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Prosidol: new piperidine-type narcotic analgesic

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Introduction

Prosidol (1-(2-ethoxyethyl)-4-phenyl-4-propionyloxypiperidine) is a new synthetic opioid developed in the Laboratory of Medicinal Compounds at the Kazakhstan Institute of Chemical Sciences in the 1990s and firstly synthesized in Novokuznetsk Chemical-Pharmaceutical Institute, Russia [1]. The drug has been patented in Russian Federation, Kazakhstan, Switzerland, Italy and Great Britain, and now it is used in medical practice.

There is a wide spectrum of piperidine derivatives that possess analgesic activity (**Fig. 1**).

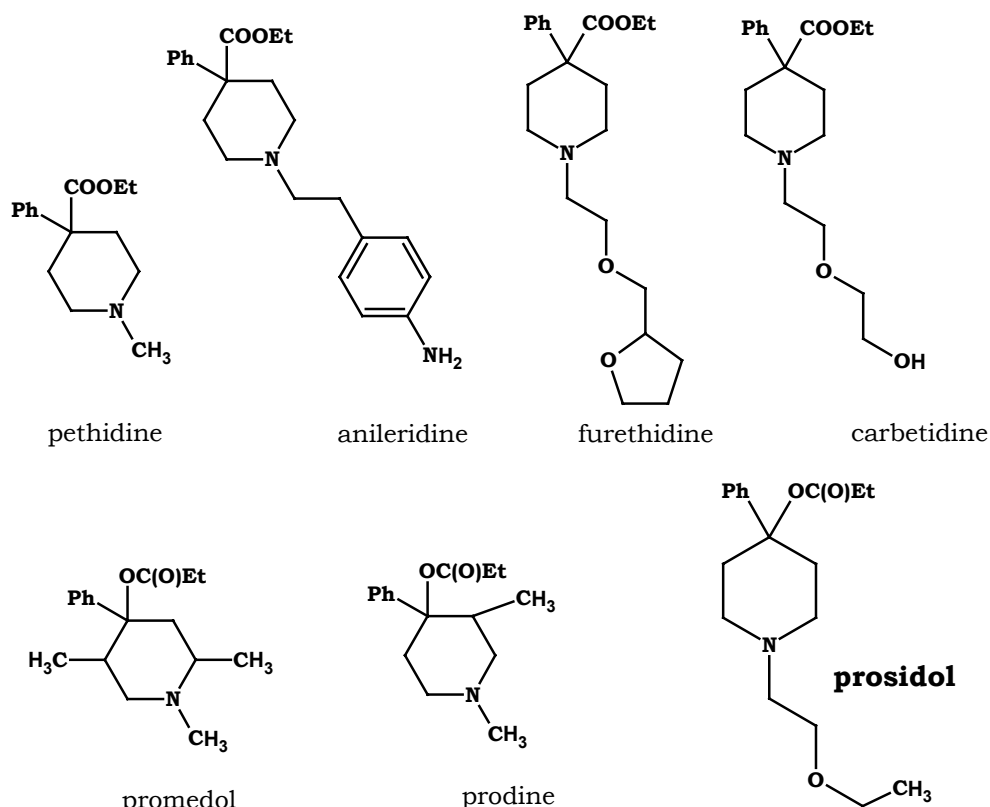


Fig. 1. Piperidine-type derivatives with opioid activity.

They all bind to opioid μ -receptors and, according to Beckett-Casey hypothesis [2], share the same pharmacophore group (**Fig. 2**):

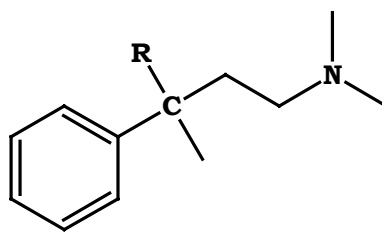


Fig. 2. Pharmacophore group for opioid analgesics according to Beckett-Casey (quaternary carbon atom with adjacent aromatic ring and tertiary nitrogen atom separated by ethylene bridge).

