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Analysis of tiadenol in human plasma by capillary gas chromatography with electron capture detection

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Abstract: A sensitive and specific method for the quantitative determination of tiadenol in human plasma is described. After addition of the internal standard, both compounds were quantitatively extracted into chloroform and then derivatized with heptafluorobutyric anhydride (the structures of both derivatives were confirmed by electron impact mass spectrometry). Quantitation was achieved by capillary gas chromatography, using a ^{63}Ni-electron capture detector. Linearity was observed in the concentration range 5–100 ng ml^{-1} and the minimum concentration of tiadenol detectable in plasma was 2.0 ng ml^{-1}. The method was successfully applied to plasma specimens collected from healthy human volunteers following a single oral administration of 800 mg of tiadenol.

Keywords: *Tiadenol in plasma; capillary GC analysis; electron capture detection; pharmacokinetics.*

Introduction

Tiadenol, 1,10-bis(hydroxyethylthio)-decane is an antihyperlipidaemic agent which is widely used for the treatment of essential hypercholesterolemia and hyperlipoproteinemia [1, 2]. However, there are no studies of its quantitative determination in plasma. The only methods available in the literature are for the quantitative determination of the pure compound by spectrophotometry [3] and coulometry [4]. Neither method is sensitive or specific. This report describes a method which satisfies these requirements. It is based on the gas–liquid chromatography (GLC) determination of a heptafluorobutyric derivative of tiadenol with electron capture detection.

Experimental

Chemicals and reagents

Tiadenol, 1,10-bis(hydroxyethylthio)-decane was obtained from Boehringer Biochemia Robin (Milan, Italy). The internal standard, 1,8-bis(hydroxyethylthio)-octane

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and tiadenol metabolites: tiadenol-monosulfoxide (1-(hydroxyethylthio),10-(hydroxyethylsulfinyl)-decane) and tiadenol-disulfoxide (1,10-bis(hydroxyethylsulfinyl)-decane) were synthesized according to the methods reported in the literature [5–7].

Chloroform and isooctane were of pesticide quality; heptafluorobutyric anhydride (HFBA) was reagent grade (C. Erba, Milan, Italy).

Instrumentation

A Hewlett–Packard Model 5710 capillary gas chromatograph, operating in the splitless mode, equipped with a ^{63}Ni-electron capture detector and a Hewlett–Packard model 3385 A data terminal was used. A Hewlett–Packard fused silica capillary column (25 m × 0.2 mm i.d.), wall-coated with 5% phenylmethylsilicone (film thickness 0.33 μm), was used. Optimal GC conditions were: temperature programming (4°C min^{-1} from 200 to 280°C); injection port and detector temperatures: 300°C. Hydrogen was used as the carrier gas at a flow rate of 0.7 ml min^{-1}; argon–methane (90:10; v/v) with a flow rate of 30 ml min^{-1} was used as the make-up gas. Samples (1 μl) were injected with an automatic injector (Hewlett–Packard model 7671 A).

Mass spectral data were obtained using a GC–MS computer system (Finnigan model 4000). Gas chromatography was performed on a glass capillary column (25 m × 0.3 mm i.d.), wall-coated with OV-1 (film thickness 0.2 μm); injection port temperature, 250°C; temperature programming, 180–280°C (5°C min^{-1}); carrier gas (helium), 1–2 ml min^{-1}. The ionization beam was 70 eV; electron multiplier voltage, 3.5 kV; trap current, 60 A; ion source temperature, 250°C.

Human studies

Six healthy, fasted female volunteers were given 800 mg of tiadenol (2 tablets of 400 mg) orally. The subjects were 20–30 years old and weighed 55–65 kg. Blood samples were collected by venupuncture into heparinized tubes before treatment and at 30 min time intervals up to 10 h afterwards. Plasma was separated by centrifugation at 2000 rpm for 10 min and frozen immediately at −20°C until required for analysis.

Extraction procedure

A 1 ml volume of plasma (blank or treated) was placed in a 15 ml centrifuge tube and 6 ml of chloroform and 100 μl of a methanol solution containing 1 μg ml^{-1} of the internal standard were added. The tube was vortexed (5 min), centrifuged at 4000 rpm (5 min) and the organic layer (5.9 ml) transferred by aspiration into a 10 ml conical tube, and concentrated to dryness in vacuo. The plasma samples were re-extracted with a further 6 ml of chloroform and processed as described. The combined organic residue was dissolved in 200 μl of an isooctane solution containing 10% of HBFA and incubated at 90°C for 10 min. The solution was evaporated to dryness under a low stream of nitrogen and the residue, dissolved in 100 μl of isooctane, was injected into the gas chromatograph (1 μl).

