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# RECENT ADVANCES IN DOPING ANALYSIS (2)

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# Coca and cocaine : what can be done in doping analyses.

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Abstract :

The traditional uses of the Coca plant in South America and its active constituents are surveyed in view of their modern therapeutic applications, food consumption as well as drug of abuse.
The pharmacological basis of their benefits during exercise is examined closer together with the stability of cocaine and its metabolites in biological fluids and other materials.
Pharmacokinetic data obtained so far with modern analytical tools have opened the possibility of detecting both Coca leaves' and/or cocaine's consumption with high confidence during much longer time period than previously thought. For example, the occurrence of cocaethylene in urine is the indicator of concomitant intake of both cocaine and ethanol. Highly sensitive techniques of drugs' detection are now more commonly available which indicate that risk of adsorptions through the skin when manipulation of cocaine or even some commonly used bank notes by trafficants or contaminations during sample collection is becoming a real problem. Additional caution for the toxicological interpretation of the analytical data is therefore mandatory.

**Key words** : *Erythroxylum*, coca leaves tea, oral intake, performance enhancement, urine analysis, derivatization, GC-MS analysis , cocaine, benzoyl-ecgonine, methyl-ecgonine, ethylcocaine, cocaethylene.

**Introduction** : The Coca plant has been a part of human affairs in South America for several thousand years. Its leaves have been identified botanically and chemically in pre-Christian area archaeological specimens from Peruvian mummy bundles.
According to the International Rules of Botanical Nomenclature, the correct generic name of coca should be *Erythroxylum*, and not *Erythroxylon*, even though the word has a Greek and not a Latin root, meaning "red wood" from the colour of its woody roots.

**Botany :** *Erythroxylum*, by far the largest of four genera of the family of Erythroxylaceae, comprise some 250 species (Plowman, 1985) which are widely distributed throughout the tropics, but with large areas of diversity in South America and Madagascar. It is evident that only the cultivated varieties are those which produce significant amount of cocaine : *E. coca* Lam. is the Amazonian and Bolivian coca. *E. novogranatense* (Morris) Hieron. is the Colombian coca and *E. novogranatense* var. *truxillense* (Rusby) Plowman is the Trujillo or Peruvian coca. Finally, an other sub-variety of *E. coca* Lam. var. *Ipadu* Plowman is specifically grown in the Amazon by the native Indians for their own everyday consumption (Plowman, 1981).

**Phytochemistry of Coca alkaloids :** Beside, cocaine, other alkaloids are to be found in cultivated *Erythroxylum* species. These alkaloids are mainly esters of the amino alcohols tropine (= tropine-3 alpha-ol), several hydroxytropines, pseudotropine (= tropine-3 beta-ol), ecgonine and their N-nor-derivatives. Whereas tropine and pseudotropine alkaloids occur in other families and are particularly widespread in Solanaceae, the ecgonine-type alkaloids seem to be restricted to Erythroxylaceae. Ecgonine is a pseudotropine bearing an additional carboxylic group in position 2. These compounds like cocaine (benzoyl-ecgonine methyl ester), are thus direct indicators of the plant species. All Erythoxylum species do not contain cocaine or other alkaloids (Plowman and Rivier, 1983), but cultivated species have shown cocaine concentrations ranging from 0.13 to 0.68 % by dry weight in the leaves while seeds contained no cocaine at all. Depending of the place of cultivation and the species, the amounts of alkaloids are quite different. For example, *E. coca* grown in Trujillo contains 0.60 % cocaine together with 0.15 % both cis and trans cinnamoylcocaine, but *E. coca* from Cuzco, an other town in Peru showed only 0.06 % cinnamoylcocaine with the same cocaine content. The ratio between the two cis/trans isomers can vary also between 1:1 to 3:1 (Turner et al., 1981). Alkaloid distribution among the leaves of various ages in *E. coca*, *E. novogranatense* and *E. novogranatense* var. *truxillense* could not be used as a chemotaxonomic marker since variation within leaves of one species exceeds those between leaves of different species (Rivier, 1981).

**Chemistry of street samples :**The bicyclic structural formula of ecgonine (as for cocaine) allows four D-forms and four L-forms and, consequently, four racemic forms. Cocaine and pseudo-cocaine are well investigated and of medical importance. The remaining two, which have been named allo-cocaine and allo-pseudo-cocaine are fairly unstable, probably due to the sterically unfavourable position of the bulky benzoyloxy group. A HPLC procedure for rapid separation and identification of the four cocaine isomers in an unknown street sample has been published (Lewin et al, 1980). In general, street samples are originating from the plant material, that is the dried coca leaves, mixed with Calcium, Potassium or Sodium

Carbonate and solvents ( kerosene for most of the time). Several steps are needed to convert the bases into methylecgonine, from which cocaine is finally made by adding benzoylanhydride. Thus, the most commonly cocaine found in sized street materials is the L-cocaine. If synthesis was made in the clandestine laboratory, one would detect all four isomers. Due to the higher cost of synthetic cocaine, natural one is the only form seen in both the legal and the non legal market.

The average cocaine concentration in the street samples we have obtained in our Institute ranges around 40 % (15 to 87 %) in 1993. Drug profiling analysis is sometimes requested to compare different samples in order to determine if they are originating from the same cocaine preparation. A rapid method for comparing illicit cocaine samples consisted in the rapid dilution of the sample it-self into Methanol followed by automated injection onto a capillary GC-NPD system. Quantitated samples were weighted to contain equivalent amounts of cocaine, allowing the cocaine to function as internal standard. Thus, illicit cocaine samples, prepared from a common source by the addition of diluents and adulterants, could be compared regardless of the cocaine concentration. The peak ration of four alkaloids (tropacocaine, norcocaine, cis-cinnamoylcocaine and trans-cinnamoylcocaine) to cocaine were calculated for each sample to produce a data base for statistical analysis. The ratios were around 0.5 %, 1 %, 5 % and 3.5 % for these four alkaloids, respectively (Janzen et al, 1994). This quite low quantities of additional alkaloids indicates that in urine, there are no chance to detect any of these four compounds, besides cocaine and its metabolites.

Cocaine hydrochloride is thus the most commonly form sold on the black market. This salt is also converted in cocaine-base, called crack ready to be smoked in specially designed pipes.

**Pharmacological and subjective effects on Human :** Psychic effects of cocaine intake are marked by a strong and rapid central stimulation. On the somatic side, one can notice anorexia, insomnia, tachicardia, arterial hypertension and dilatation of the pupils. As potent vasoconstrictor, cocaine has been used as anaesthetic agent in chirurgical ORL called Bonain's liquid preparation. Dependence is marked with more psychic than physical deficiency states in between drug's intakes.

