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Detection of Tamoxifen Metabolites in Urine by GC/MS

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Introduction

Tamoxifen is an anti-estrogen, which is generally used in the treatment of breast cancer and non-malignant breast disorders. Due to its stimulating effect on the secretion of the pituitary gonadotropic hormones, it is also used in the treatment of anovulatory infertility. In males it may cause an increase of the endogenous production of androgens. Because of certain pharmacological properties it is supposed to be misused in some "risk sports" [1]. Athletes may be encouraged to treat the adverse effects of an extensive abuse of anabolic-androgenic steroids (suppression of androgens, gynaecomastia). Tamoxifen is strongly bound to plasma proteins and undergoes enterohepatic circulation. It is extensively metabolized by N-demethylation, hydroxylation and conjugation [2,3]. The metabolites are excreted mainly in faeces; only small amounts appear in urine [4]. The aim of this study was to find metabolites of tamoxifen, which are suitable for the detection in urine utilizing the screening procedures commonly performed in doping control.

Experimental

Excretion Study
A single dose of tamoxifen citrate (equivalent to 40 mg of tamoxifen) was administered orally to a male volunteer. Urine samples were collected after 3, 6, 9, 16, 24, 36, 48, 96 and 144 hours.

Sample Preparation
- XAD-2 extraction of 5 ml urine samples
- internal standards used (each 100 ng/ml urine):
  tamoxifen, $19D_3$-epitestosterone, cholesteryl propionate
- enzymatic hydrolysis with ß-glucuronidase from E. coli (3 hours at 50 °C)
- extraction with diethyl ether at pH 9
- selective derivatization of the dried extracts (40 µl of MSTFA; 10 µl of MBTFA)

Instrumental Parameters
Samples were analyzed on a Hewlett/Packard GC/MS system (HP 5890/HP 5989). Parameters are listed below.

- GC: 17 m HP-1 capillary column (0.21 mm ID, 0.33 µm film thickness)
  constant column flow 0.6 ml/min (helium)
  oven temperature programmed from 180 to 310 °C at a rate of 10 grd/min
  2 µl splitless injection

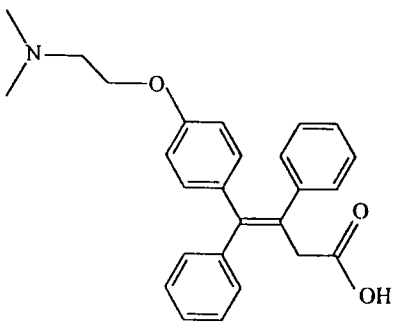
- MS: EI mode : 70 eV electron energy, scan range 50 - 600 amu
  PCI mode : methane as reagent gas, scan range 100 - 600 amu

Results

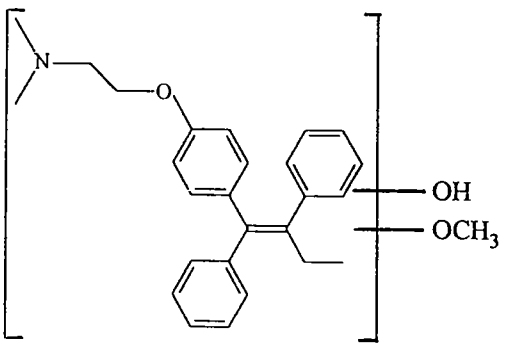
Preliminary investigations revealed that tamoxifen itself does not appear in urine unchanged. Therefore it seemed to be a suitable additional internal standard. The EI mass spectrum of tamoxifen with a low abundant but distinguishable molecular ion m/e 371 is dominated by the dimethylaminoethoxy side chain fragments m/e 58 and m/e 72 (fig.1). Assuming the metabolites to exhibit a similar fragmentation pattern we compared the extracted ion profiles (EIP) m/e 58 of urine samples before and 16-24 hours after administration (fig. 2). After administration, two additional major peaks appeared, showing similar EI mass spectra in the low mass range and presumable molecular ions of m/e 473 and m/e 489, respectively, at the high end (fig. 3). In order to confirm the molecular ion information the samples were re-analyzed using positive chemical ionization (PCI). The PCI mass spectra clearly show the [M+H] ions m/e 474 and m/e 490, respectively (fig. 4 and 5). Taking into account at least one TMS group for each metabolite the net mass increase of 30 and 46 may result from the formation of a carboxylic acid (metabolite M1) and a methoxyhydroxy derivative (metabolite M2) [5,6]. This suggestion is supported by the fact, that metabolite M1 is excreted unconjugated whereas metabolite M2 is obviously a conjugated one. Unfortunately it was not possible to fix the expected N-demethylated and/or hydroxylated metabolite(s) from the full scan recordings. Monitoring dedicated ions in EI-SIM mode (fig. 6) we received hints of a mono-hydroxylated metabolite (TMS derivative with molecular ion of m/e 459, marked with *). The biologically active N-desmethyl metabolite could not be detected; it is assumed to be further metabolized and subsequently excreted in faeces. The analytical data of the detected metabolites are summarized in Table 1.