Prosidol is claimed to be as highly active analgesic as morphine but less addictive and much more tolerable, although the side effects are typical for opioids and include addiction, abstinence syndrome, respiratory depression, bradycardia *etc.* Prosidol can be administered intravenously, intramuscularly, subcutaneously (as 1% solution), orally (25 mg tablets) or buccally (10 and 20 mg tablets). Maximal therapeutic dose is 250 mg per day. The analgesic effect starts in 20 min and lasts for several hours [3].

The Prohibited List specifies only restricted number of narcotic analgesics, although there is a wide spectrum of compounds with such activity. Logically, application of any narcotic analgesic should constitute a doping violation. Therefore, it seems reasonable to monitor if the athletes abuse pharmaceuticals that are not formally prohibited but provide the same physiological effects as typical opioids like morphine.

We have performed the excretion study of prosidol after buccal administration of single 20 mg tablet. The drug and two main metabolites identified as *O*-desethyl and *O*-desethyl-*O'*-despropionyl were found non-conjugated in urine. In addition, 2 minor conjugated metabolites, supposedly hydroxyprosidol and its desethyl analog, were detected by GC-MS EI as trimethylsilyl derivatives.

Experimental

Urine samples were collected 2 and 8 hrs after buccal administration of 20 mg to a cancer patient who was receiving prosidol by medical prescription. No blank urine was available. A small part of prosidol tablet was dissolved in methanol (not quantitatively) and analyzed by GC-MS directly and after silylation with MSHFBA-TMSI-TMCS.

Urine was subjected to standard screening procedures for volatile stimulants and total anabolic steroids [4]. Following the first procedure, to 5 ml of urine 30 µl of diphenylamine internal standard solution, 300 µl of 5M KOH, 5 ml of diethyl ether and 1 g of anhydrous

Na₂SO₄ were added followed by vortexing and centrifugation to separate layers. Ether layer was evaporated to dryness at 60°C and reconstituted in 50 µl of methyl *tert*-butyl ether. Two µl aliquot was injected into GC-NPD Agilent 6890N and GC-MS Agilent 6890N/5973inert in split mode 1:10 at injection port temperature of 250°C. Separation was performed on a HP-1 Ultra quartz capillary column 12 m × 0.2 mm × 0.33 µm, temperature program was as follows: 110°C (2 min), 15°C/min to 300°C (2 min). Helium carrier gas flow rate 1.1 ml/min (GC-NPD) and 0.8 ml/min (GC-MS).

Following the second procedure, to 3 ml of urine 50 µl of methyltestosterone internal standard solution, 1 ml of 1M acetate buffer (pH 5.5) and 30 µl of β-glucuronidase from *Helix Pomatia* were added and enzymolysis was performed at 57°C for 3 hrs. After enzymolysis pH was adjusted to 9 by adding carbonate/bicarbonate buffer, and then *ca.* 2 g of anhydrous Na₂SO₄ were added followed by liquid-liquid extraction with 5 ml of diethyl ether. After centrifugation ether layer was evaporated to dryness at 60°C and the residue was derivatized with 50 µl of MSTFA / NH₄I / dithiothreitol (1000:2:4 v/w/w) or MSHFBA / TMSI / TMCS (100:2:5 v/v/v) at 70°C for 20 min. After cooling and transferring to vial, 3 µl aliquot was injected in GC-MS Agilent 6890N/5973inert in split mode 1:10 at injection port temperature of 280°C.

Separation was performed on a HP-1 Ultra quartz capillary column 12 m × 0.2 mm × 0.33 µm, temperature program was as follows: 190°C (0 min), 2°C/min to 234°C (0 min), 12°C/min to 300°C (4.5 min). Helium carrier gas head pressure was 10.1 psi.

