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## Excretion studies with dextropropoxyphene

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### Introduction

Dextropropoxyphene, (1S,2R)-(1-Benzyl-3-dimethylamino-2-methyl-1-phenylpropyl)-propionat, (Fig. 1), which was first synthesized in 1953, is an analgesic opioid and was regarded till 1997 as a banned doping substance belonged to the group of **narcotics**.

*'The term narcotic was derived from the Greek word for stupor. At one time, it referred to any drug that induced sleep, but then became associated with the strong opiate analgesics. It is now used increasingly in a legal context to refer to a wide variety of abused substances. Although the term is not likely to disappear, it is no longer useful in a pharmacological context.'* (Goodman and Gilman's - *The pharmacological basis of therapeutics, 9th edition, 1996 [1]*)

The more precise pharmacological name of this group ought to be **Opioid Analgesics** or **Opiates**, which are drugs isolated from opium such as morphine and codeine. Opiates are also synthetic morphine analogous and covers all drugs which have morphine-like activity.

In 1997 the Medical Commission of the IOC cancelled propoxyphene and dextropropoxyphene from the list of banned substances. The reason was that both analgesics are weak acting opioids with less addictive effects. Several positive cases in the past with dextropropoxyphene seemed to be caused mainly by the use of dextropropoxyphene in the training period for treatment of analgesic. When the drug was taken off too late before a competition the detection of the drug and its metabolite at competition, even in traces, constituted a positive doping test.

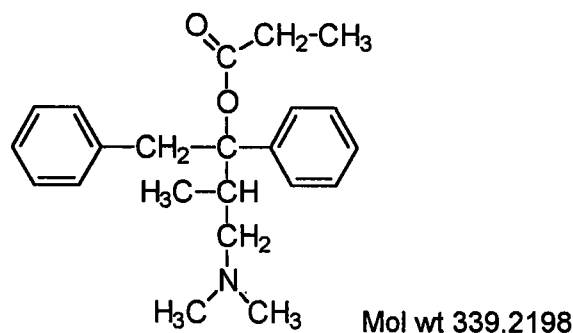


Fig.1 Structure formula of dextropropoxyphene (propoxyphene)

From the four possible isomers of propoxyphene only the  $\alpha$ -dl- (racemate) and the d-diastereoisomer (dextropropoxyphene) have analgesic actions whereas the  $\beta$ -diastereoisomers are completely inactive [1].

Dextropropoxyphene is structurally related to methadone. It is one-half to two-third active than codeine (orally) (Table 1). Dextropropoxyphene and codeine in comparison to morphine do not develop tolerance and physical dependence.

Dextropropoxyphene is therapeutically used to manage mild-to-moderate pain [2]

Table 1 Comparison of analgesic effects of propoxyphene, codeine and aspirin [1]

Substance	Equivalent effect
Propoxyphene (oral)	120 mg
Codeine (oral)	60 mg
Aspirin (oral)	600 mg

### Metabolism of dextropropoxyphene

Dextropropoxyphene is metabolized in human mainly to nordextropropoxyphene (Fig.2).

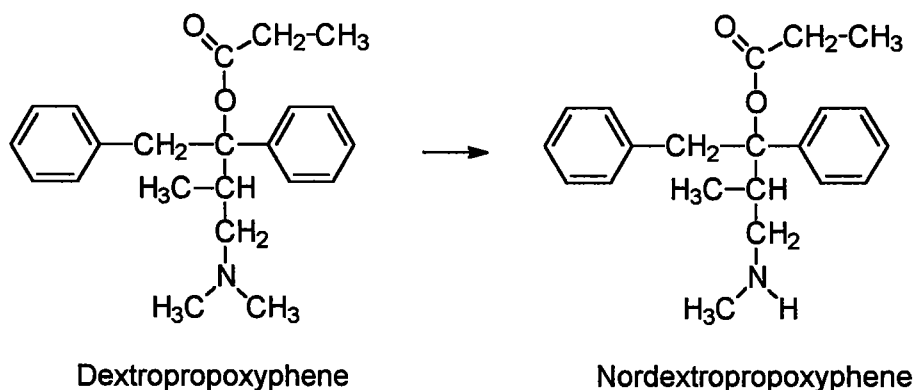


Fig.2 Main metabolite of dextropropoxyphene

Plasma elimination half-life of dextropropoxyphene is reported with 6-12 hours and for the metabolite norpropoxyphene with approximately 30 hours [2].

