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SENSITIVE METHOD FOR THE DETERMINATION OF 6-OXO-PROSTAGLANDIN $F_{1\alpha}$ BY GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE AND MASS-FRAGMENTOGRAPHIC DETECTION

É. TOMORI, G. CSEH and M. VARGEK
Institute For Drug Research, H-1325, P.O. Box 82, Budapest (Hungary)

SUMMARY

A selective and sensitive quantitative method is described for the determination of the endogenous prostacyclin content of the aorta of rats, treated with GYKI-11679, a new hydrazone derivative with antihypertensive action. The thoracal aorta from three rats was excised and pooled, 1 h after treatment with 10 mg/kg of GYKI-11679. The prostacyclin, transformed to 6-oxo-$PGF_{1\alpha}$, was extracted. For gas chromatographic electron-capture detection (GC–ECD), 6-oxo-$PGF_{1\alpha}$ was converted into the O-methyloxime tris(trifluoroacetyl) hexafluoroisopropyl ester derivative. The GC–ECD properties, stability and mass-spectrometric characteristics of this new derivative allow the quantitative analysis of 6-oxo-$PGF_{1\alpha}$ by using nor-$PGF_{2\alpha}$ as internal standard.

INTRODUCTION

In recent years, much attention has been devoted to the optimization of the gas chromatographic (GC) analysis of 6-oxo-prostaglandin (PG) $F_{1\alpha}$. The derivatization published by Pace-Asciak[1] involved esterification, followed by methoximation and trimethylsilylation to yield a methyl ester methoxime tris(trimethylsilyl) (ME-MO-TMS) derivative. This derivative is especially useful for GC–mass spectrometric (GC–MS) methods. Gas chromatography with electron-capture detection (GC–ECD) seems an ideal answer to some problems in biochemical trace analysis, as the detection limit allows the measurement of picogram amounts of prostaglandins[2–6]. The hexafluoroisopropyl ester derivative was used for measuring homovanillic acid and γ-aminobutyric acid[7,8]. This derivative has good volatility and electron-capturing properties.

In our method, the methyloxime tris(trifluoroacetyl) hexafluoroisopropyl ester (MO-TFA-HFIP) is employed for the measurement of 6-oxo-$PGF_{1\alpha}$ by GC–ECD and GC–MS. The new derivative is sufficiently stable, the excess of reagent and solvent can be evaporated and the derivative can be reconstituted in a minimum volume of solvent for improved sensitivity.

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MATERIALS AND METHODS

Methoxyamine hydrochloride was obtained from Pierce (Rockford, IL, U.S.A.), trifluoroacetic anhydride (TFAA) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) from Merck (Darmstadt, F.R.G.). All organic solvents were of analytical grade. The 6-oxo-$PGF_{1\alpha}$ and nor-$PGF_{1\alpha}$ were gifts from Chinoin (Budapest, Hungary). The OV-17 and OV-1 stationary phases coated on Gas Chrom Q (80–100 mesh) were purchased from Applied Science Labs. (State College, PA, U.S.A.). The glass capillary column (18 m × 0.25 mm), coated with OV-101, was prepared by the Research Laboratory for Inorganic Chemistry, Hungarian Academy of Sciences, Budapest. The 0.25-mm (20 cm × 20 cm) thin-layers plates (silica gel 60 F-254) were from Merck.

Chromatographic conditions

System A. An Hewlett-Packard Model 5713 A gas chromatograph was equipped with a 15-mC ^{63}Ni electron-capture detector. Packed columns were used: a 90 cm × 4 mm I.D. column of 3% OV-17 on Gas Chrom Q (80–100 mesh) at a flow-rate of 45 ml/min, and a 90 cm × 2 mm I.D. column of 10% OV-1 on Gas Chrom Q (80–100 mesh) at a flow-rate of 45 ml/min. The injection port was maintained at 220°C, the detector temperature was 300°C and the column temperatures were 200 and 190°C. An argon–methane mixture was used as the carrier gas.