Tab. 1: Urinary metabolites of tamoxifen *(proposed)*

metabolite	RRT (Tam)	RRT (ChP)	M	M [TMS]	ions EI	ions PCI
tamoxifen acid = M1	1.308	0.779	401	473	58, 72, 473	474
methoxyhydroxytamoxifen = M2	1.351	0.805	417	489	58, 72, 489	490



tamoxifen metabolite M1



tamoxifen metabolite M2

In order to get an imagination of the time-course of tamoxifen elimination, the metabolites M1 and M2 were semi-quantified (related to tamoxifen as internal standard) in all samples of the excretion study (fig. 7). Surprisingly the collection period of 6 days turned out to be too short to cover the duration of detectability of tamoxifen metabolites in urine after a single oral dose of 40 mg.

Conclusions

Tamoxifen yields at least two significant metabolites in urine. Evaluating the analytical data the type of these metabolites is proposed as tamoxifen acid for metabolite M1 and methoxy-hydroxytamoxifen for metabolite M2. Metabolite M2 seems to be the major one; it can be detected in urine for at least 6 days. The detection of tamoxifen metabolites can be easily included into screening procedure 4 (total steroid fraction), monitoring the ions 58, 72, 473 and 489.

References

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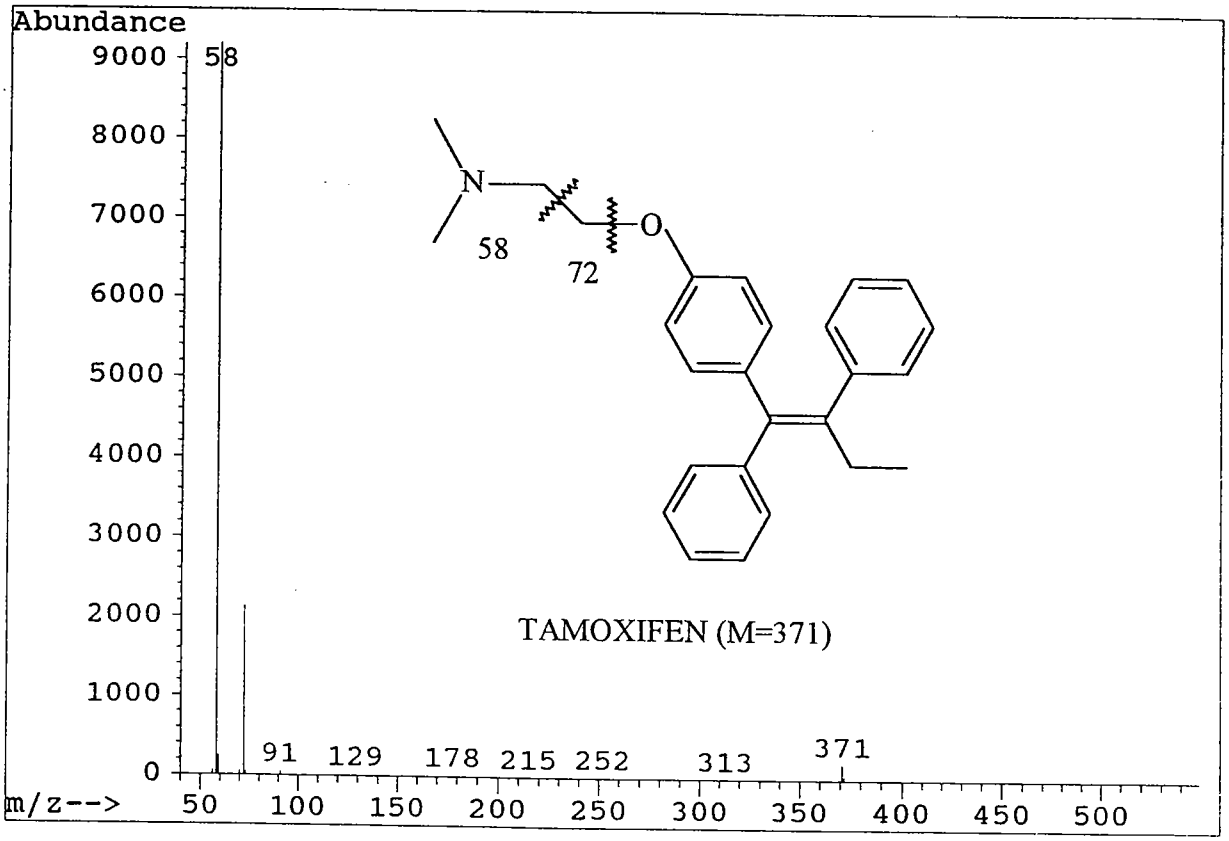
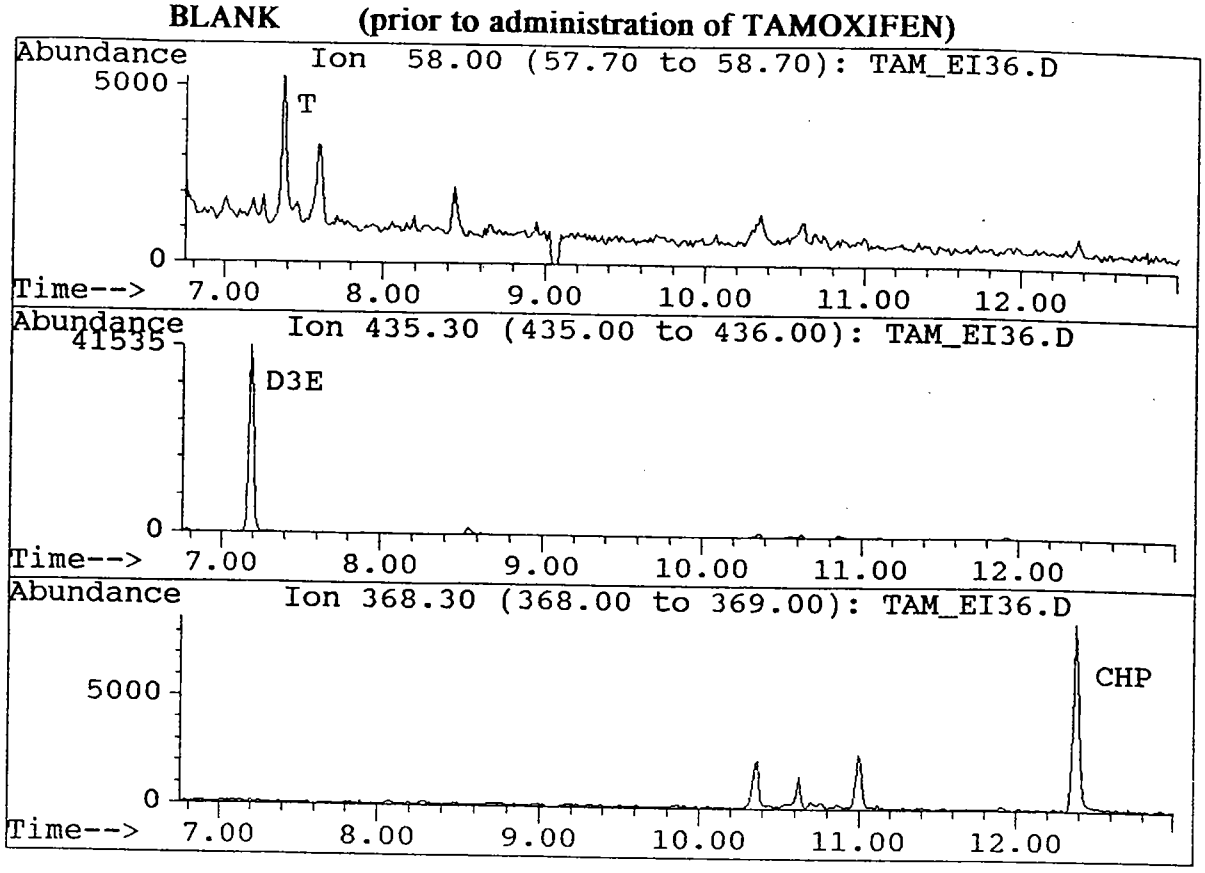