Results and Discussion

Tablet profile

We analyzed a methanolic solution of prosidol tablet by GC-MS without derivatization. It was found that the pharmaceutical contains a number of impurities structurally related to prosidol, and some of them cannot be deduced from the synthesis layout presented by manufacturer. Based on mass spectral data, the following structures were attributed to three main tablet constituents: 1-(2-ethoxyethyl)-1,2,3,6-tetrahydropyridine (estimated content 21%, **Fig. 3a**), 1-(2-ethoxyethyl)-3-phenylpyrrolidine (9%, **Fig. 3b**), and prosidol (68%, **Fig. 4**). There were also detected 6 minor impurities including even brominated prosidol (!).

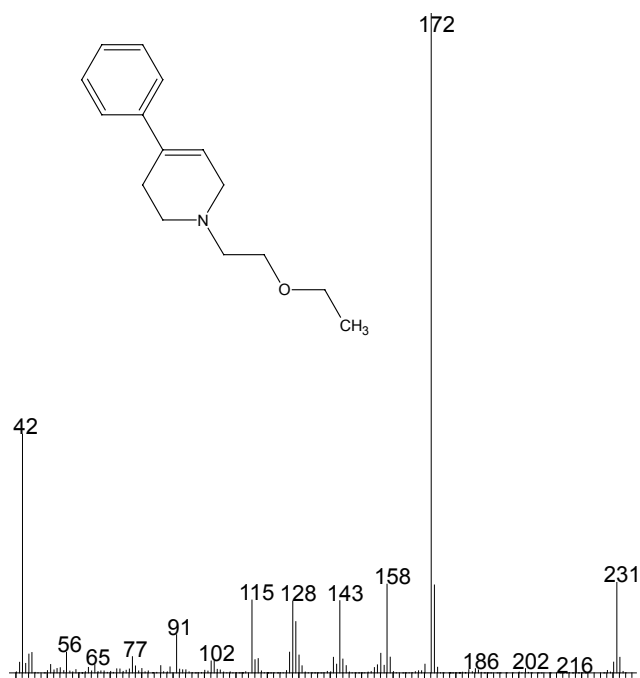


Fig. 3a. Impurity in prosidol tablet

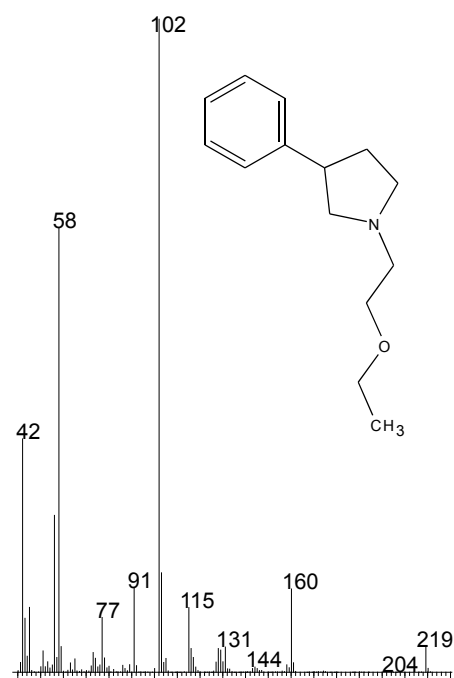


Fig. 3b. Impurity in prosidol tablet

Prosidol gives characteristic electron ionization mass spectrum with prominent ion at m/z 172 by successive elimination of propionyl and ethoxy groups. This ion is common for all prosidol-related structures.

