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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 unstability. 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 μm 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 μg internal standard and 2 g solid natrium chloride were added. After being shacked 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 μL 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 natrium chloride and 4 mL diethyl ether (without internal standard), followed by being shacked 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 μL of MSTFA/NH_4I(0.2%, w/v) for 10 min at 80 °C. Aliquots (2 μL) 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.

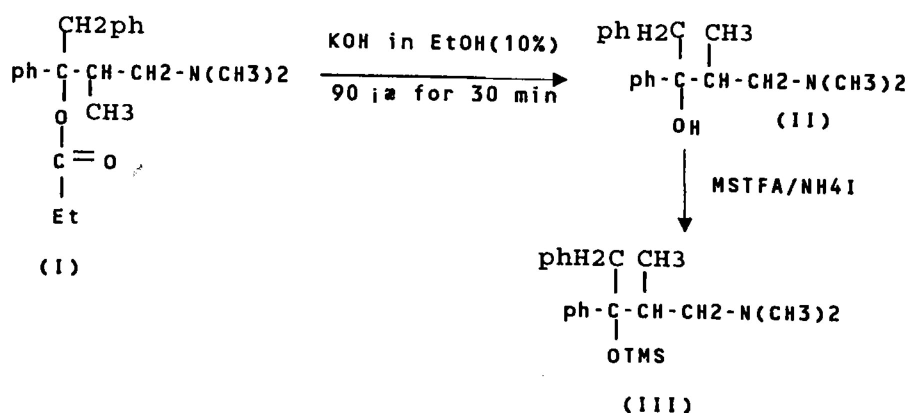


Fig.1 Reaction pathways of PP

PP(I) was hydrolyzed in potassium hydroxide anhydrous ethanolic solution to its corresponding alcohol(II). MSTFA/NH_4I 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.

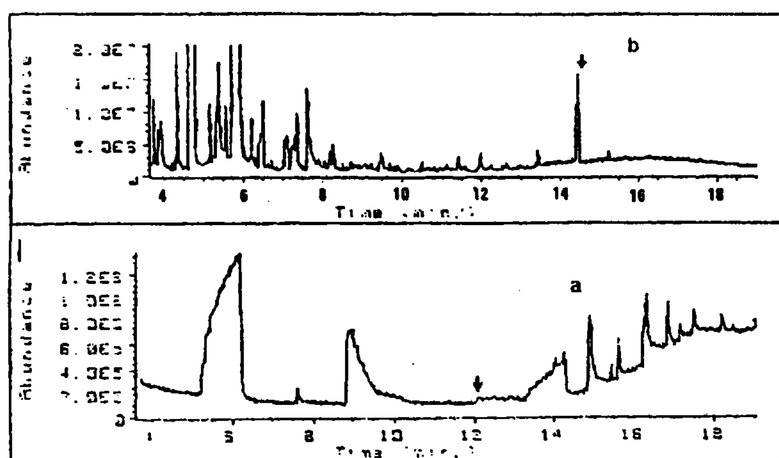


Fig 2 Total ion chromatograms of I(a) and III(b)

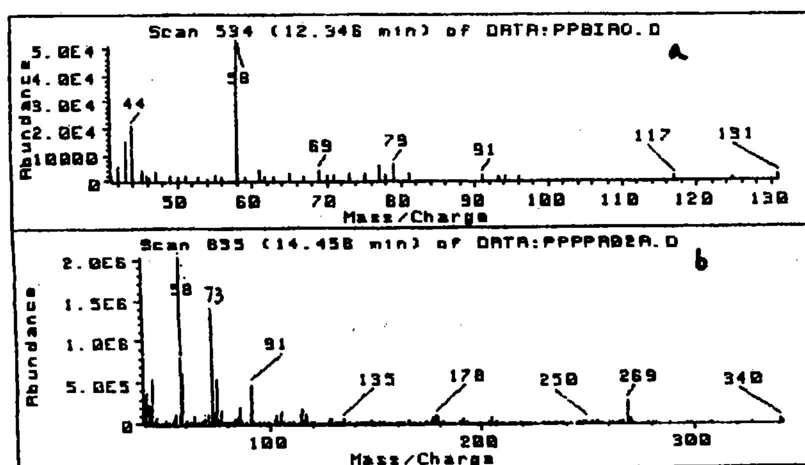
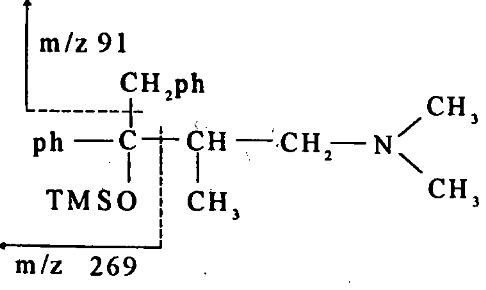