Fig. 1. Structure and EI mass spectrum of tamoxifen



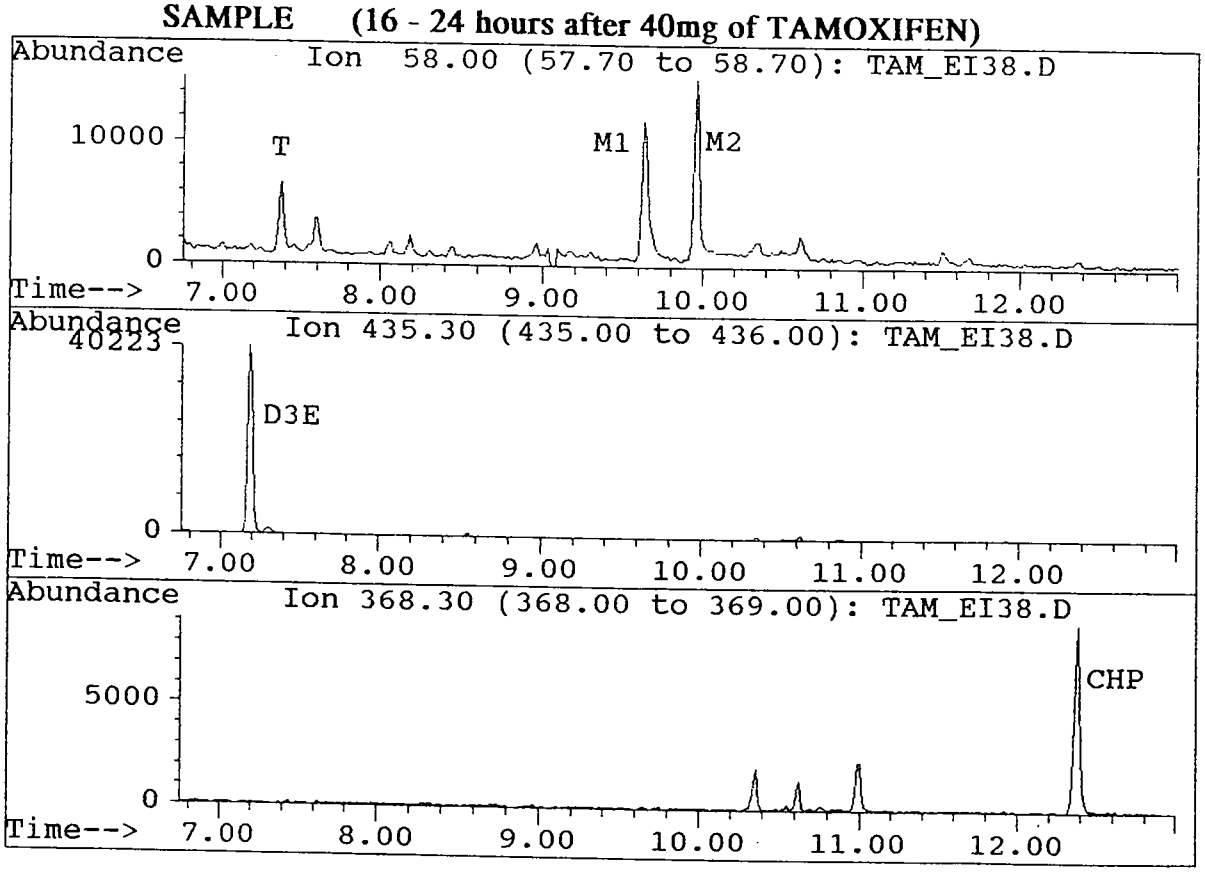
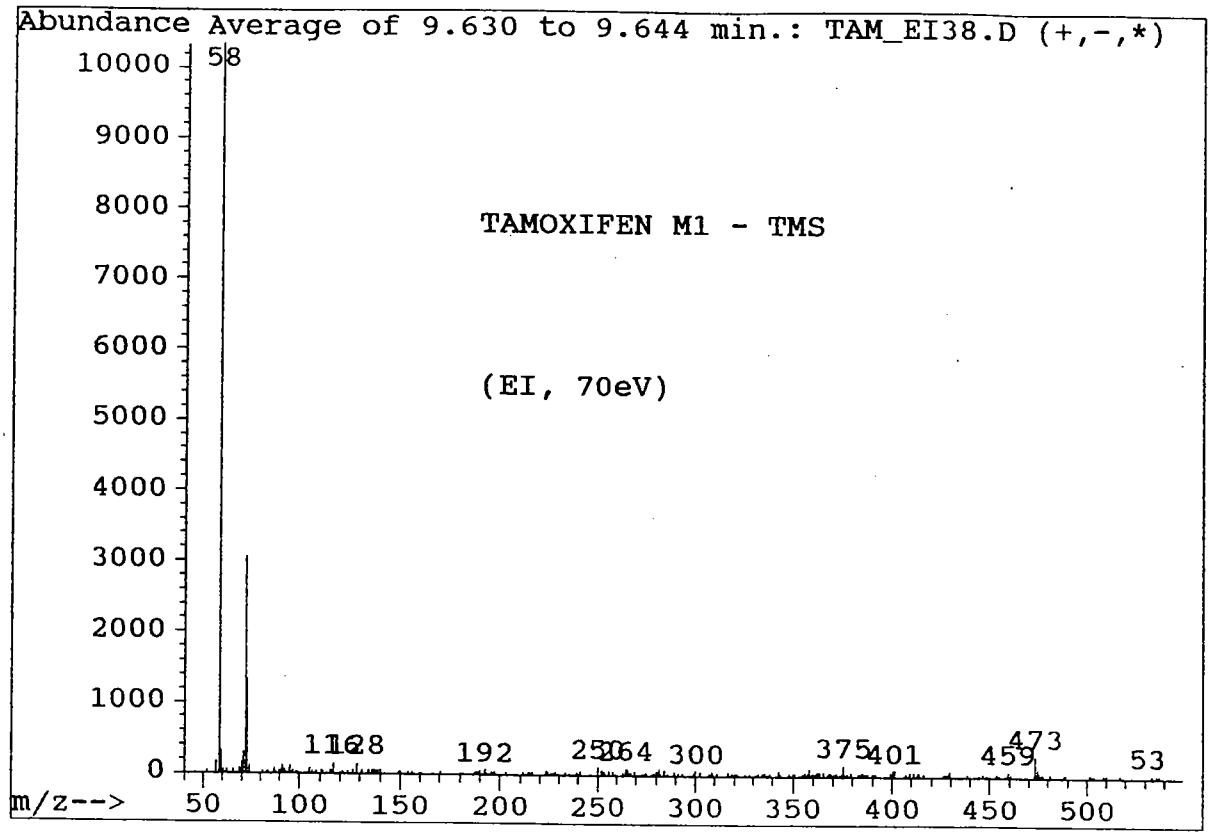


Fig. 2. Extracted ion profiles (EI, 70 eV) m/e 58, m/e 435 and m/e 368 obtained from samples before and after administration of tamoxifen (40 mg)
annotation: T - tamoxifen; D3E - 19D_3-epitestosterone; CHP - cholesteryl propionate; M1, M2 - tamoxifen metabolites