As dextropropoxyphene was banned only in competition and not in the 'out of competition' period athletes who were medically treated with this opioid had to stop administration of dextropropoxyphene before competition. Based on the published plasma elimination half-life for dextropropoxyphene and its metabolite nordextropropoxyphene we expected the detection of these opioids for several days.

Excretion studies with dextropropoxyphene were performed to determine the time period for detection of dextropropoxyphenene and its metabolite under routine dope control conditions after a single oral application.

#### **Excretion studies**

Two male volunteers ( person A, 45 years, 77kg; person B, 40 years, 62 kg) obtained orally 150 mg of dextropropoxyphene hydrochloride (Develin® retard from Gödecke, Berlin, Germany). Urine samples were collected until 12 days after application and stored at 4°C.

#### **Sample preparation**

Sample preparation followed the routine screening procedure for stimulants. To 5 ml of urine are added 0.5 ml 5N KOH, 2 ml of t-butylmethyl ether, 25 µg of N,N-diisopropyl-n-dodecane (Dipa 12) as internal standard and ca. 3 g of sodium sulfate. The mixture is shaken for 20 minutes, centrifuged at 2500 rpm and the ether layer is transferred into vials for an automatic liquid sampler or manual injection. 4 µl of the solution are injected.

#### **GC/MS-determination**

GC 5890/MS 5971A, GC-column: 20m capillary column HP 5 MS, fused silica, 0,25 mm I.D., 0,25 µm film thickness; carrier gas: ca. 1 ml helium at 180°C; split: ca. 1:10, temperature program: 100°C, +20°C /min, 2 min at 320°C, injector: 280°C; detector: 300°C. The injection port and transfer line are heated to 300°C and 320°C respectively. Dextropropoxyphene and its metabolite are analyzed using full scan mode with 70 eV EI ionization. Retention time of dextropropoxyphen: 7,18 min, norpropoxyphene alkaline rearrangement product N-propionyl amide: 8,75 min, and Dipal2 (internal standard): 5,12 min.

The EI mass spectra of dextropropoxyphene and nordextropropoxyphene alkaline rearrangement product are shown in Fig.3 and 4. The EI fragmentation of dextropropoxyphene (mol wt 339) generates after  $\alpha$ -cleavage to the nitrogen the most abundant ion m/z 58 as base peak, whereas all other fragments are less intense (Fig.3). As norpropoxyphene differs structurally only slightly from dextropropoxyphene (minus CH<sub>3</sub> at the nitrogen atom) a similar EI spectrum would be expected with a shift of m/z 14 for all nitrogen containing fragment ions. Interestingly the obtained spectrum is completely different and several abundant ions are formed. Nordextropropoxyphene undergoes a rearrangement process when treated with alkali as it is performed during the extraction step. The conversion of norpropoxyphene and dinorpropoxyphene to the

corresponding N-propionyl amides via an intramolecular migration of the propionyl group has been described [3].