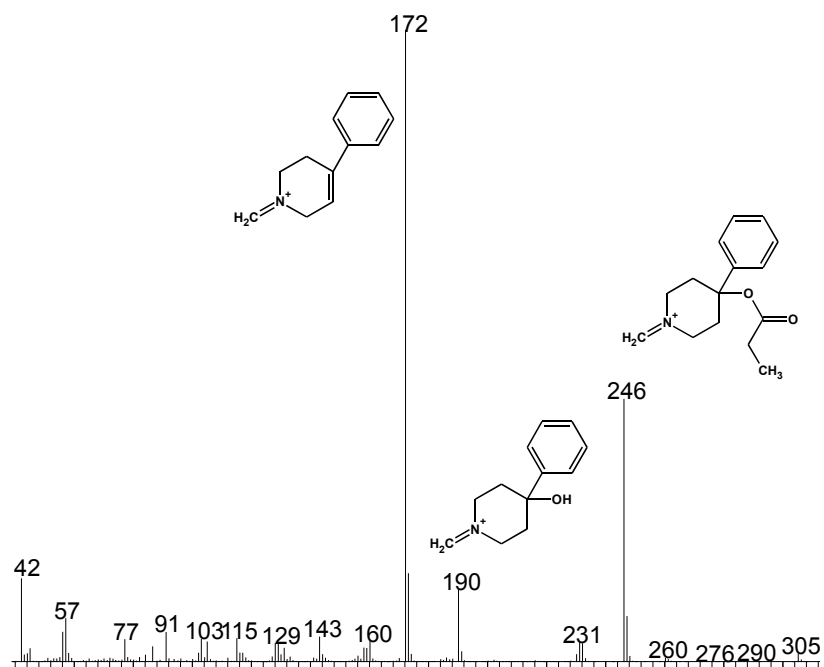


Fig. 4. Mass spectrum of prosidol (the molecular ion is at m/z 305).

It is worth mentioning that urine matrix has only slight interference with ion at m/z 172 and therefore it can be easily detected.

Prosidol metabolism

Prosidol is readily metabolized to produce mostly desethyl (**I**) and desethyl despropionyl (**IV**) metabolites, which can be found in free fraction, and two minor hydroxy metabolites excreted as conjugates (desethyl hydroxyprosido, **II**, and hydroxyprosido, **III**, Fig. 5). Structure of the metabolites was proposed from electron ionization mass spectrometry data; no confirmatory synthesis was performed.

Prosidol can be detected only in the initial period of a few hours after administration, and after 8 hrs it has been almost completely metabolized (Fig. 6). It is interesting to notice that metabolite **II** was not detected in the initial period of 2 hrs.