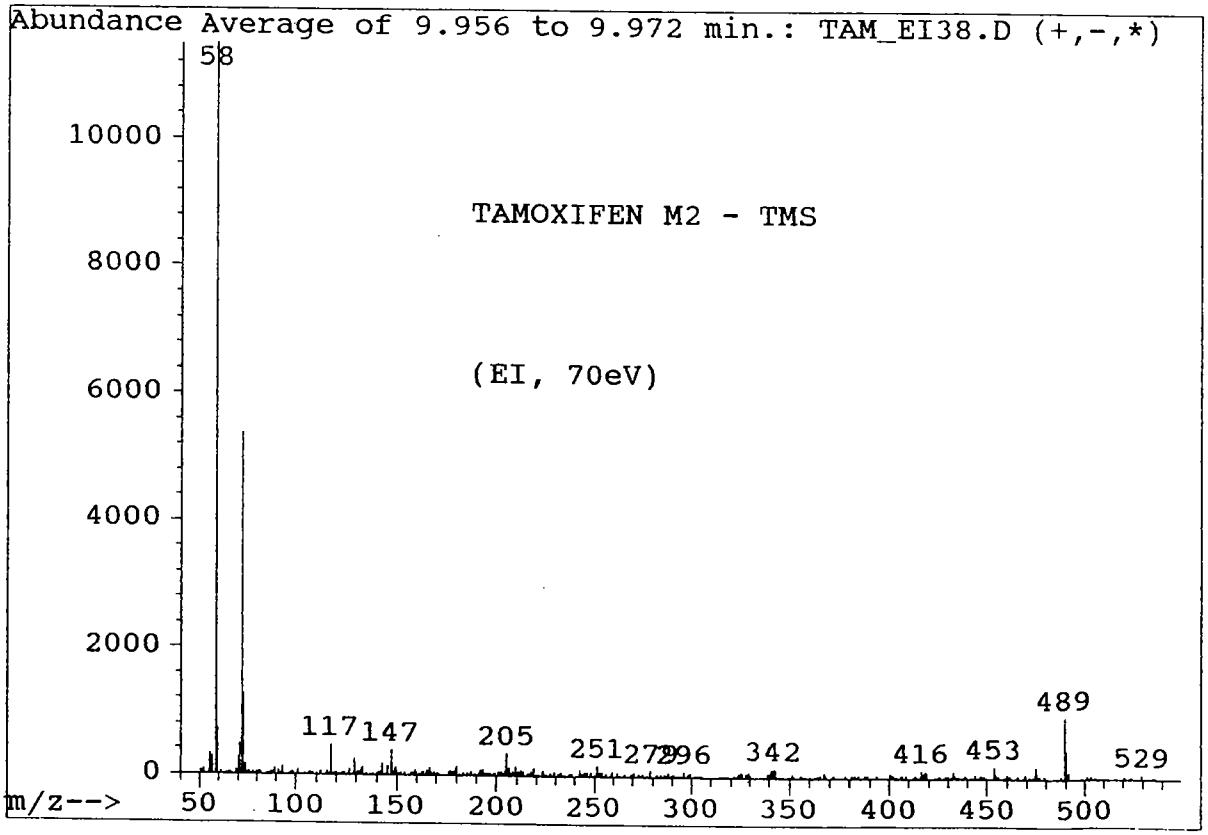


Fig. 3. EI mass spectra of detected metabolites as TMS derivatives

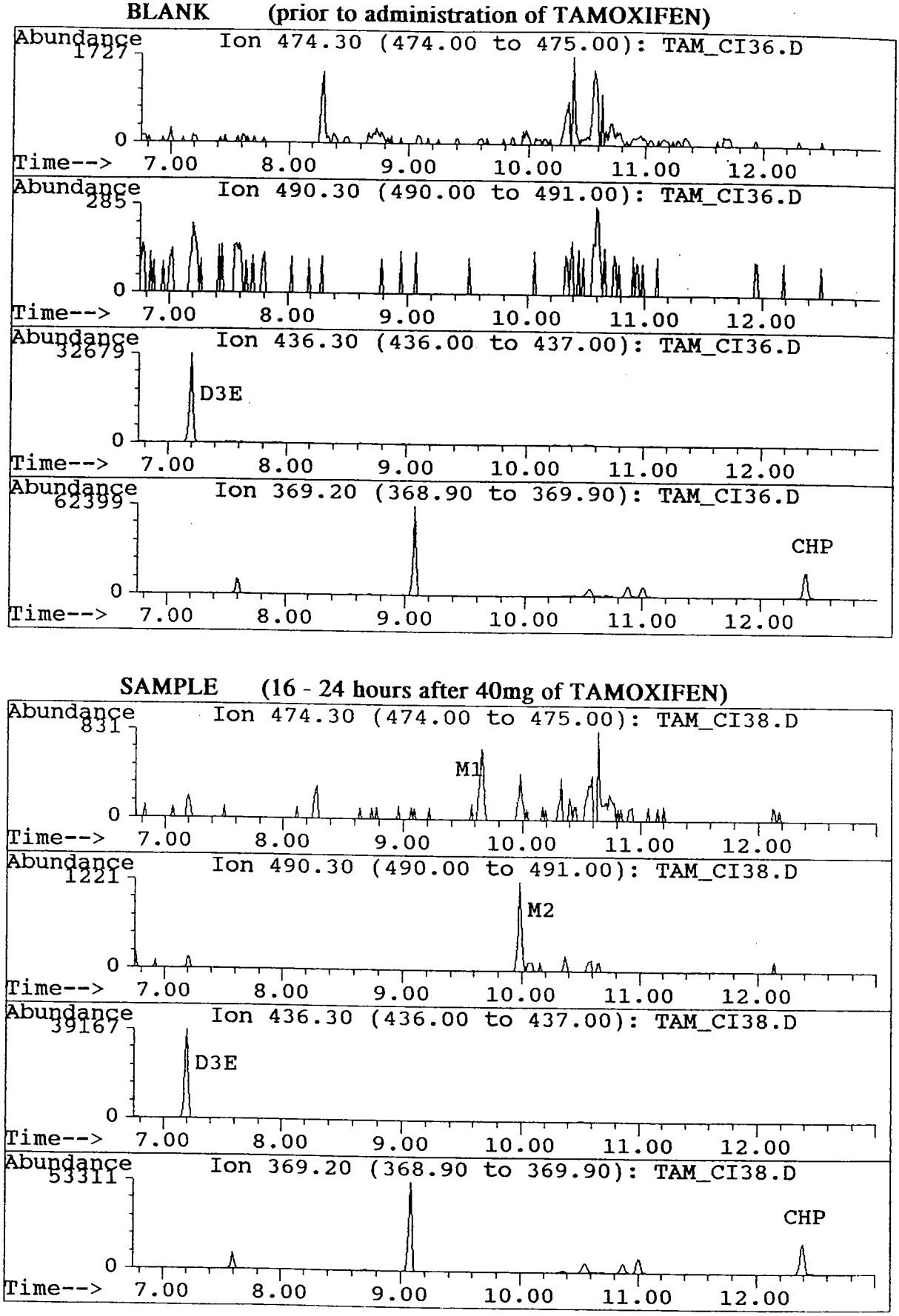
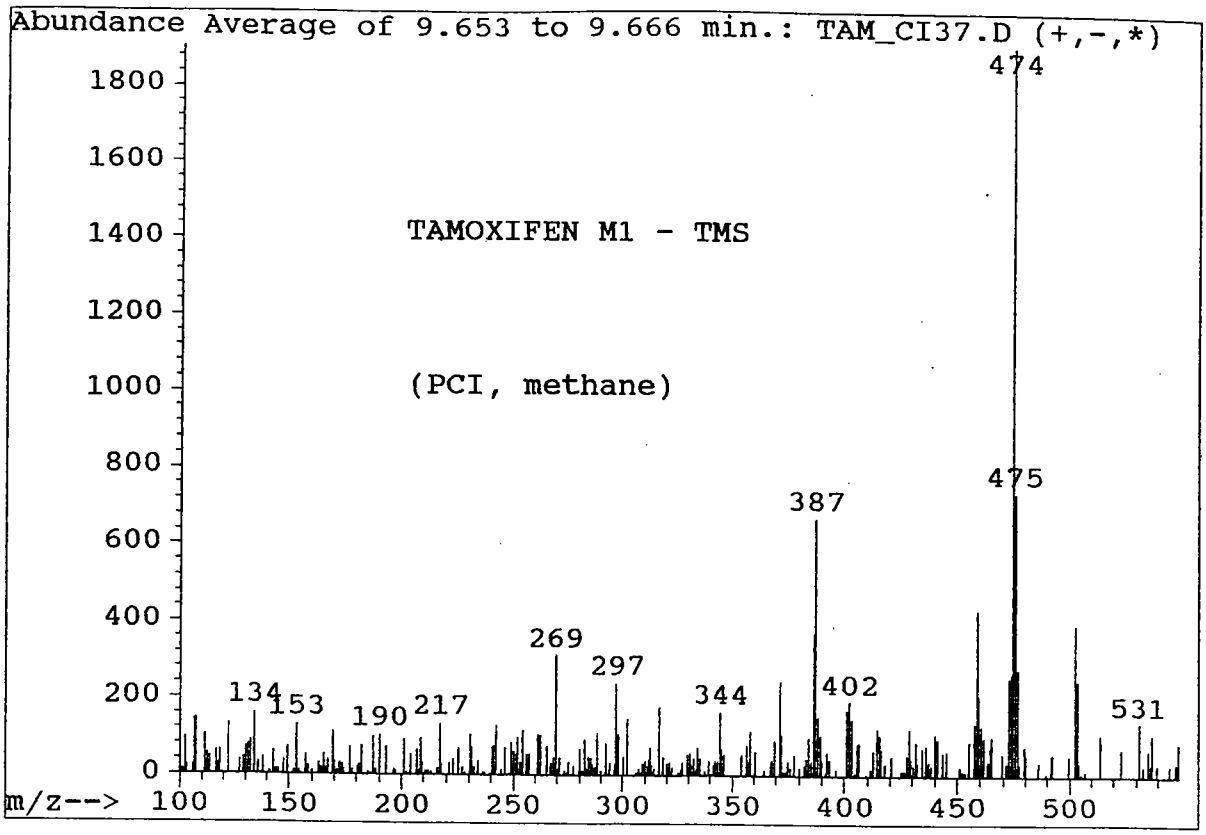


Fig. 4. Extracted ion profiles (PCI) m/e 474, m/e 490, m/e 436 and m/e 369 obtained from samples before and after administration of tamoxifen (40 mg)