In the Western World, cocaine is more often sniffed, smoked or injected, sometimes together with heroine. It seems that its use is of the recreational and occasional type, concerning people from the show business, journalism, political world, etc.

**Traditional uses of coca leaves :** A completely different picture emerges from its traditional use : the chewing of the leaves of *Erythroxylum coca.*

Throughout the centuries, the chewing of coca leaves has remained an integral part of Andean culture. It is not only in the highlands that coca was and still is employed : it plays a vital role in many Indian societies in the western Amazon. Despite its great age and widespread existence, the custom of chewing coca is poorly understood and it is considered noxious by some authorities. Other believe it to be physiologically beneficial for Andean Indians in their adaptation to hunger, cold fatigue at high altitudes. Since the time of the Spanish Conquest, observers have wondered about the performance and endurance of the Indians under the influence of coca. The fast runners of the Inca who travelled the roads in the highlands to bring messages used to measure distances in "cocadas"; i.e. distances covered under the influence of one quid of coca. One "cocadas" is said to be about three kilometres. There have naturally been no control experiments, and scientific evidence for an increased performance is lacking even today (Holmstedt, 1981). The difference of stimulation obtained, as judged subjectively, between using the whole coca leaves, coca tea and taking cocaine by local application in the nose or by intravenous injection or even by smoking cocaine base seem to lie essentially in means of administration and dosage. Preliminary work by Buchard indicated that coca protects against development of hyperglycaemia and reactive hypoglycaemia following oral glucose load in Andean Indians (Buchard, 1975). If all this is verified, it may result in a new approach to the management of high starch utilisation and such coca use might be of interest for the intense physical exercise which is commonly practicized by top athletes. In modern terms, coca appears to be useful treatment for various gastrointestinal ailments, motion sickness, and laryngeal fatigue. It can be an adjunct in programs for weight reduction and of physical fitness. It may also be used as a fast-acting antidepressant (Weil, 1981). It has been advocated without success so-far that Coca could be administrated as a chewing gum or lozenge containing whole extract of the leaf, including alkaloids, natural flavours, and nutrients. However, it has never been possible to convince the health authorities of Western countries to accept such uses, and attempts to promote simple oral coca leaf consumption through the importation of tea bag preparations had failed.

**Coca-cola :** A French chemist made the first commercially available drink in 1861 named "Vin Mariani". This alcoholic tonic vine received immediate and important success. In the U.S.A., J.S. Pemberton, a pharmacists invented a stimulating drink recipe to be used against headache which was sold in 1891 to A.G. Candler. This latter founded the next year the Coca-cola company for the large scale production of the very successful drink. Until 1906, Coca-cola drinks did contain cocaine as it was not yet forbidden at that time in the U.S.A. Later, cocaine was replaced by caffeine, but the actual drink still contain flavours from coca leaves extracts.

**Coca leaf tea :** Coca leaf tea is commonly offered in Peru, Ecuador and Bolivia to travellers who are coming from low lands and suffer from altitude sickness and other minor disorders when arriving at high altitudes. Manufactured coca tea bags have been reported to be imported to the United States' market under the trade name "Health Inca Tea" and its ingredients are listed as "decocainized coca leaves". Analyses of "Health Inca Tea" bags showed an average cocaine content of 4.8 mg per bag (Siegel et al., 1986). The presence of cocaine in coca tea has raised the question of whether it is possible for a coca tea drinker to show positive for cocaine and or metabolites in an urine test. GC-MS analyses have shown that even after 29 hours, a tea drinker will still be with a benzoylecgonine concentration higher than 100 ng/ml after consuming one cup of "Health Inca Tea" (ElSohly et al., 1986). Although the selling of such tea bags is forbidden in the U.S.A. and most probably in many other countries, it is still very common in South America. On the other hand, the increase of illicit use of cocaine in western societies over the last 20 years has focused attention on this problem's drug and the rapid onset of dependence of the cocaine consumer is problematic on several point's views : on the one hand it is a serious health problem, and on the other hand, one cannot but worry about the true physical and intellectual independence of a competitor being hoocked on cocaine.

These situations are indicative to the problems that not only toxicologists and forensic scientists, but also doping regulatory specialists could face when interpreting data from urine analyses. The general knowledge of all ways of drug intakes and their respective pharmacological data should provide a sound base for the detection of coca or cocaine consumption and for decision making in doping controls.

## Experimental :

**Confection of a coca tea:** 1 liter of boiled water was added to ten grams of dry coca leaves (*E. coca var. coca L.*). In order to improve the extraction of cocaine from the vegetal material, the juice of one lemon was also added to the solution which was absorbed by the volunteers at least 15 min after the preparation.

At that time, 1 ml of the coca tea was collected for cocaine quantification. The mean concentration values of cocaine in this tea were found to be between 34 and 41 mg/liter of tea.

**Excretion studies:** The volunteers were drinking 1 liter of coca tea each with some sugar in it. The time of absorption was limited to 30 min. Time 0 was defined as the beginning of drinking and each urine emitted was then collected for the 72 following hours.

The urine samples were stored at 4°C after adding 1g/l of sodium azide. The sample analyses were performed less than two weeks after the excretion studies.

## Analyses of the samples

**Screening:** In our laboratory, screening of cocaine and metabolites is performed through several procedures.
Our screening procedure V for diuretics is based on extractive methylation (Lisi et al, 1992). It allows to detect in the same signal cocaine and methylated benzoylecgonine.
The screening procedure II for heavy volatile nitrogen compounds adapted from Cologne laboratory procedure (Donike et al, 1988), is also used to screen cocaine and its other metabolite, ecgonine methylester, the latter detected in its TMS-derivative form. (see Figure 1 for the TMS derivatives of cocaine metabolites).

**Confirmation:** 2 ml of borate buffer 1 M (pH=9.0) was added to an aliquot of 2 ml of urine and vortexed for 10 s. Extraction was performed with 3 ml of chloroform / isopropanol (3:1). Samples were then centrifugesd and the organic phase was transferred and evaporated to dryness under a nitrogen stream at 40°C. The residue was dissolved with 100 µl of Pentafluoropropionyl anhydre / Pentafluorpropanol (PFPA / PFP, 2:1). The mixture was gently vortexed and heated at 60°C for 20 min. After cooling, to room temperature, it was evaporated to dryness and the residue was redissolved in 50 µl of ethylacetate. 1 µl of this solution was injected into the GC-MS system (see Figure 2 for the PFP derivatives of cocaine metabolites).

