood concentrations to toxicity-an autopsy study of 99 cases. J Forensic Sci 43:41–45
13) Jenkins AJ, Levine B, Titus J et al. (1999) The interpretation of cocaine and benzoylecgonine concentrations in postmortem cases. Forensic Sci Int 101:17–25
14) Hamilton HE, Wallace JE, Shimek EL et al. (1977) Cocaine and benzoylecgonine excretion in humans. J Forensic Sci 22:697–707
15) Amber J (1985) The urinary excretion of cocaine and metabolites in humans: a kinetic analysis of published data. J Anal Toxicol 9:241–245
16) Moffat AC, Jackson JV, Moss MS et al. (eds) (1986) Clark's Isolation and Identification of Drugs, 2nd edn. The Pharmaceutical Press, London, pp 489–490
17) Vitti TG, Boni RL (1985) Metabolism of cocaine. In: Barnett G, Chiang CN (eds) Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs. Biomedical Publications, Foster City, CA, pp 427–440
18) Amber J, Fischman M, Ruo T-I (1984) Urinary excretion of ecgonine methyl ester, a major metabolite of cocaine in humans. J Anal Toxicol 8:23–25
19) Amber J, Ruo TI, Nelson J et al. (1988) Urinary excretion of cocaine, benzoylecgonine, and ecgonine methyl ester in humans. J Anal Toxicol 12:301–306