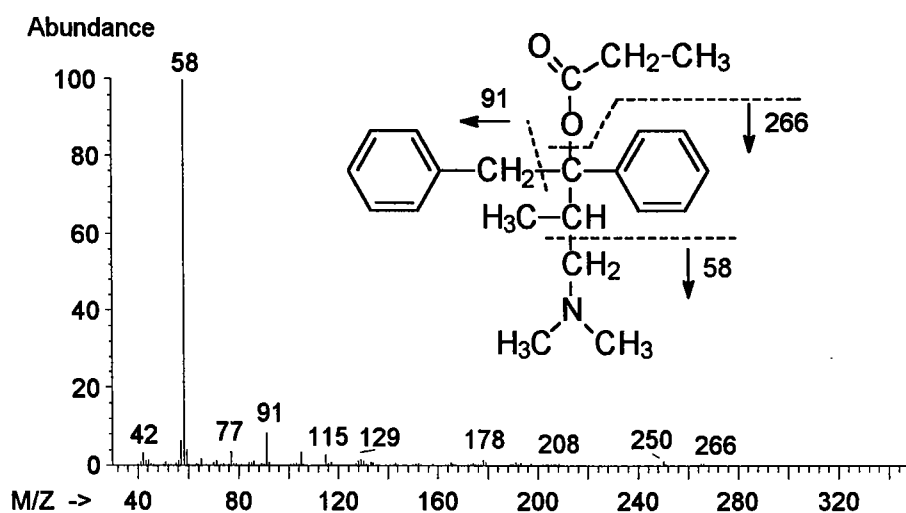


Fig.3 EI mass spectrum of dextropropoxyphene,  $M^+$  339

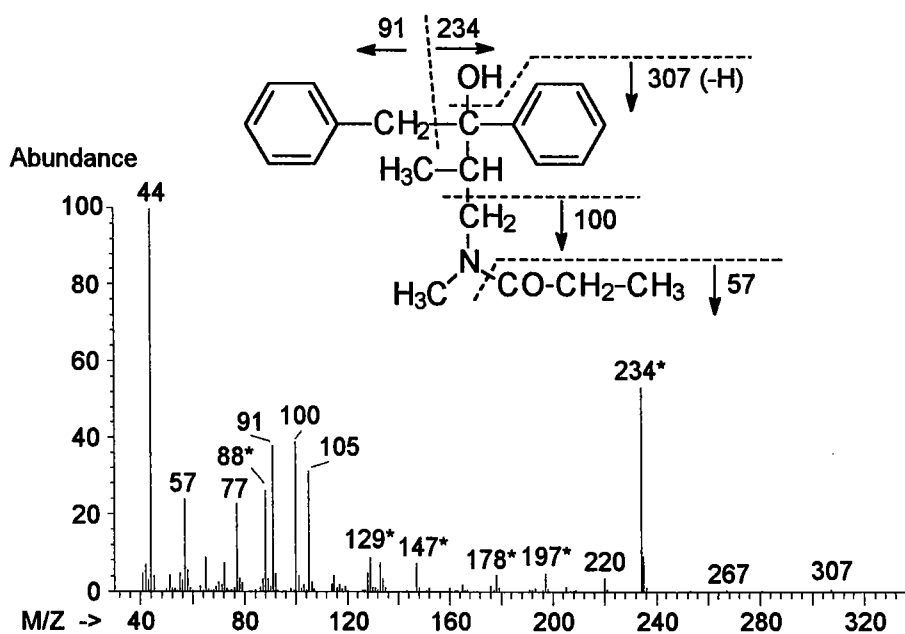


Fig.4 EI mass spectrum of nordextropropoxyphene alkaline rearrangement product N-propionyl amide,  $M^+$  325, \* marked ions are shifted (+ 1 amu) when the urinary extract is solved in deuterio methanol (MeOD)

The EI mass spectrum of the N-propionyl amide in Fig.4 can be explained following typical fragmentation pathways. The fragment  $m/z$  234 is generated by loss of  $m/z$  91 (benzyl group) from the molecular ion 325 (not detected). The ion  $m/z$  307 is proposed to be formed by loss of water,  $m/z$  197 is attributable to  $[C_6H_5CH_2COHC_6H_5]^+$ ,

$m/z$  105 to  $[\text{COC}_6\text{H}_5]^+$ ,  $m/z$  100  $[\text{CH}_2\text{NCH}_3\text{COC}_2\text{H}_5]^+$ ,  $m/z$  88  $[\text{H}_2\text{NCH}_3\text{COC}_2\text{H}_5]^+$ ,  $m/z$  57  $[\text{COC}_2\text{H}_5]^+$ ,  $m/z$  44  $[\text{CH}_2\text{NHCH}_3]^+$  ( $m/z$  44 is a typical fragmentation for secondary underivatized methyl-amines but also for the acylated amine). When the isolated metabolite (N-propionyl amide) was dissolved in deuterio methanol (MeOD) the EI mass spectrum displays shifts for ions  $197 \rightarrow 198$  and  $m/z$   $234 \rightarrow 235$  confirming the proposed structure and fragmentation patterns.