**GC-MS analysis for screening and confirmation:** GC/MS from Hewlett-Packard was used (GC 5890 serie II was fitted with a HP7673A autosampler and connected to an MS 5971. The separation was carried out using a 5% phenylmethylsilicone fused capillary column (HP, Ultra 2, 25 m, 0.2 i.d., 0.33 µm film thickness).
The injector (splitless) and the interface were operated at 270 °C and 280°C resp. The temperature program was: initial temperature 100°C for 0.5 min , then to 320°C at the rate of 20°C/min and maintained for 7 min at 320°C. Helium was used as carrier gas at a flow rate of 1 ml/min. The mass spectrometer was operated with electron impact ionization (EI 70 eV) in the scan mode from 50 to 500 amu at 0.95 scans per second).

**Quantification procedure:** For the quantification of cocaine and its main metabolites, deuterated internal standards (N-trideuterated compounds from Radian, USA) for each substance were used in order to establish calibration curves in the appropriate range of concentration. Analyses were performed on the PFP derivatives in the SIM mode with two specific ions per substance.

## Results

### GC-MS analysis

**Cocaine** is not derivatizable in our procedures. The mass spectrum of cocaine after screening II or confirmation process is of course the same. The Figure 3 shows that the signal abundance is quite similar in both procedures with the same amount of starting material. In that case an urine collected two hours after beginning of coca leaves tea absorption.
In our screening procedure, the ions m/z=303 and m/z=182 are extracted from a scan analysis to screen cocaine.

**Ecgonine methylester** in its TMS-derivative form ($M^+$= 271) is producing a mass spectra with high signal in the lower masses (m/z=82 for the base peak and m/z=97) and some interesting fragments in the higher masses, the molecular ion being around 10% of the base peak abundance (Figure 4 C and D).
For the screening procedure, ion m/z=182 and the molecular ion, because of their better specificity, are extracted to screen ecgonine methylester TMS derivative.
The PFP derivative obtained after the confirmation procedure (Figure 4 A and B) is showing specific ions in the higher masses (base peak m/z=182 and the molecular ion m/z=345) with a relative higher abundance and lower fragmentation when compared with the TMS derivative.

**Benzoylecgonine** is more difficult to extract than the two first compounds with the screening procedure II. The Figure 5 C and D shows that the signal abundance on the same sample is much lower after this extraction than in the confirmation procedure (Figure 5 A and B), although, tthis is not due to the type of derivatization.
In our laboratory, benzoylecgonine is not screened through the procedure II. But after extractive methylation in the procedure V, any benzoylecgonine in the sample will be methylated and show a cocaine signal.

Proposed fragmentation pathways for the different derivatives are shown in the Figure 6.

### Pharmacokinetic profiles :

After drinking 1 liter of coca leaves tea, cocaine is detectable in urine for less than 10 hours (see Figure 7) with an excretion peak of 1.5 µg/ml after 2 hours.
The ecgonine methylester (EME) is found in its higher concentration after 4 hours, whereas benzoylecgonine (BZE) concentration peak of about 30 µg/ml comes only 13 hours after tea absorption.
Both metabolites are still detectable in urine 70 hours after intake, the concentrations being of 600 ng/ml and 100 ng/ml (for BZE and EME

respectively). These results show that one could be still positive to cocaine metabolites more than two days after consomption.
The presence of cocaine itsself in urine shows a relatively recent intake, certainly less than ten hours.
These results are possibly dose-dependant. Further experiments with other dosages should be set up to investigate this point.

## Discussion:

**Exposition to and metabolism of cocaine:** Some controversy has recently been raised with the detection of ultra-trace amounts of cocaine on the surface of more than 75% of the one dollar US bills commonly being exchanged in most of the major cities in the USA (Cone, 1994). Adsorption of cocaine through the skin is possible specially when in solution and positive finding of trace amounts of benzoyl-ecgonine in urine might be attributed to exposure to cocaine and not only to ingestion (see Figure 8).

Peak plasma concentration of cocaine depends on the way of administration, 10 to 20 min after injection, 60 min after ingestion or inhalation. Cocaine is rapidly biotransformed at the liver and by blood's esterases into non-psychoactive metabolites benzoyl-ecgonine (30 to 50 % of the dose) and methyl-ecgonine (20 to 40 % of the dose) and other minor compounds like ecgonine (1 to 10 % of the dose) or nor-cocaine. Moreover, when taken together with ethyl alcohol, cocaine is partially converted by liver enzymes to its ethyl homologue called cocaethylene or ethylcocaine (Figure 8). Ethylcocaine is psychoactive and also more toxic than cocaine itself. Since ethylcocaine has never been detected in street samples, its presence in body fluids can be considered as conclusive of concurrent cocaine and ethanol intake (Giroud et al. 1994). This appearance of a new psychoactive compound formed in vivo from two exogenously applied substances is remarkable as ethylcocaine can be used as excellent evidence for cocaine ingestion. It has not been possible for us to detect so far the presence of ethylecgonine or norethylcocaine in urine sample from overdose cases. Typically, elimination in urine takes 48 to 72 hours, approximately.

**Stability of cocaine and its metabolites in biological fluids :** Urine pH should be checked as at high pH, cocaine and benzoylecgonine are unstable. Also addition of an inhibitor of esterases (2% Sodium Fluoride or Physiostigmine) is recommended in particular for blood or plasma samples in order to maintain the original levels of cocaine and each metabolite.
In order to evaluate the importance of dependence of a person, laboratories are asked to give hints on metabolism. Long term exposure to drugs of abuse allows now their detection in hair if the appropriate technology is applied. This also true for cocaine although care should be taken to remove

any possibly contaminating cocaine out-side the hair matrix. Usually, hair are growing at 1 to 1.5 cm each month. Some are normally growing and other stop their elongation during some weeks or others definitively. Hair analyses might also be of some help in order to understand and evaluate the extend of cocaine intake during the few last months depending of the length of the collected hair.

**Doping issues :** In the doping context, a positive urine for cocaine and/or metabolites means that a drug of abuse has been taken. There are no way at the present time to assess the stimulating effect of the drug by urine analyses. Blood is then requested, but when the results of the urine test are known, it is too late to take any blood sample. Usually, the controlling agency is then concerned more likely with a health problem that with an performance enhancing problem. To which extend is that person dependent on cocaine is the next question often asked. The laboratory can answer partly to this in proposing additional urine and hair analyses, besides of course, a carefully made medical examination. The intensity of the cocaine intake will of course determine the correct treatment. In conclusion, once a positive cocaine case is discovered, one has to continue the examination of the specimen in order to classify the importance of the dependence to this drug. It is hoped that this paper will serve as guide to the analyst in order to find out the best approach for answering the controlling agency.