Calibration curve

The calibration curve was prepared from blank plasma samples (1 ml) spiked with 5–100 ng ml^{-1} of tiadenol and 100 ng ml^{-1} of internal standard. The regression equation for the ratios of the area of derivatized tiadenol to the area of derivatized internal standard versus concentrations of tiadenol was used in determining concentrations of tiadenol in human plasma.

Results and Discussion

Chromatography

Several sample clean-up procedures, including washing the chloroform extracts with buffers of various pH values or chromatography on a silica gel column were investigated in order to eliminate the endogenous interfering materials extracted from the plasma. All these procedures gave low recovery and poor reproducibility. However, by temperature programming from 200 to 280°C at 4°C min^{-1}, most of the interfering materials were eluted within the first 14 min, and the rest of the chromatogram was essentially free from interfering peaks. Typical chromatograms of plasma blank and plasma spiked with tiadenol (I) and the internal standard (II) are shown in Fig. 1. The retention times under the conditions described were 16 and 20 min for I and II, respectively.

Internal standard and derivatization

Both I and II react completely with HFBA within 10 min at 90°C and the derivatives formed are stable for 1 week at room temperature.

The interference of the biological material on the derivatization efficiency of tiadenol and of the internal standard was checked by comparing the peak areas of both

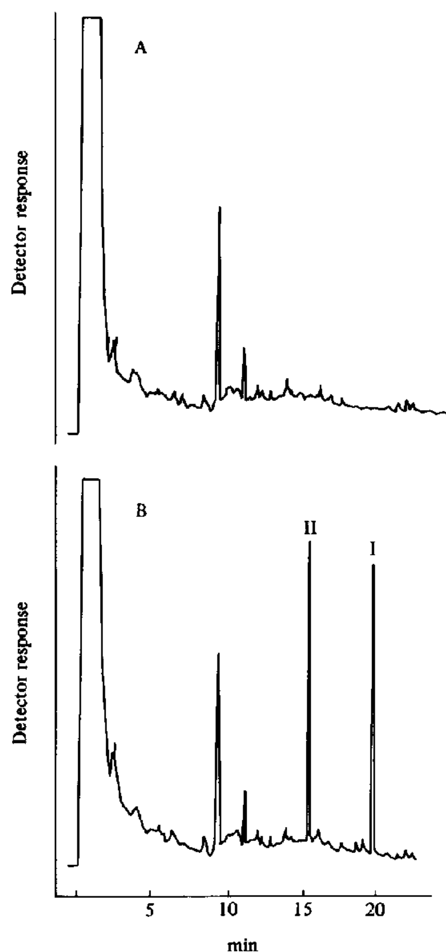

Figure 1
Gas liquid chromatogram obtained from a blank plasma sample (A) and a plasma sample (B) containing 10 ng ml^{-1} of tiadenol (I) and of the internal standard (II) (attenuation 16×).

compounds when derivatized in the absence and in the presence of blank plasma extracts. No loss in derivatization efficiency was observed for either compound.

Confirmation of derivative structures

Plasma samples collected from human volunteers given tiadenol orally were pooled. The internal standard was added and the plasma extracted and analyzed by GC–MS after derivatization. Evidence for the esterification of I with HFBA was shown by the characteristic fragments at *m/z* 69, 119 and 169 and by the total mass of the molecular ion at *m/z* equal to 686, corresponding to formation of the di-heptafluorobutyril derivative. Other characteristic peaks were at *m/z* 473 (due to a loss of a heptafluorobutyril residue), at *m/z* 414, 241 and 171.

For the internal standard, the mass spectrum showed a molecular ion at *m/z* 658, corresponding to the formation of the di-heptafluorobutyril derivative. The other characteristic peaks were identical to those for tiadenol.

Recovery

The recovery of tiadenol from human plasma was determined by adding varying concentrations of the drug to blank plasma samples. The samples were then extracted and derivatized as described. The percentage recoveries were calculated by comparing the extracted samples with the standard curve obtained by directly injecting derivatized I without extraction.

Tiadenol recovery from plasma averaged 95.48 ± 5.0% and was constant over the concentration range examined (5–100 ng ml^{-1}). The same procedure was adopted for the determination of the recovery of the internal standard and found to be 94.32 ± 4.8%.

Precision, accuracy and linearity

The precision and accuracy of the method were determined by analysis of each spiked plasma calibration solution (5–100 ng ml^{-1}) ten times. In view of the range of % RSD obtained and shown in Table 1, sample plasma solutions were analyzed in triplicate to improve precision. Additionally, a two-point calibration curve of I in the range required and a plasma blank were prepared each day to take into account day-to-day variation in instrument sensitivity.