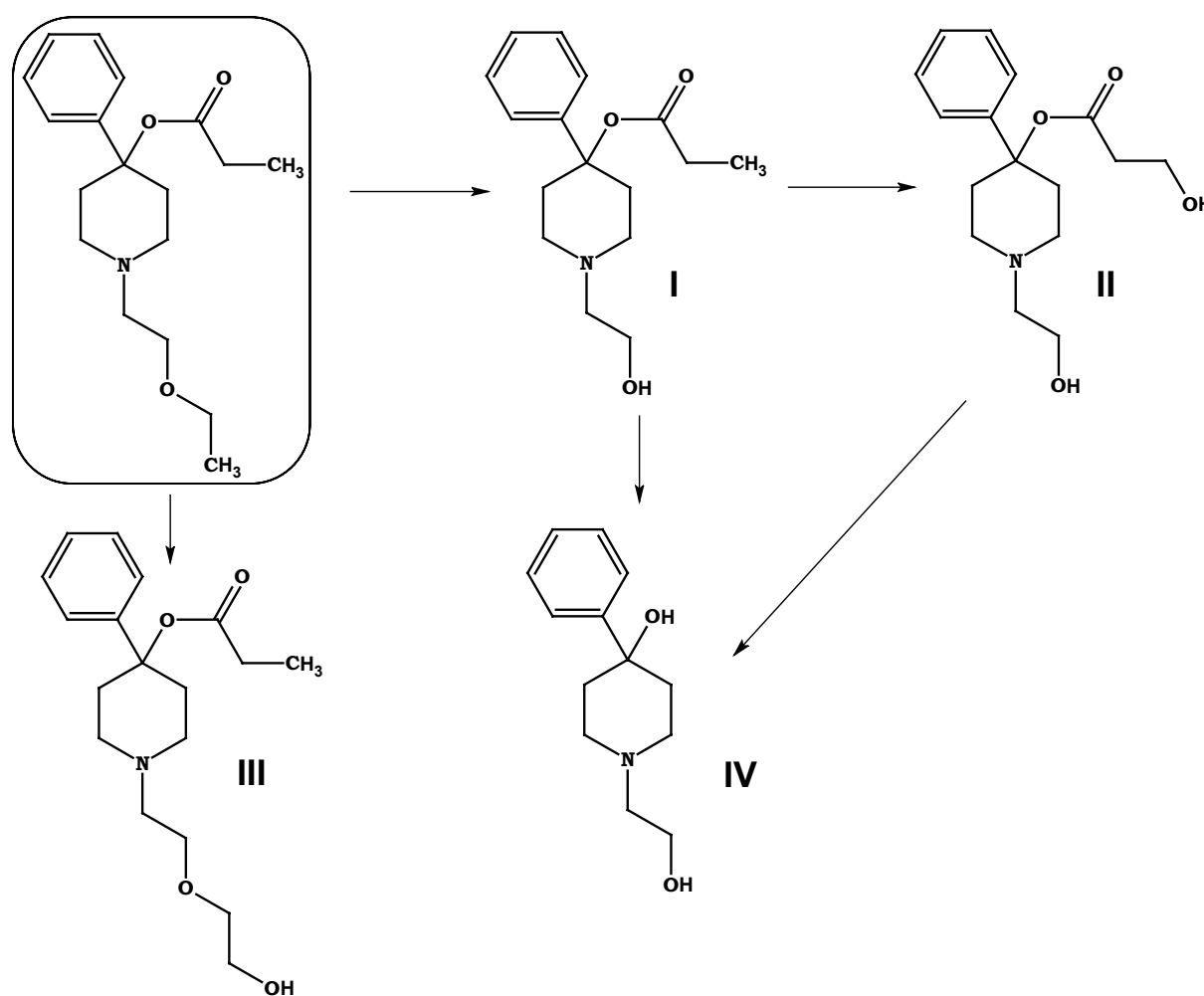


Fig. 5. Metabolism of prosidol.

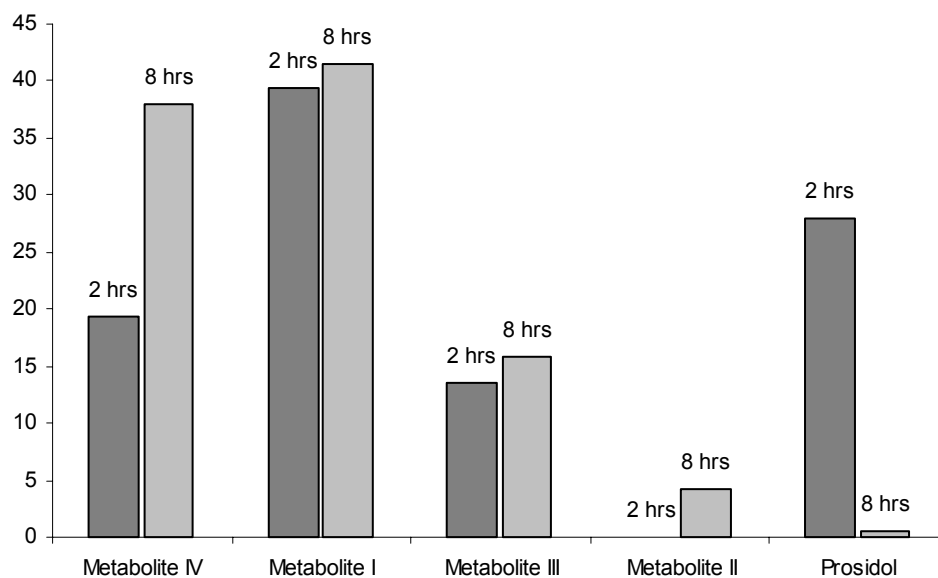


Fig. 6. Metabolic distribution of prosidol and its metabolites (%) vs. time.