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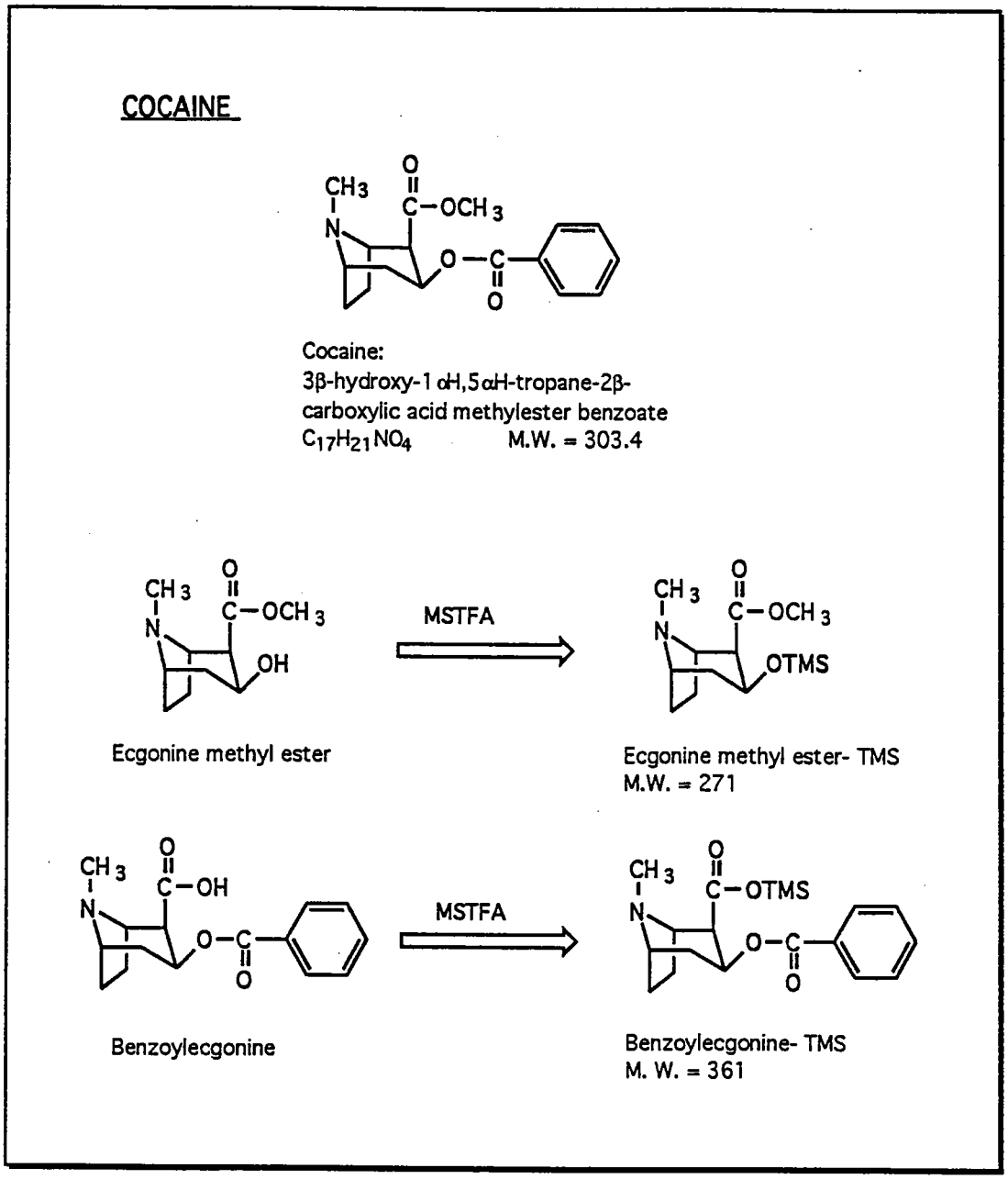