### Excretion studies

The results of both excretion studies are presented in Fig.5 and Fig.6.

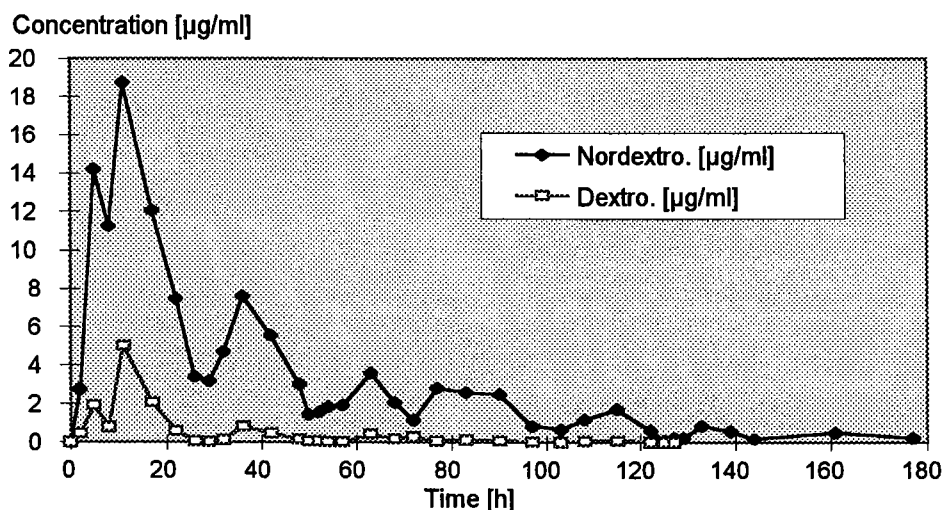


Fig. 5 Person A: Urinary excretion of dextropropoxyphene and d-norpropoxyphene after oral application of 170 mg of dextropropoxyphene hydrochloride

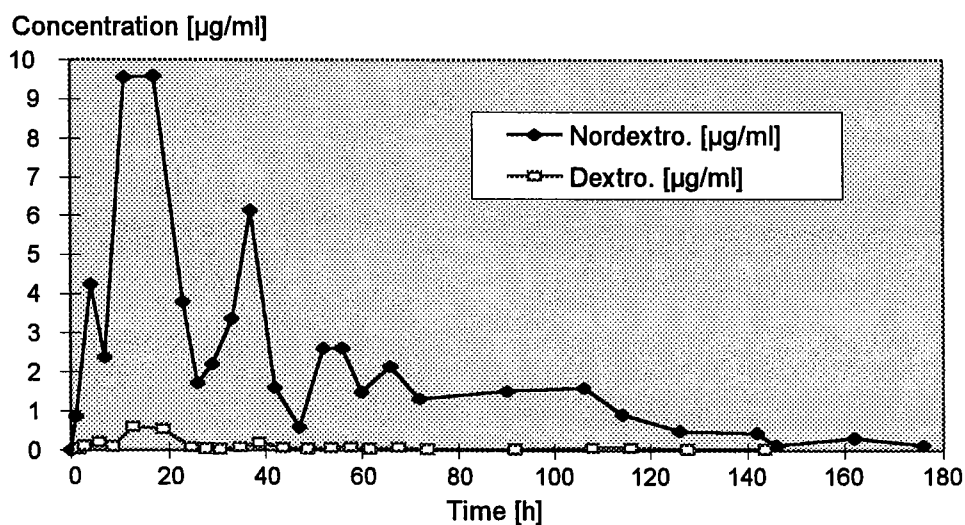


Fig. 6 Person B: Urinary excretion of dextropropoxyphene and d-norpropoxyphene after oral application of 170 mg of dextropropoxyphene hydrochloride

Table 2 Total amount of excreted dextropropoxyphene and nordextropropoxyphene after oral administration of 170 mg of dextropropoxyphene hydrochloride ( $\approx$  153,6 mg dextropropoxyphene).