Detection of prosidol

Prosidol as well as metabolites **I** and **IV** are stable and volatile enough to be detected by gas chromatography without derivatization. The mass spectra of these metabolites are given in Fig. **7a** and **7b** (the metabolites hereinafter are presented in their elution order).

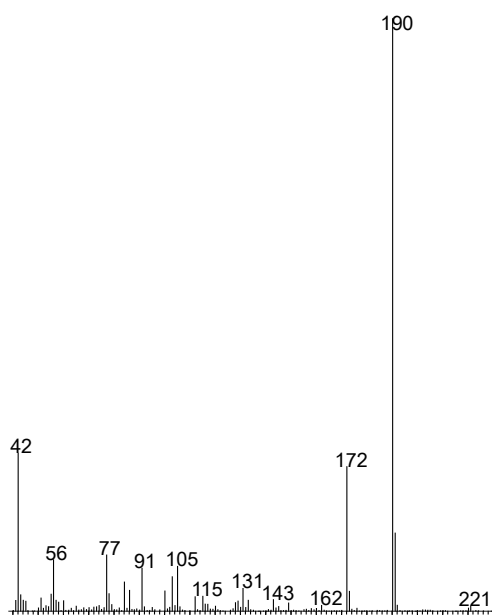


Fig. 7a. Mass spectrum of **IV** (MW 221).

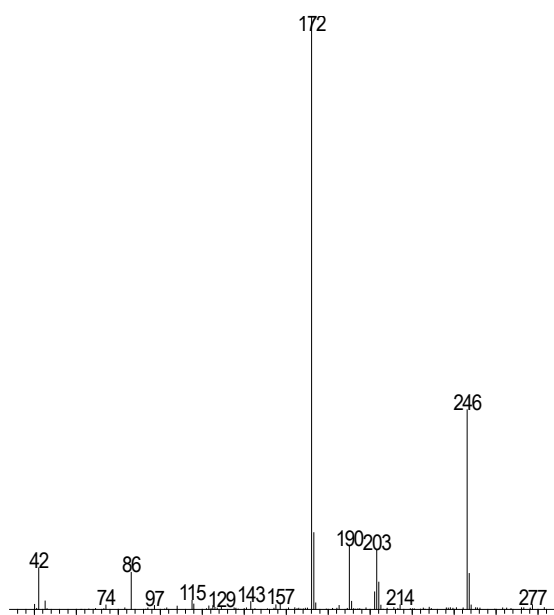


Fig. 7b. Mass spectrum of **I** (MW 277).

To detect the other prosidol metabolites, urine should be subjected to enzymolysis followed by extraction and silylation. Interestingly, when the mixture of MSHFBA / TMSI / TMCS is used, prosidol and all its metabolites may be detected (for mass spectra see **Fig. 8a** –

8d, respective mass chromatogram plotted for m/z 172 is presented in **Fig. 9**). On the contrary, using the common in doping analysis derivatization mixture of MSTFA / NH_4I / dithiothreitol that promotes enolization, the parent molecule and its metabolites, except metabolite **IV** (desethyl despropionyl prosidol), are converted to *prosidol artifact*, namely, desethyl desoxyprosidol. Therefore, only two peaks can be detected in the chromatogram (same urine, **Fig. 10**): desethyl desoxyprosidol (structure and mass spectrum as TMS derivative are given in **Fig. 11**, retention time relatively to methyltestosterone – 0.1677) that reflects contribution of all metabolites except metabolite **IV**, and metabolite **IV** which is detected as is (RRT 0.2243).