There was a linear relationship when the ratios of peak area of derivatized tiadenol to peak area of derivatized internal standard were plotted against the various concen-

Table 1
Precision and accuracy of the method for tiadenol in plasma

Tiadenol added (ng ml^{-1})	Tiadenol found (ng ml^{-1})	RSD (%)	Relative Error† (%)
5	4.78 ± 0.32*	6.69	8.74
10	9.70 ± 0.52	5.36	7.00
20	20.25 ± 1.08	5.33	6.96
40	39.70 ± 1.84	4.63	6.05
60	60.80 ± 2.34	3.84	5.02
80	79.50 ± 2.98	3.74	4.88
100	99.10 ± 3.37	3.40	4.44

* Mean ± SD.
† Triplicate injections.

trations of I (mean area ratio: 0.037 = 5 ng ml^{-1}; 0.076 = 10 ng ml^{-1}; 0.150 = 20 ng ml^{-1}; 0.285 = 40 ng ml^{-1}; 0.425 = 60 ng ml^{-1}; 0.568 = 80 ng ml^{-1}; 0.715 = 100 ng ml^{-1}). Linear regression analysis showed that the best fit through the data points is described by $y = 0.004 + 0.007\,x$, with $r^2 = 0.9993$.

Specificity and sensitivity

Evidence for the specificity of the method is provided by the following considerations: (a) The absence of interfering peaks in the region of the chromatogram containing drug and internal standard (Fig. 1); (b) The TLC analysis of chloroform extracts from pooled plasma samples of treated subjects showed that the tiadenol metabolites present in human plasma were tiadenol-monosulfoxide and tiadenol-disulfoxide. When GLC analysis was performed on synthetically obtained metabolites the results indicated that these compounds would not interfere with the determination of tiadenol, since they were not eluted under the conditions employed; (c) Other hypolipidaemic agents (clofibric acid and congeners or nicotinic acid) which could be eventually co-administered with the drug, were not extracted at neutral pH.

The absolute sensitivity of the electron capture detector was 20 pg so that it is possible to detect 2 ng of tiadenol in 1 ml of plasma.

Human studies

The method described here was applied to the determination of tiadenol in the plasma of 6 healthy female volunteers given a single oral dose of 800 mg. Peak plasma levels of 52.5 ± 7.8 ng ml^{-1} occurred 60 min after administration. An example of the time course of the tiadenol concentrations in plasma in one subject is shown in Fig. 2.

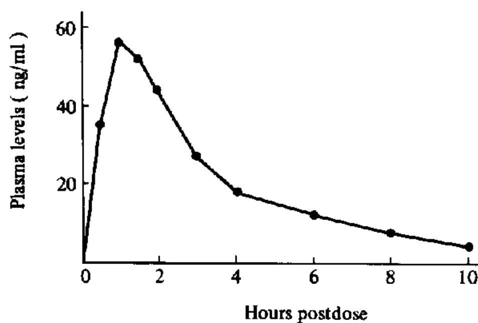

Figure 2
Plasma levels of I in a human subject after a single oral 800 mg dose.

Conclusions

An adequately sensitive and selective method for tiadenol monitoring in human plasma is needed in order to study the pharmacokinetics of the drug. Sensitivity is particularly important because tiadenol is extensively metabolized in man. More than 70% of the orally administered dose is eliminated as S-oxidized and C-oxidized highly polar metabolites [8] in the urine of the first 12 h [9]. It has been demonstrated that in chronic therapeutic trials several patients have developed tolerance to the drug owing to an inductive effect of tiadenol on the enzyme system cytochrome P-450, which is responsible for its metabolism by sulfoxidation [10].

An HPLC–UV determination of tiadenol would be difficult unless a suitable derivative was available, since the absorbance of tiadenol is very low ($E^{1\%}/_{1cm}$ = 21 at

212 nm). Therefore GLC seemed to be the technique chosen for this purpose, especially considering the sensitivity required.

The results indicate that the GC procedure developed is sensitive enough to detect plasma levels of tiadenol 10–12 h post dosing, thus allowing a complete kinetic study of the unchanged drug. The lack of interference by metabolic products of the drug or by other co-administered hypolipidaemic agents makes the method selective. The relatively long duration of the total chromatographic analysis time (3 × 20 min) is offset by the short time required for sample manipulation.

These results make this procedure, at present successfully applied to plasma specimens collected from healthy human volunteers, particularly useful for the therapeutic monitoring of the drug.

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