System B. An Hewlett-Packard 5713 A instrument was modified for glass capillary GC. An all-glass, solvent-free injector[9] was mounted in the heated injector block. Argon–methane (9:1), mixed with the column effluent gas before entry into the detector, was used as make-up gas. The inlet pressure was 6 p.s.i. (1 ml/min) and the pressure of the make-up gas was 14 p.s.i. (20 ml/min). The injector block and detector temperatures were as in system A. The column temperature was 190°C. The column was an OV-101 glass capillary, 18 m × 0.25 mm I.D.

System C. GC–MS analyses were performed on an Hewlett-Packard 5992 A instrument. Other components of the system were: an OV-1 packed column (90 cm × 4 mm I.D.), a jet separator, an OV-101 glass capillary column (as in system B) and a solventless injector[9]. The capillary column was coupled directly to the mass spectrometer. The helium flow-rate for the packed column was 20 ml/min, and the inlet pressure for the capillary column was 10 p.s.i. The temperature of the injection port was 250°C, that of the column was 190°C. The electron-impact energy was 70 eV and the electron multiplier voltage was 2800 V.

Extraction of 6-oxo-$PGF_{1\alpha}$ from tissue

The thoracal aorta from three rats was excised and pooled (*ca.* 100–150 mg) 1 h after treatment with 10 mg/kg of 1-(6-morpholino-3-pyridizinyl)-2-[1-(*tert.*-butoxycarbonyl)-2-propylidene]hydrazine[10] (GYKI-11679). The aorta was homogenized and incubated at 37°C for 10 min with 3 ml of 0.05 *M* Tris–HCl (pH 8.0) in which 50 ng of nor-$PGF_{1\alpha}$ were present as an internal standard. The fluid was acidified to pH 3 with 200 μl of 4% (v/v) formic acid and extracted three times with 4 ml of diethyl ether–ethyl acetate (1:4). The supernatant was mixed with 200 μl of 1 *M* ammonia and 1 ml of methanol and the solvent was evaporated to dryness at 37°C. The residue, dissolved in chloroform–methanol (9:1, v/v), was applied as a 12-cm

long band on a 0.25-mm silica gel thin-layer plate. The chromatogram was developed with the organic phase of the system ethyl acetate–isooctane–acetic acid–water (11:5:2:10). The 6-oxo-$PGF_{1\alpha}$ band (R_F = 0.4) was localized with the help of nor-$PGF_{1\alpha}$ and 6-oxo-PGF_1 standards (8 μg) spotted on both sides of the plate and visualized by spraying with 10% phosphomolybdic acid in ethanol, at 120°C. The zones were scraped off and eluted from the silica gel with 1 ml of methanol.

Preparation of MO-TFA-HFIP and TFA-HFIP derivatives

After evaporation of methanol under a stream of nitrogen, the dry residue was treated with 50 μl of a 50 mg/ml solution of methoxyamine hydrochloride in anhydrous pyridine. The mixture was left overnight at room temperature and excess of solvent was evaporated under a stream of nitrogen. The residue was reconstituted in a mixture of 20 μl of methanol, 0.5 ml of 0.9% saline and 5 μl of 1 *M* citric acid and extracted two times with 0.5 ml of ethyl acetate. The ethyl acetate extract was transferred to a 2-ml reaction vial and evaporated under a stream of nitrogen. To the dry residue, 80 μl of freshly distilled TFAA and 40 μl of HFIP were added for derivatization. The vial was stoppered and kept at room temperature for 60 min. The reagent was then removed under a stream of dry nitrogen and the dry residue was dissolved in 50 μl f ethyl acetate.

RESULTS AND DISCUSSION

The linearity of the method over the range 500–15 000 pg TFA-HFIP derivative of nor-$PGF_{2\alpha}$ on an OV-1 packed column with ECD (conditions as in system A, amounts plotted against peak areas) was very good (correlation coefficient, r = 0.9990). Similar results were obtained on an OV-17 column and on a capillary column coated with OV-101.