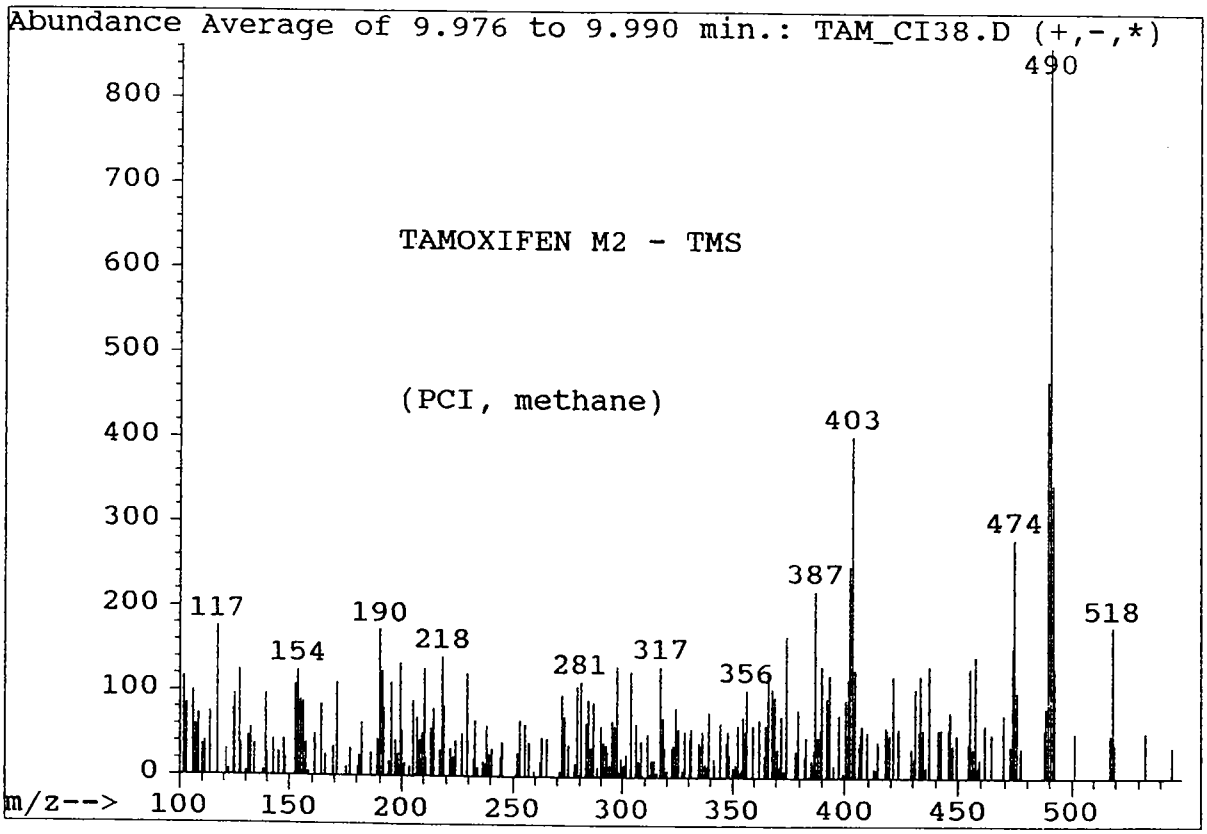


Fig. 5. PCI mass spectra of detected metabolites as TMS derivatives

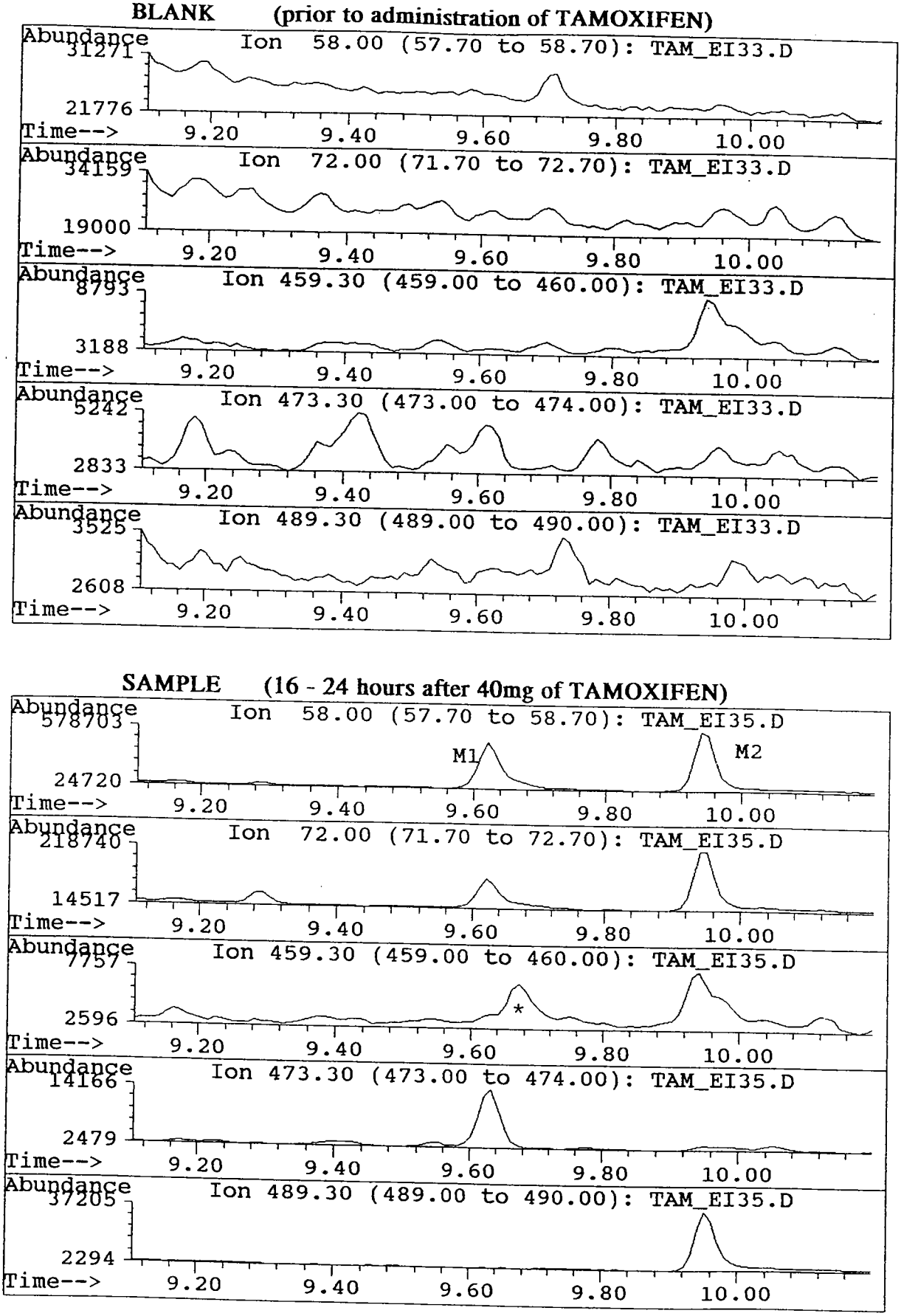


Fig. 6. SIM chromatograms (EI, 70 eV) m/e 