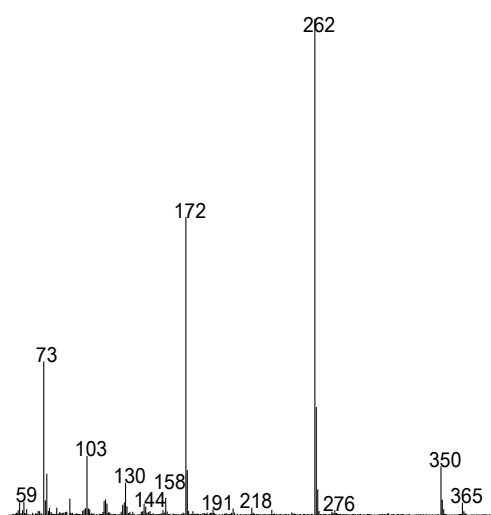


Fig. 8a. Mass spectrum of **IV** as bis-TMS (MW 365).

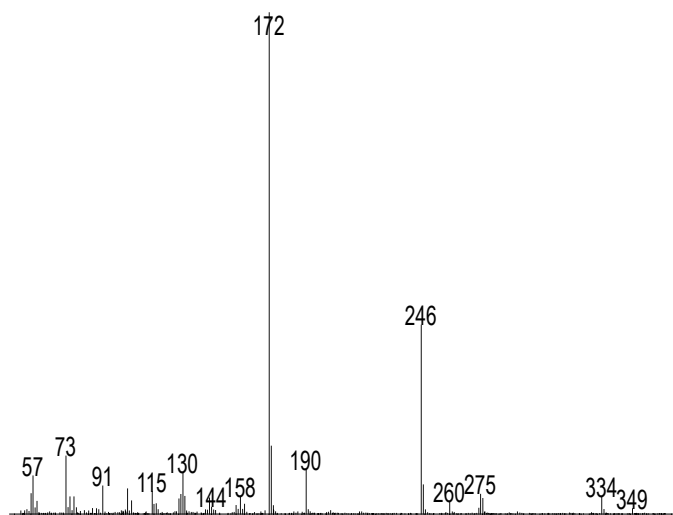


Fig. 8b. Mass spectrum of **I** as TMS (MW 349).

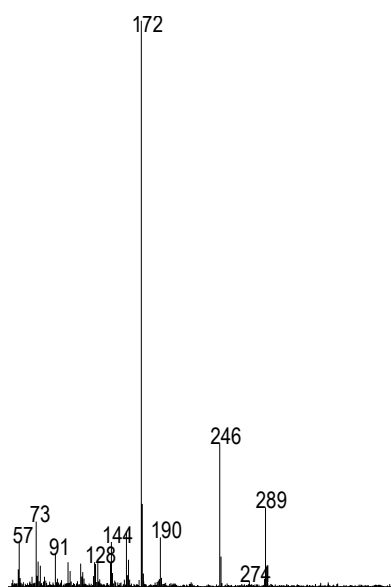


Fig. 8c. Mass spectrum of **III** as TMS (MW 393).

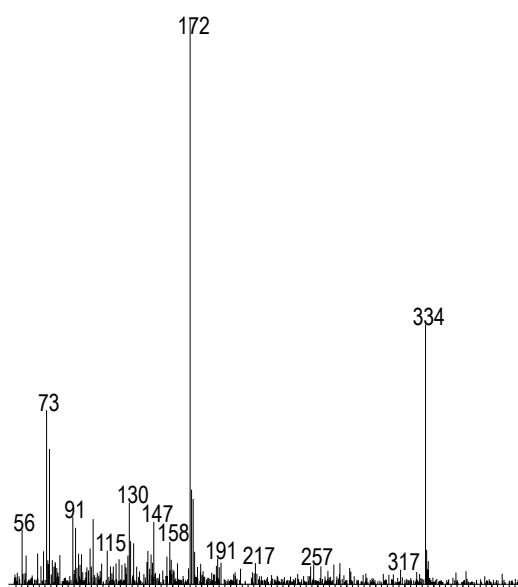


Fig. 8d. Mass spectrum of **II** as bis-TMS (MW 437).