The response to the MO-TFA-HFIP derivative of 6-oxo-$PGF_{1\alpha}$ was also tested. The correlation coefficient obtained in the range of 1–100 ng was 0.9989. The lower limits of detection were 100 pg for nor-$PGF_{2\alpha}$ and 200 pg for 6-oxo-$PGF_{1\alpha}$, respectively (signal-to-noise ratio = 4:1). Calibration curves obtained after extraction of 6-oxo-$PGF_{1\alpha}$ from the aorta wall of rats (50–200 ng added to about 100 mg wet weight tissue, 50 ng of nor-$PGF_{2\alpha}$ as internal standard) resulted in the following correlation coefficients: 0.9951 for OV-1, 0.9964 for OV-17 and 0.9962 for OV-101. The detection limit for endogenous 6-oxo-$PGF_{1\alpha}$ after extraction and derivatization was 500 pg by the GC–ECD method.

The preparation of the most common derivatives of PG examined for GC–ECD involves the derivatization of the hydroxy groups to form, *e.g.*, heptafluorobutyrate esters[11,12]. However, the thermal instability of the heptafluorobutyrate derivatives results in the formation of multiple peaks and renders them unsuitable for GC–ECD analysis[12]. Methyl ester methoxime triacetyl derivatives of the PGF series also gave broad and distorted peaks, indicating degradation on the column[13]. Methoxime derivatives of 6-oxo-$PGF_{1\alpha}$ and other PGs containing keto groups were found to be stable during GC–MS[11–16]. Pentafluorobenzyl methoxime trimethylsilyl ether or pentafluorobenzyloxime methyl trimethylsilyl ether[5,17] derivatives afforded very low detection limits (1–10 pg) for PG. Christ-Haselhof and Nugteren also used silyl-

ated pentafluorobenzyloxime methyl ester derivatives for the measurement of prostacyclin in blood at the pg level.

Clayes *et al.*[18] pointed out the advantage of first executing the methoximation. This sequence was adopted in our procedure. It was followed by hexafluoroisopropylation and trifluoroacetylation in a single step. The advantage of this new procedure is that it is simple and fast, and the derivatives can be analyzed even after one month of storage.

The new derivatives were tested by GC–MS. Figs. 1 and 2 show the mass spectra of the MO-TFA-HFIP ester of 6-oxo-$PGF_{1\alpha}$ (compound I) and the TFA-HFIP ester of nor-$PGF_{2\alpha}$ (compound II). In Table I, the relative abundances of the principal fragments of the derivatives of 6-oxo-$PGF_{1\alpha}$ and nor-$PGF_{2\alpha}$ are shown.

The molecular ion is not observed in the spectra of compounds I and II. The base peak in the spectrum of I appears at *m/z* 464 and that of II at *m/z* 221. The evidence for the presence of the hexafluoroisopropanol derivative was provided by the ion at *m/z* 670 $[M-{\cdot}O{-}CH(CF_3)_2]^+$ for compound I and at *m/z* 221 $^+CH=CH{-}C(O){-}O{-}CH(CF_3)_2$ for compound II. The characteristic ions appeared in the high mass region at *m/z* 609 $[M-2\ CF_3COOH]^{+\cdot}$, at *m/z* 578 $[M-2\ CF_3COOH-{\cdot}OCH_3]^+$ and at *m/z* 495 $[M-3\ CF_3COOH]^{+\cdot}$ for compound I. The fragmentation of this derivative was in good agreement with that of the corresponding ME-MO-Ac derivative of 6-oxo-$PGF_{1\alpha}$[13]. The ion at *m/z* 664 $[M-CF_3COOH]^{+\cdot}$ was formed by elimination of trifluoroacetic acid from compound II. The ion at *m/z* 379 was formed by the elimination of 3 CF_3COOH and the side chain of $(C_{16-19})^{\cdot}$ from the molecular ion. The ion at *m/z* 213 was formed by elimination of three trifluoroacetic acid residues and the side chain h $[M-3\ CF_3COOH-h+2H^+]$. The