**Figure 1:** Cocaine and metabolites before and after TMS derivatization

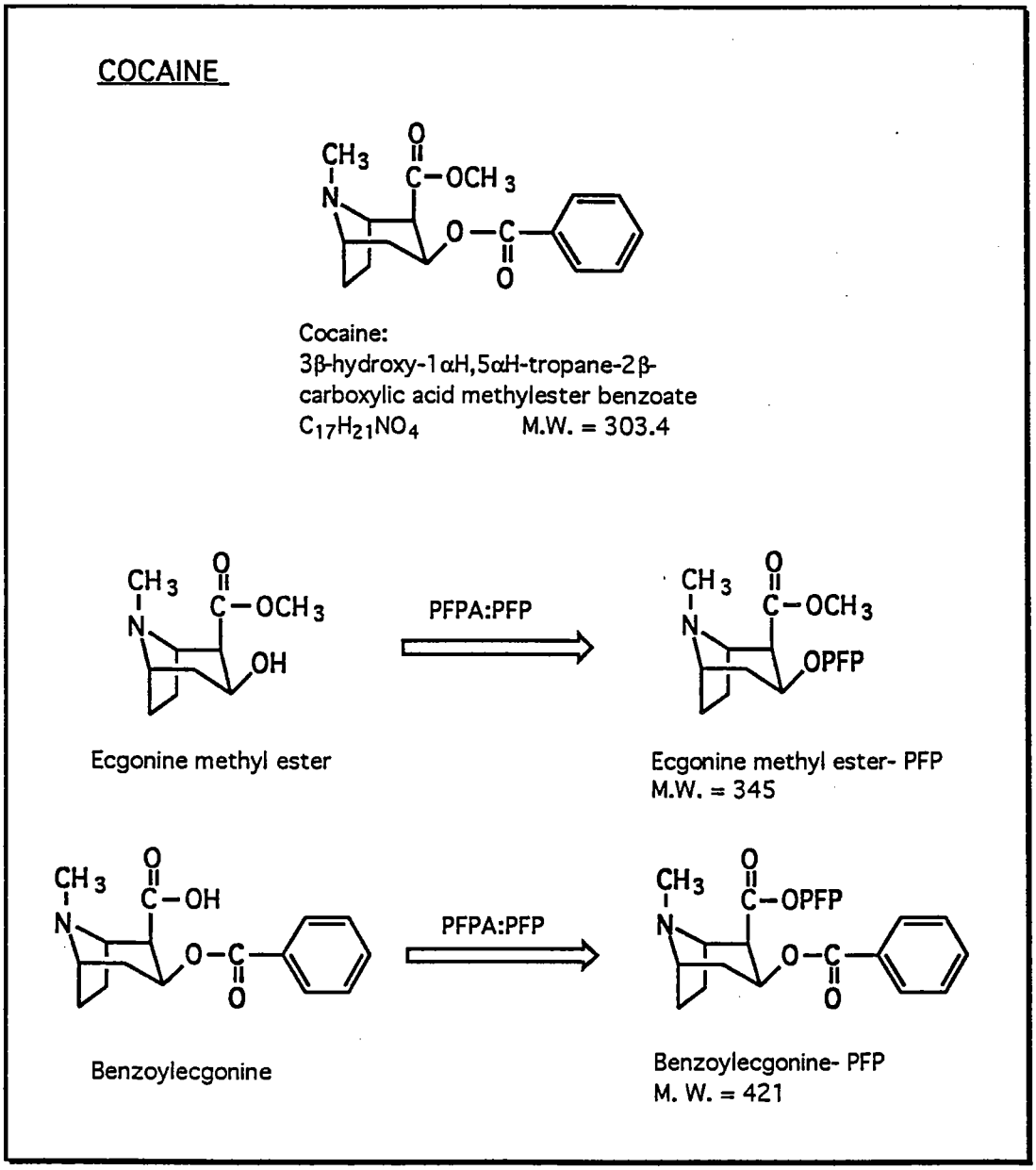


**Figure 2:** Cocaine and metabolites before and after PFP derivatization

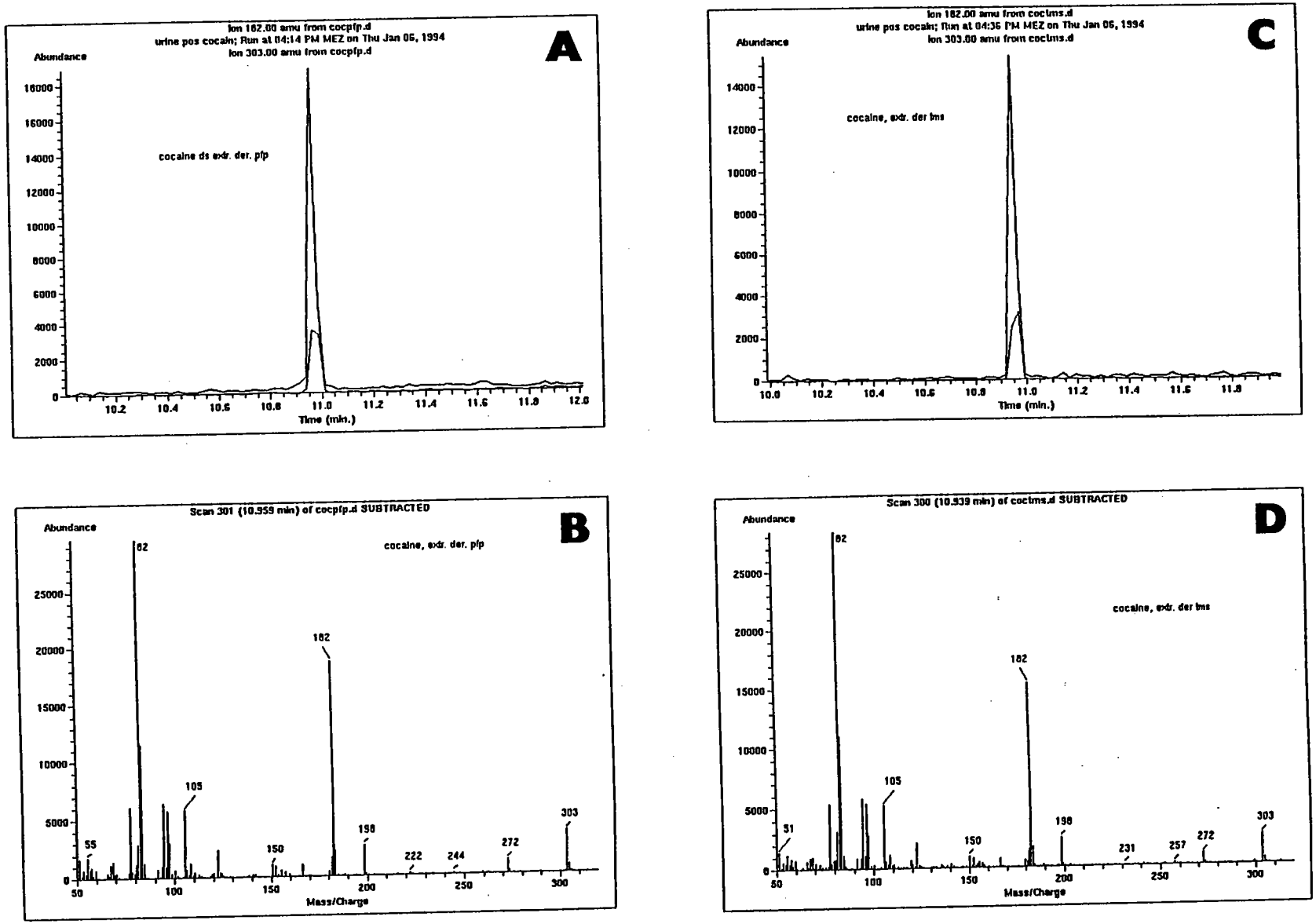


**Figure 3:** **Urine sample, 2 hours after coca leaves tea intake.**
Extracted ion traces m/z=182 and m/z=303 and mass spectrum at cocaine R.T. of :
A and B: Procedure II extract with TMS derivatization (cocaine is not derivatized)
C and D: Confirmation procedure extract with PFP derivatization (cocaine is not derivatized)