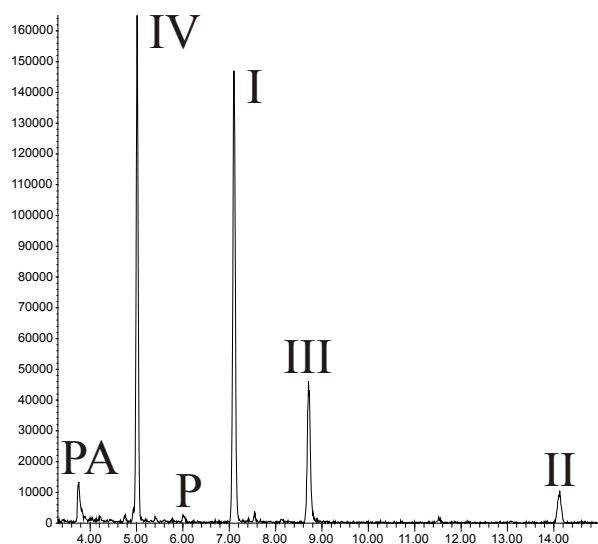


Fig. 9. Derivatization with MSHFBA / TMSI / TMCS (*PA* – prosidol artifact, *P* – prosidol, roman numerals correspond to respective metabolites).

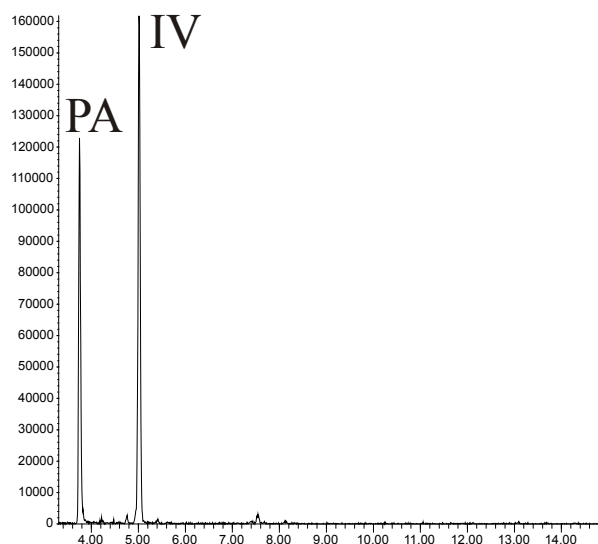


Fig. 10. Derivatization with MSTFA / NH₄I / dithiothreitol (*PA* – prosidol artifact, *IV* – metabolite **IV**).

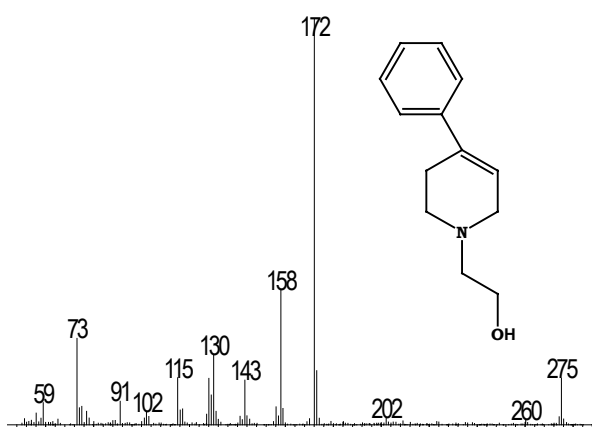


Fig. 10. Mass spectrum and structure of prosidol artifact (MW 275 as TMS).

Conclusion

Metabolism of a new piperidine-type narcotic analgesic prosidol was studied. In humans prosidol is readily metabolized to produce four metabolites, two of which (desethyl and desethyl despropionyl prosidol) are excreted free and volatile enough to be detected by GC as is, and other two (hydroxy prosidol and desethyl hydroxy prosidol) are conjugated.

As to doping control analysis, prosidol may be easily determined in urine either in screening for volatile stimulants or anabolic steroids. For confirmation purposes enzymolysis followed by extraction and silylation with MSHFBA / TMSI / TMCS is preferable as it would enable detecting all the metabolites of prosidol.

References

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