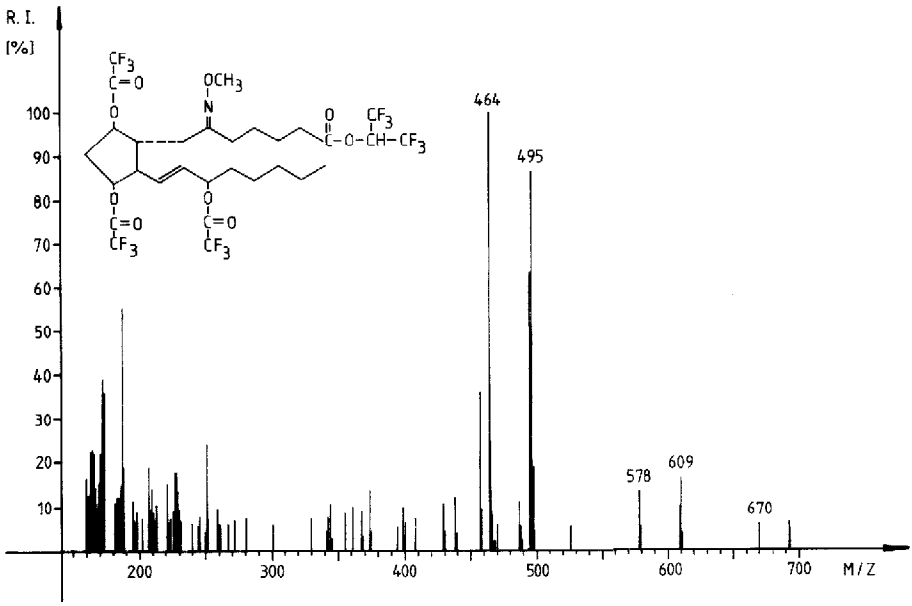

Fig. 1. Mass spectrum of the MO-TFA-HFIP derivative of 6-oxo-$PGF_{1\alpha}$ on an OV-101 capillary column. Experimental conditions as in system C. R.I. = Relative intensity.

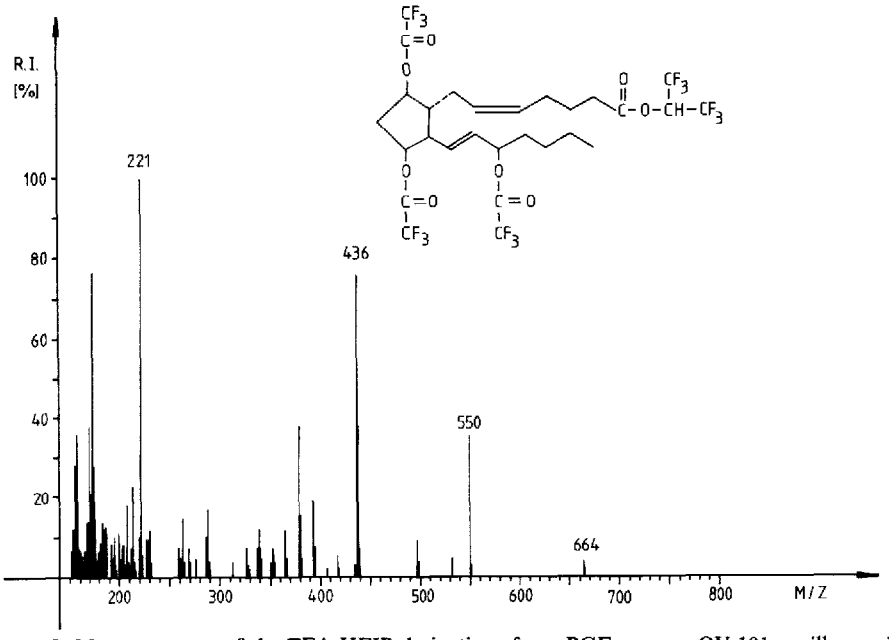

Fig. 2. Mass spectrum of the TFA-HFIP derivative of nor-PGF$_{2\alpha}$ on an OV-101 capillary column. Experimental conditions as in system C. R.I. = relative intensity.

fragmentation pathway of the HFIP-TFA derivative of nor-PGF$_{2\alpha}$ was also in a good agreement with that of the corresponding tris(heptafluorobutyrate) methyl esters[4] or trimethylsilyl methyl esters or acetyl methyl ester[14] derivatives.

The new derivative has good mass spectrometric properties and the sensitivity of mass fragmentography was suitable for measurements at the ng level of the prostacylin content in rat aorta walls.