	Dextropropoxyphene	Nordextropropoxyphene
Person A	2,5 mg	24,0 mg
Person B	0,5 mg	16,4 mg

In both studies dextropropoxyphene could be detected for 5 days and the main metabolite nordextropropoxyphene for at least 10 days after administration.

### Summary

As the IOC has cancelled dextropropoxyphene from the list of banned substances this opioid analgesic can be used for therapeutic reasons without causing any problem in doping control as it has been in the past, based on the long elimination half-life mainly of nordextropropoxyphene. As dextropropoxyphene and its metabolite are detectable in the screening procedure for stimulants the medical use of dextropropoxyphene can be monitored.

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## **Studies on the Analysis of Dextropropoxyphene and its Metabolite in Human Urine**

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### *Abstract*

The structure of Dextropropoxyphene(PP) was modified to improve its GC properties due to its thermal instability. The modification reaction involved a hydrolysis in ethanol and a trimethylsilation. The product was much sensitive than PP by GC analysis and suitable for the confirmation of PP positive cases.

Keywords: Dextropropoxyphene, metabolite, GC/MS

### *1. Introduction*

Dextropropoxyphene(PP) is one of the narcotic analgesics in clinic often used. It is also used in sports to relieve pain raised from trauma so that the Athletes could go on with their competitions. It is in the International Olympic Committee(IOC) banned list. According the statistic of positive cases made by IOC in 1992, PP was the second oftenst used by athletes and only less used than codeine.

The chemical structure of PP is showed in Fig 1. This structure is thermal unstable, which could be degraded in gas chromatographic injector(1,2) then results in multy peaks and decrease the sensitivity of detection. Some authors although used the technique of "on column injection"(3) they could not avoid the degradation of PP and produce more specific mass spectra. Further more this method could not meet the request of systematic analysis in doping control laboratories. Our paper presents a method, in which the chemical structure of PP is to be partly modified to the thermal stable product, so that the sensitivity for analyzing PP in human urine could be increased to almost 100 fold.

### *2. Experimental*

#### 2.1 Chemicals and reagents

Solvents used were of HPLC quality and chemicals analytical grade. The standard of dextropropoxyphene was kindly supplied by INRS-Sante Laboratory, Canada. Methaqualone was used as internal standard and purchase from Sigma (U.S.A.). N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was from Sigma (U.S.A.). All other solvents and chemicals were chinese products. diethyl ether should be distilled with anhydrous calcium hydride and stored in the dark at -20°C.



## 2.2 Instruments and Experimental Conditions

All GC-MS analysis were carried out with a HP 5970 B MSD (Hewlett-Packard, U.S.A.) coupled to a HP 5890 A gas chromatograph (Hewlett-Packard, U.S.A.). GC was performed on a HP-5 column (25mx0.2mm I.D., cross-linked with 5% phenyl-methylsilicone, 0.33  $\mu\text{m}$  thickness, Hewlett-Packard, U.S.A.) in splitless mode. The initial temperature was 100°C and increased by 20°C/min to 180°C, then increased by 5°C/min to 220°C, finally increased by 20°C to 280°C, this temperature was held for 10 min. Injector was carried out at 250°C with an autosampler. The GC-MS interface temperature was 290°C. The carrier gas was helium with the rate of 0.98 mL/min at 180°C. The GC-MS system was operated in the electron impact mode with electron energy 70 eV and ion source temperature 200°C. Before work the instrument was tuned daily with perfluorotributylamine (PFTBA). The full scan (scan) was performed at 40-400 amu, or selected ion monitoring(SIM) with m/z 219 and 269.

## 2.3 PP positive Human Urine

A healthy voluntary male took orally once PP-HCl 10 mg. His urine during 90 hr. after administration was collected and stored at -20°C.