58, m/e 72, m/e 459, m/e 473 and m/e 489 obtained from samples before and after administration of tamoxifen (40 mg)

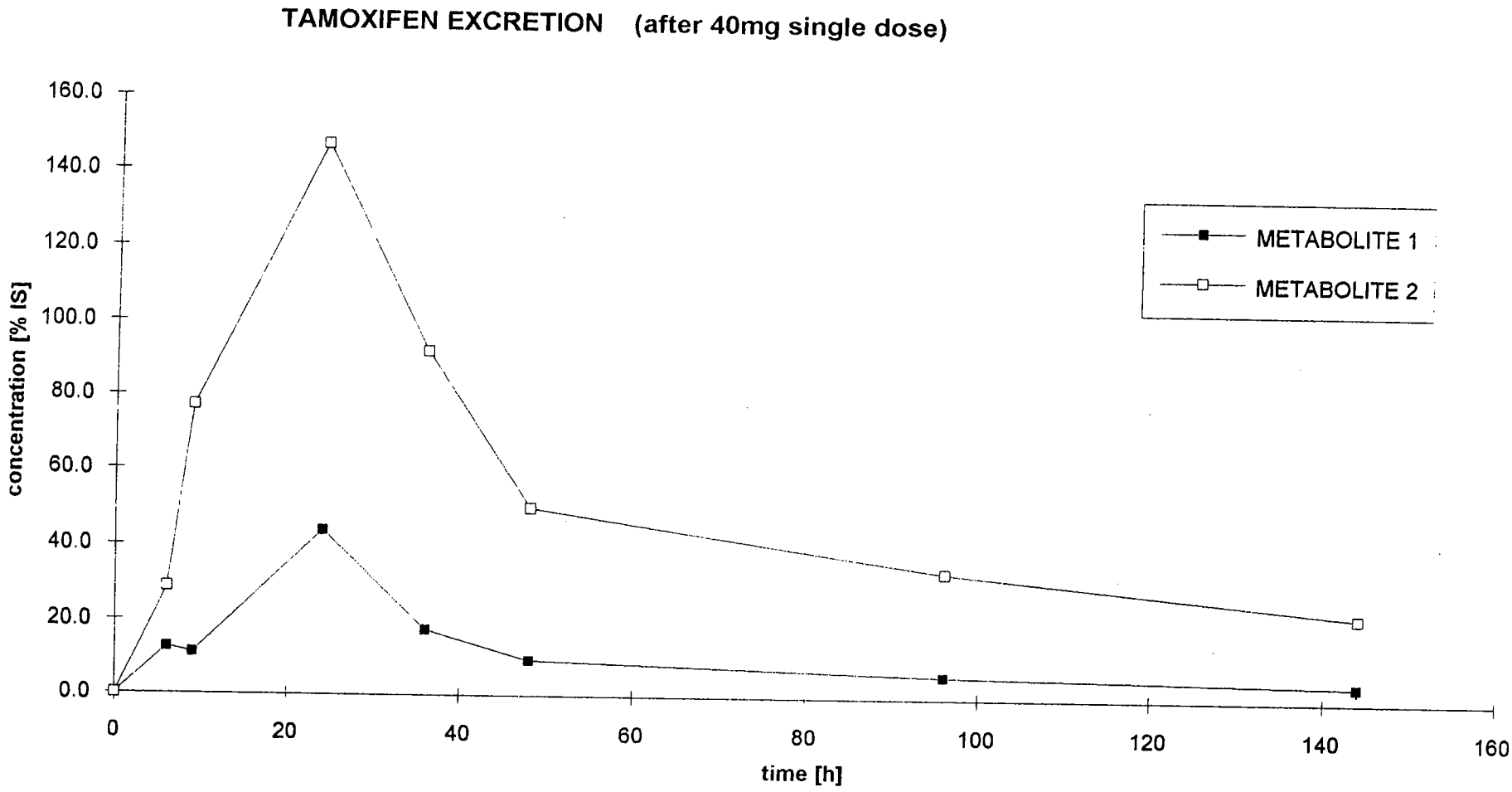


Fig. 7. Time-course of tamoxifen excretion