Fig 3 Mass spectra of I(a) and III(b)

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.

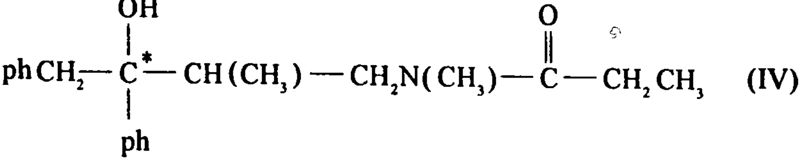


m/z 269 — CH_2ph=178; TMS=73; M^+-15=340

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°C for 3 hr the trimethylsilation was still incomplete; if a little amount of NH_4I was added to MSTFA as catalytic agent, the trimethylsilation was finished at 80°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 NH_4I 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 NH_4I could get back.

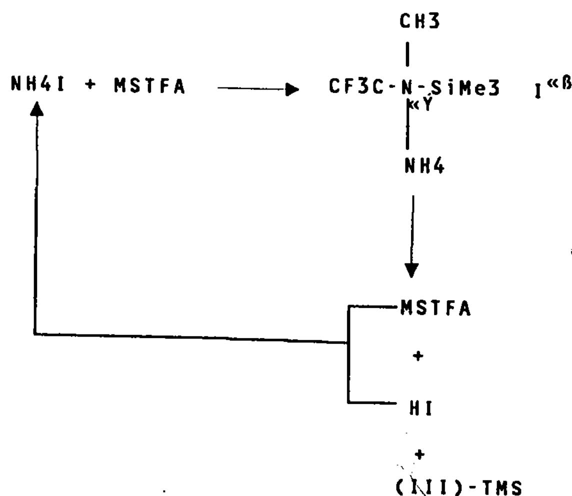


Fig 5 Possible mechanism of the derivatization reaction

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%±6.0(n=4) (the concentration of PP before extraction was 2 μg/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.

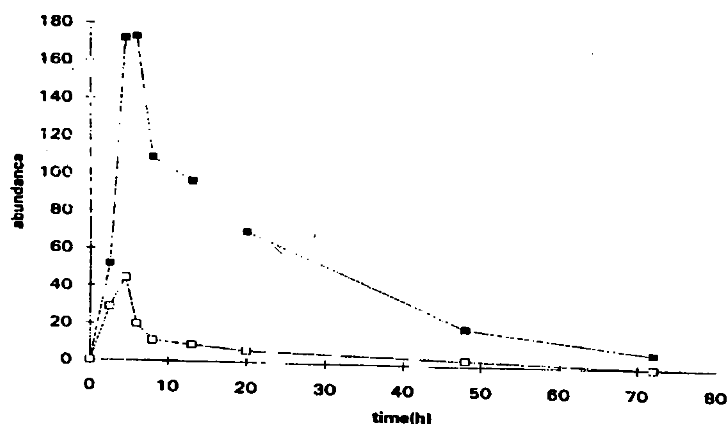


Fig 6 Variation of PP and its metabolite concentration in human urine
■: m/z 219 ($x10^6$) metabolite □: m/z 269 ($x10^5$) parent

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