## 2.4 Extraction and Derivatization

To 5 mL of urine 0.5 mL potassium hydroxide solution (5 mol/L KOH in water), 4 mL diethyl ether including 8  $\mu\text{g}$  internal standard and 2 g solid sodium chloride were added. After being shaken for 10 min and centrifuged for 5 min (2500 rpm) the organic layer was separated and evaporated to dryness in a stream of nitrogen. To the residual 200  $\mu\text{L}$  of potassium hydroxide in anhydrous ethanol solution(10%, w/v) was added, followed by reaction at 90 °C for 30 min. After reaction the mixture was evaporated to dryness in a stream of nitrogen at 60°C and mixed with 1 mL distilled water, 0.5 g solid sodium chloride and 4 mL diethyl ether (without internal standard), followed by being shaken for 10 min and centrifuged for 5 min (2500 rpm). After separation and evaporation of the organic layer to dryness, the TMS-derivatives prepared by treatment with 50  $\mu\text{L}$  of MSTFA/ $\text{NH}_4\text{I}$ (0.2%, w/v) for 10 min at 80 °C. Aliquots (2  $\mu\text{L}$ ) were injected into the gas chromatograph-mass spectrometer.

## 3. Results and Discussion

### 3.1 Improvement of the PP Chemical Structure

The results of the chemical treatment can be expressed structurally as in Fig 1.

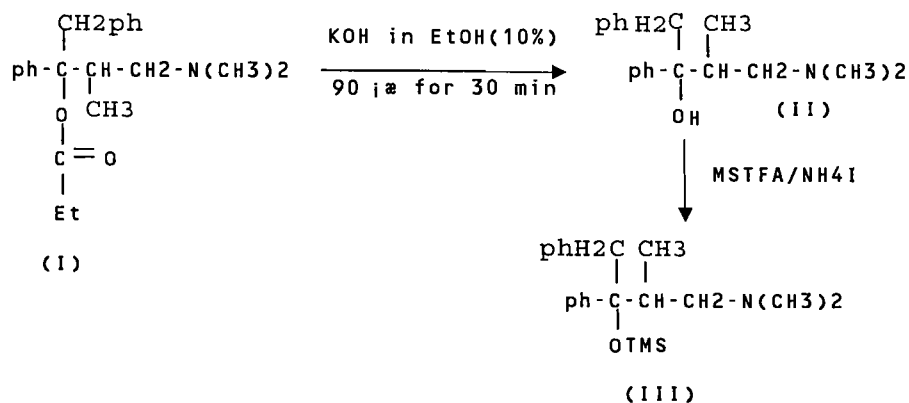


Fig.1 Reaction pathways of PP

PP(I) was hydrolyzed in potassium hydroxide anhydrous ethanolic solution to its corresponding alcohol(II). MSTFA/NH<sub>4</sub>I converted the product(II) to its trimethylsilated product (III). To compare the chromatographic and mass spectrometric behaviors of PP and the product (III), 10 µg of PP standard was diluted to 100 µL and 2 µL of the diluted standard was directly injected into GC/MS without any pretreatment; meanwhile another aliquot of 10 µg of PP was treated as described in the section 2.4 and injected. The chromatography and mass spectrum for both without and with pretreatment PP were shown in Fig 2 and Fig 3 respectively.

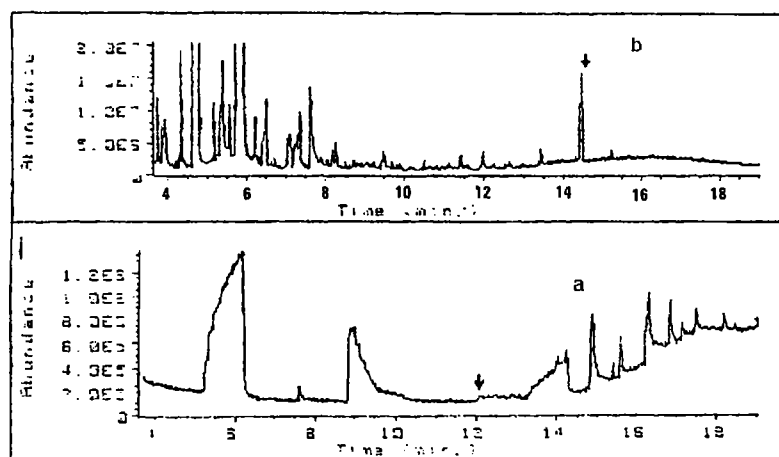


Fig 2 Total ion chromatograms of I(a) and III(b)