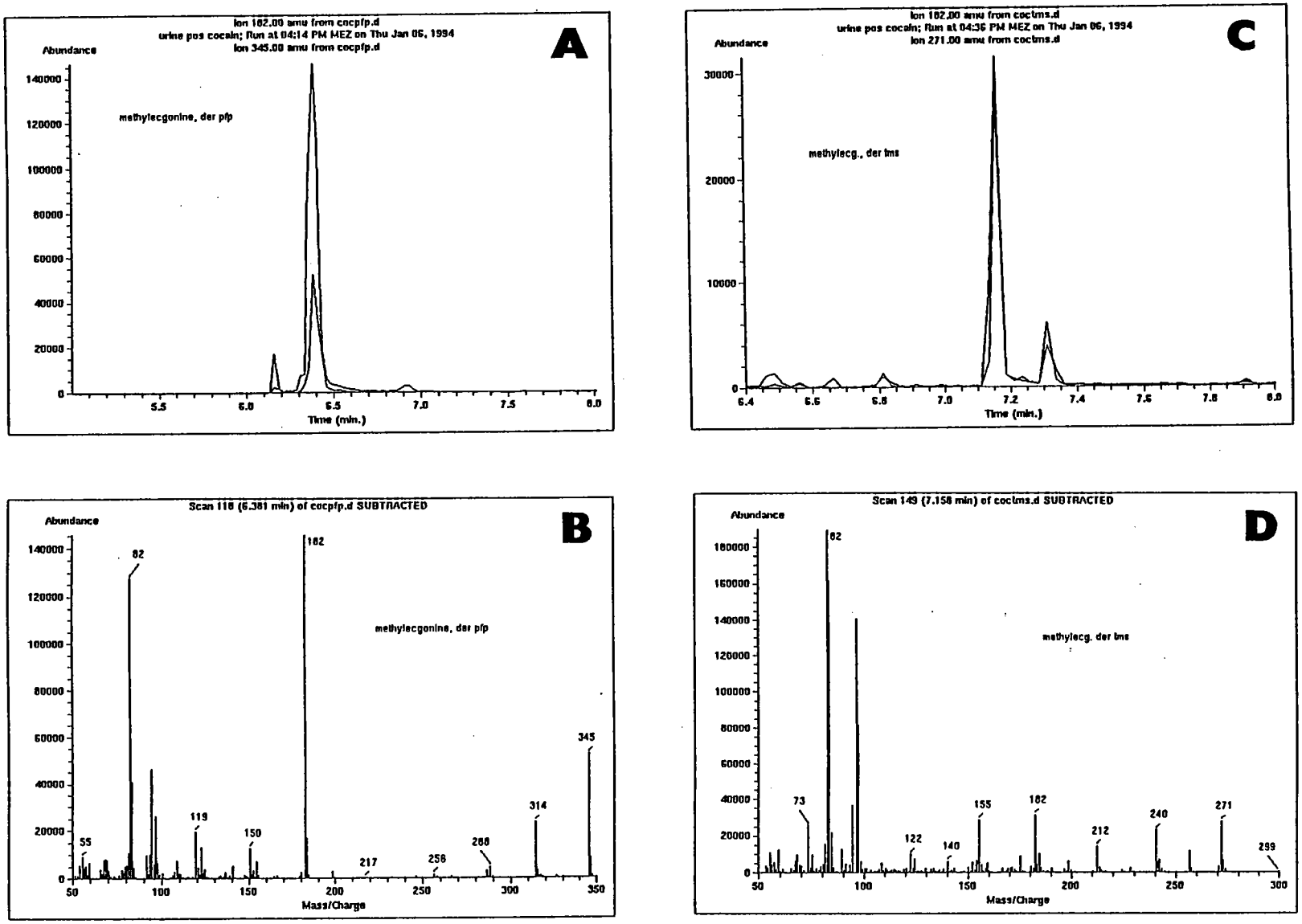


**Figure 4:** **Urine sample, 2 hours after coca leaves tea intake.**
A and B:Extracted ion traces m/z=182 and 345 and mass spectrum at EME- PFP derivative R.T.
C and D:Extracted ion traces m/z=182 and 271 and mass spectrum at EME-TMS derivative R.T.

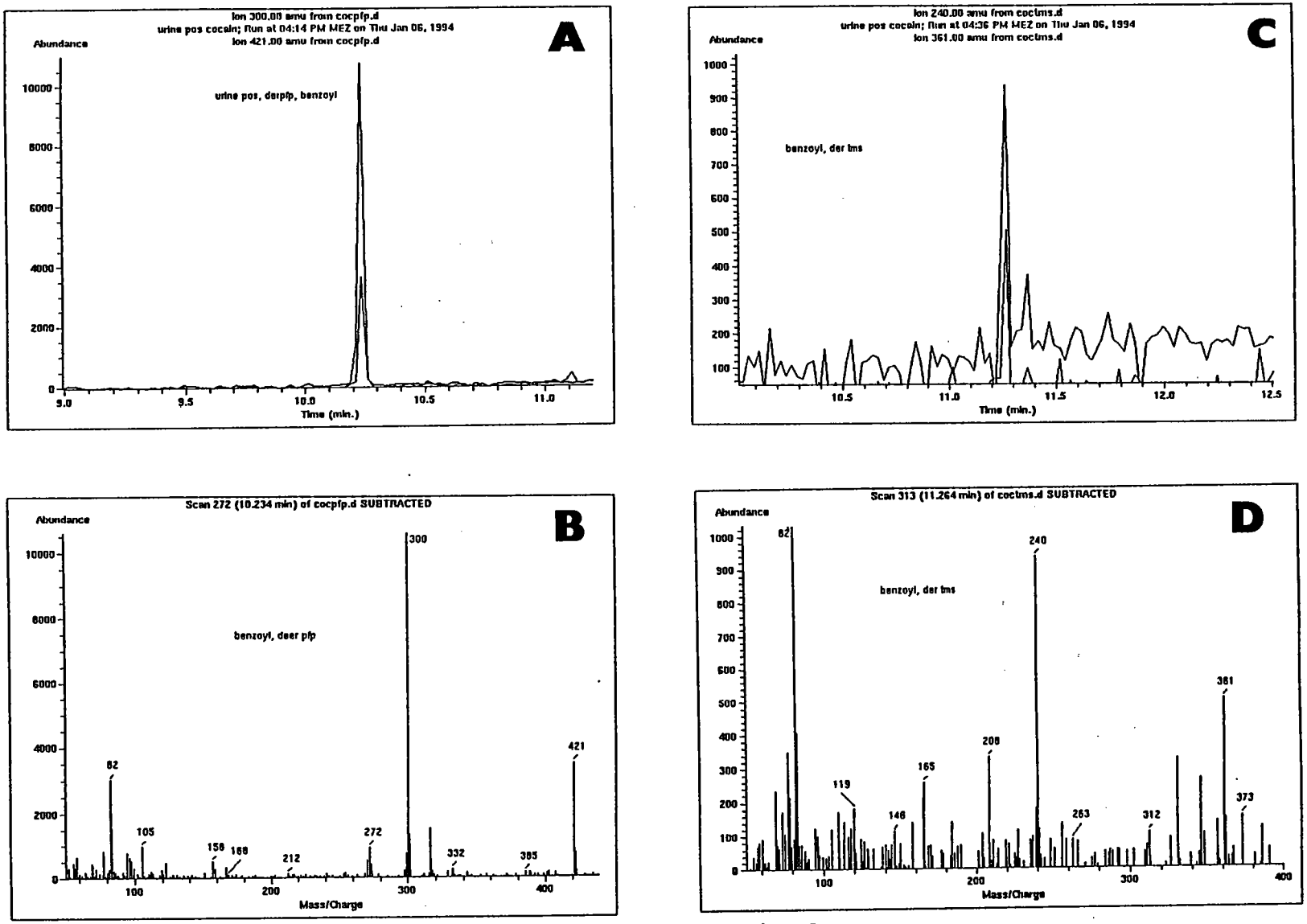


**Figure 5:** **Urine sample, 2 hours after coca leaves tea intake.**
A and B:Extracted ion traces m/z=300 and 421 and mass spectrum at BZE- PFP derivative R.T.
C and D:Extracted ion traces m/z=240 and 361 and mass spectrum at BZE-TMS derivative R.T.