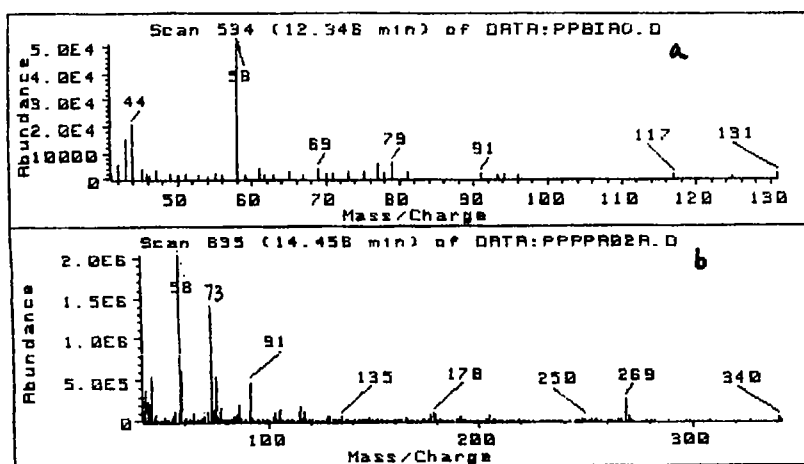
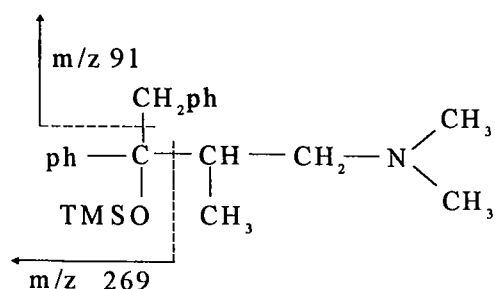


Fig 3 Mass spectra of I(a) and III(b)

In Fig 2 the peak of PP in original form was very small but the peak of its decomposed compound was relative stronger with a poor chromatographic peak shape, which could not be put into any analytical use. The mass spectra of PP in original form had no specific m/z peak, only a unspecified strong peak with m/z 58. After chemical structural improvements of PP the

product (III) was not only well chromatographic behavior with good peak shape, but also well response in MS with higher sensitivity (almost 100 fold stronger than that of PP in original form). Furthermore the product (III) provides much more specific mass fragments with  $m/z$  58, 250, 269, and 340 ( $M^+$ ). A logical explanation of mass fragments of product (III) was shown in Fig 4.

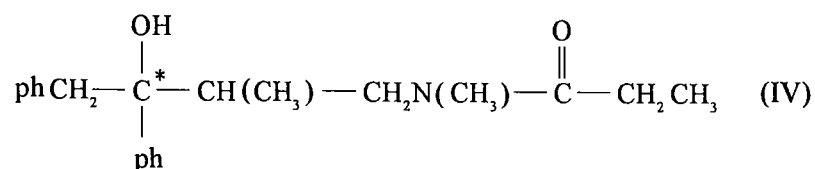


$$m/z\ 269 - CH_2ph = 178; TMS = 73; M^+ - 15 = 340$$

Fig 4 Characteristic ion fragmentation of III

### 3.2 Trimethylsilation of the Metabolite of PP

The metabolite of PP, norpropoxyphene, was rearranged during alkaline extraction to norpropoxyphene amide (4) with the following chemical structure:



Our experiment showed that this structure(IV) was not changed during the reaction with potassium hydroxide solution in anhydrous ethanol (10%, w/v), but could be trimethylsilated with MSTFA. In fact the atom  $C^*$  in product (IV) has larger steric hindrance so that if only pure MSTFA reacts with product (IV) at  $80^\circ\text{C}$  for 3 hr the trimethylsilation was still incomplete; if a little amount of  $\text{NH}_4\text{I}$  was added to MSTFA as catalytic agent, the trimethylsilation was finished at  $80^\circ\text{C}$  in 10 min. Combining the results from Masse et. al.(5,6) and our experiments we found the possible reaction mechanics listed in Fig 5. During the reaction  $\text{NH}_4\text{I}$  reacts first with MSTFA to produce a quaternary ammonium salt(V). This salt (V) could react much easy with hydroxyl compounds to the formation of MSTFA, HI and a correspondent trimethylsilated product. MSTFA reacts with HI so that the catalyst  $\text{NH}_4\text{I}$  could get back.

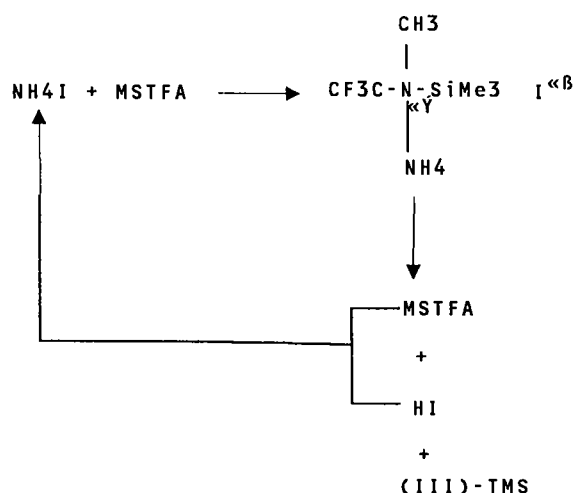


Fig 5 Possible mechanism of the derivatization reaction

### 3.3 Recovery and Detection Limit

Because of the lack of standards (II) and (III), the reaction yield could not be estimated. We used the changes in concentration of PP before and after extraction in order to get the recovery of the solvent extraction to be about  $90.4\% \pm 6.0 (n=4)$  (the concentration of PP before extraction was  $2 \mu\text{g/mL}$ ). After a series concentrations of PP standard was extracted, hydrolyzed and derivatized, the detection limit for PP in SIM mode with single  $m/z$  269 was 50 pg.

### 3.4 Urinary Excretion Profiles of PP and its Metabolite

Urinary excretion profiles of PP and its metabolite were showed in Fig 6. PP was rapidly metabolite in human body and difficult to be detected in its original form without chemical structural improvement. With the method presented here the PP in original form could be detected in 48 hr. after oral administration; meanwhile the metabolite could be found in 72 hr. after administration. The detection of metabolite of PP has significance in doping control.

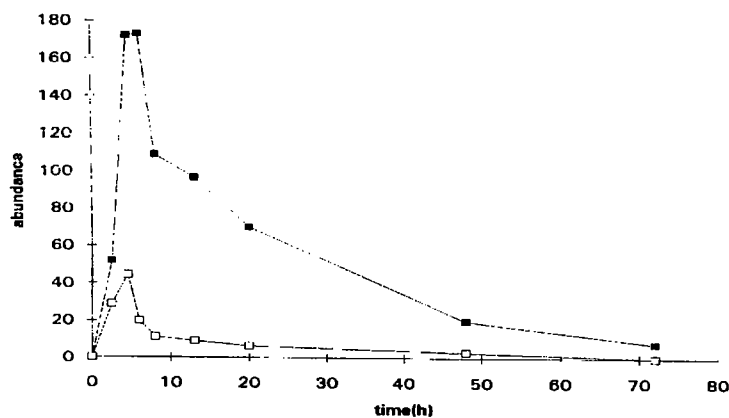


Fig 6 Variation of PP and its metabolite concentration in human urine  
 ■:  $m/z$  219 ( $\times 10^6$ ) metabolite □:  $m/z$  269 ( $\times 10^5$ ) parent

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