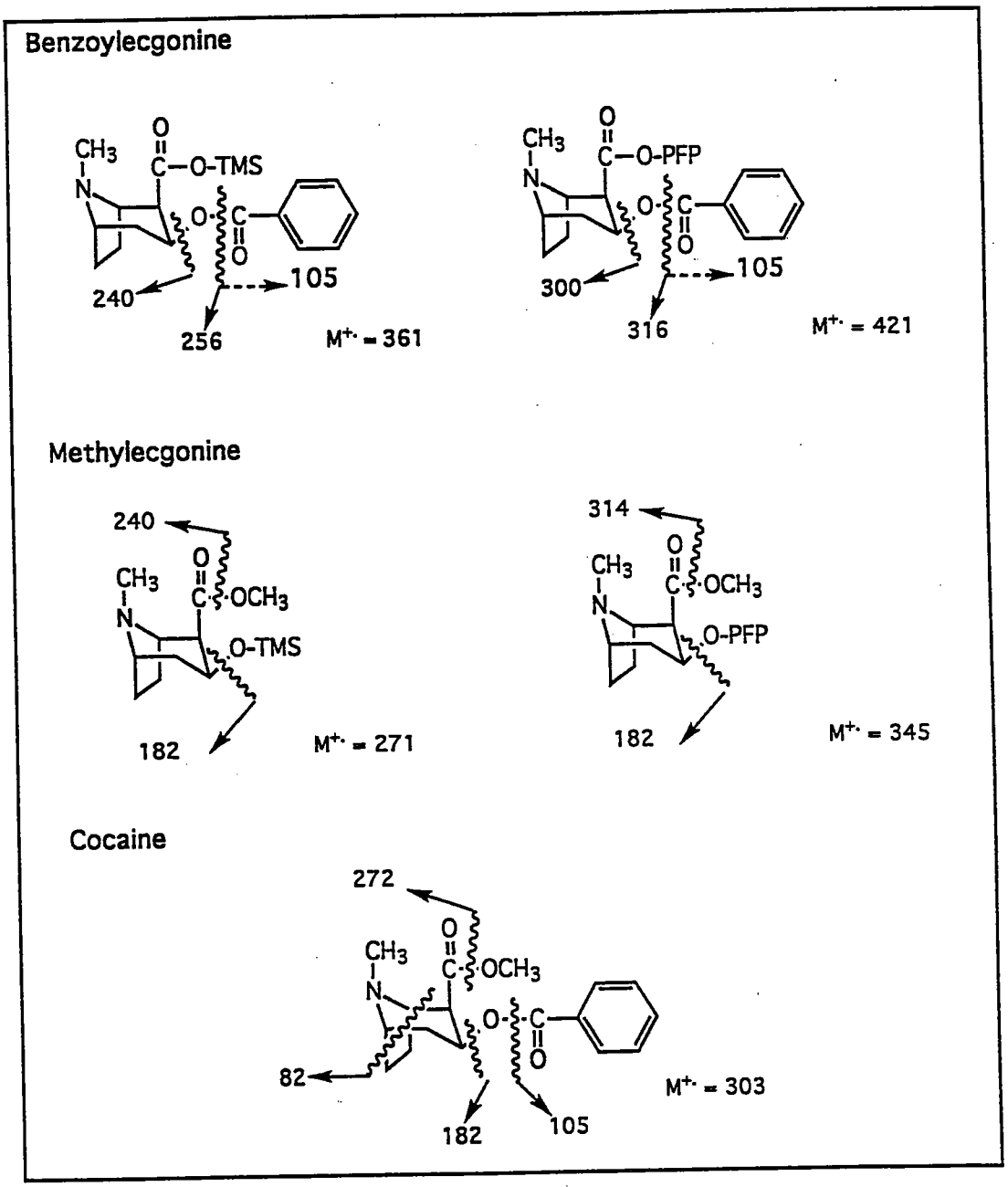


**Figure 6:** Proposed fragmentation pathways for cocaine and metabolites in their different derivative forms

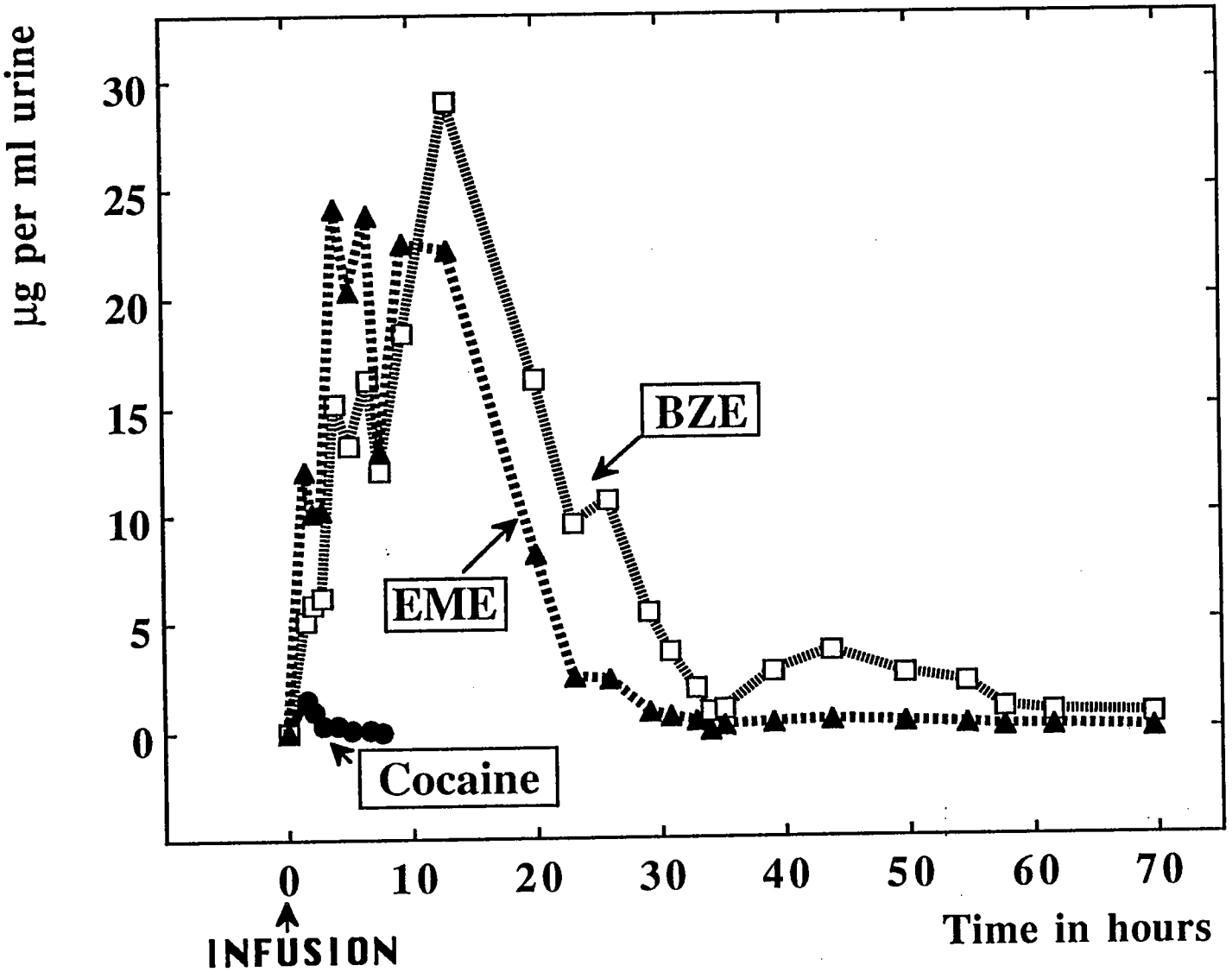


**Figure 7:** Excretion of cocaine and metabolites after coca leaves infusion intake

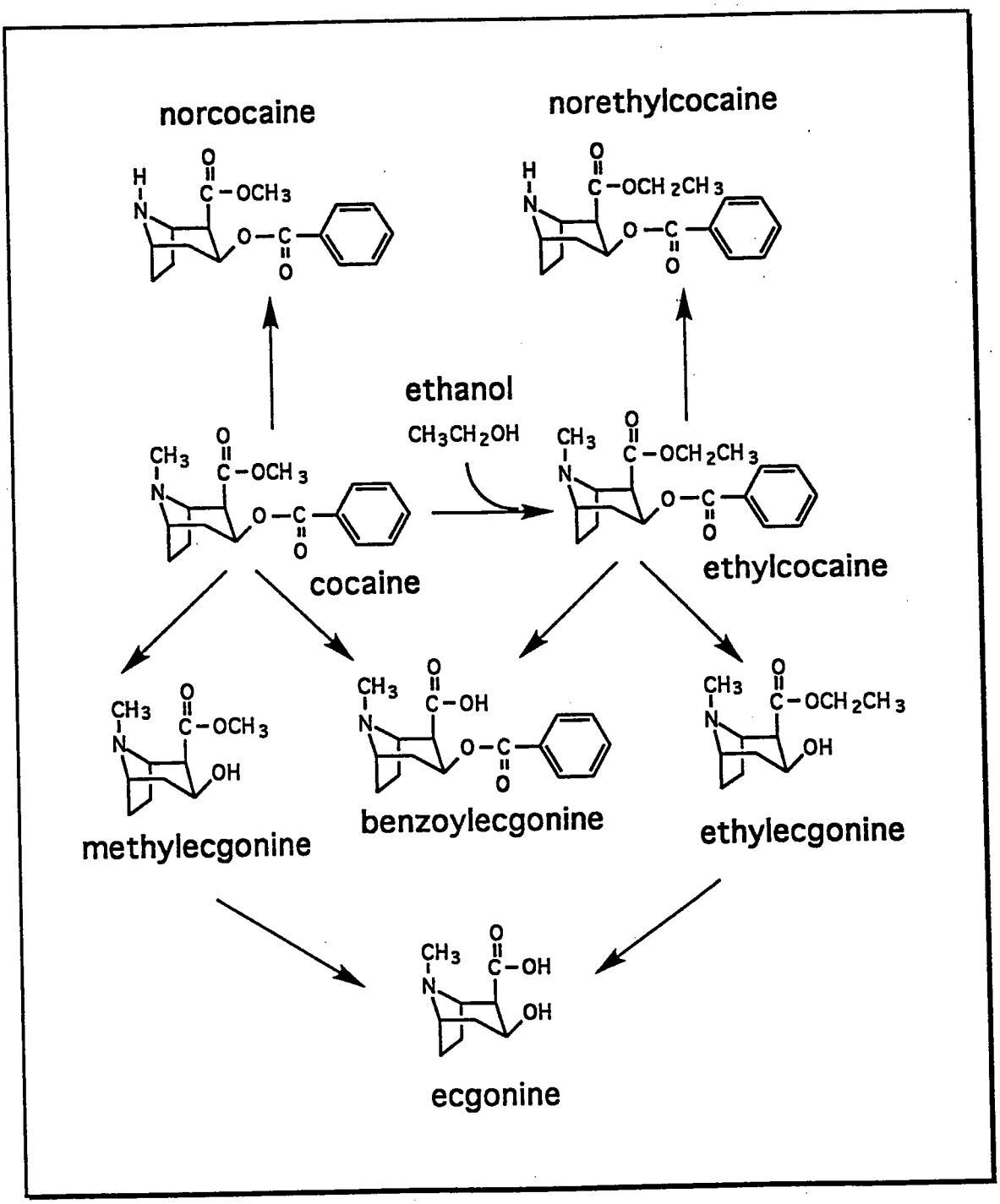


**Figure 8:** Routes of metabolisation of cocaine with or without concomitant alcohol absorption.