The procedure for the calibration curves (selected ion monitoring, SIM) was the same as that used for calibration with GC–ECD. The ions at *m/z* 464 for MO-TFA-HFIP-6-oxo-PGF$_{1\alpha}$ and at *m/z* 550 for TFA-HFIP-nor PGF$_{2\alpha}$ were monitored.

TABLE I

RELATIVE ABUNDANCES OF PRINCIPAL FRAGMENTS OF MO-TFA-HFIP DERIVATIVES OF 6-oxo-PGF$_{1\alpha}$ AND THE TFA-HFIP DERIVATIVE OF nor-PGF$_{2\alpha}$

Compound	*Molecular ion*	*m/z and relative intensities* of some fragment ions***
6-oxo-PGF$_{1\alpha}$ (MO-tris-TFA-HFIP)	837 (0)	670[a](8), 609[b](20), 578[c](17), 495[d](83), 464[e](100),
nor-PGF$_{2\alpha}$ (tris-TFA-HFIP)	778(0)	664[f](5), 550[b](35), 436[d](76), 379[g](38), 221[h](100)

* Relative intensities in parentheses.

** Notation: a = $[M-{}^{\cdot}O-CH(CF_3)_2]^+$; b = $[M-2\ CF_3COOH]^{+\cdot}$; c = $[M-2\ CF_3COOH-{}^{\cdot}OCH_3]^+$; d = $[M-3\ CF_3COOH]^{+\cdot}$; e = $[M-3\ CF_3COOH-{}^{\cdot}OCH_3]^+$; f = $[M-CF_3COOH]^{+\cdot}$; g = $[M-3\ CF_3COOH-(C_{16-19})^{\cdot}]^+$; h = $^+CH=CH-C(O)-O-CH(CF_3)_2$.

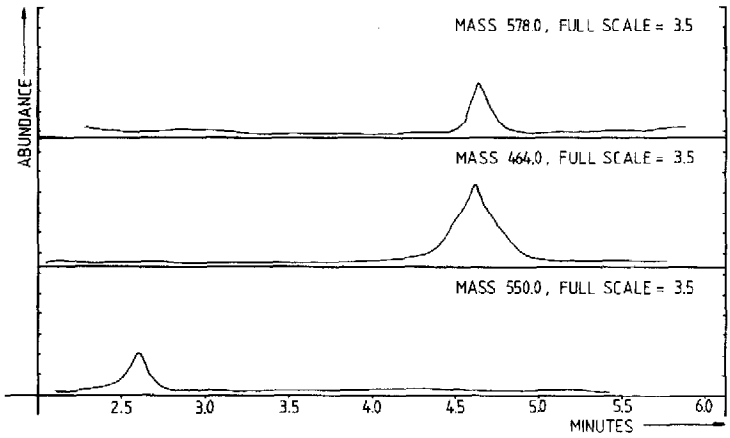

Fig. 3. Selected ion monitoring of the derivatives of 6-oxo-$PGF_{1\alpha}$ and nor-$PGF_{2\alpha}$, obtained from an aorta extract, chromatographed on an OV-1 packed column. For experimental conditions see system C.

The dependence of the response ratio (for MO-TFA-HFIP-6-oxo-$PGF_{1\alpha}$ at m/z 464 to the internal standard at m/z 550 for TFA-HFIP-nor-$PGF_{2\alpha}$) on the amount of 6-oxo-$PGF_{1\alpha}$ was linear (correlation coefficient, $r = 0.9532$) on an OV-101 capillary column. For GC–MS measurements the MO-HFIP-TFA derivatives resulted in less sensitivity than the ME-MO-TMS derivatives[14,15]. However, the ions selected in the higher mass region at m/z 609 $[M-2\ CF_3COOH]^{+\cdot}$ were very selective and specific for 6-oxo-$PGF_{1\alpha}$ and at m/z 550 $[M-2\ CF_3COOH]^{+\cdot}$ for nor-$PGF_{2\alpha}$, respectively. Considering the mass spectrum of MO-TFA-HFIP-6-oxo-$PGF_{1\alpha}$ (Fig. 1), the ion at m/z 464 is the base peak, but when monitoring this ion in the lower mass region other ions may interfere with the quantification. The detection limit for HFIP-

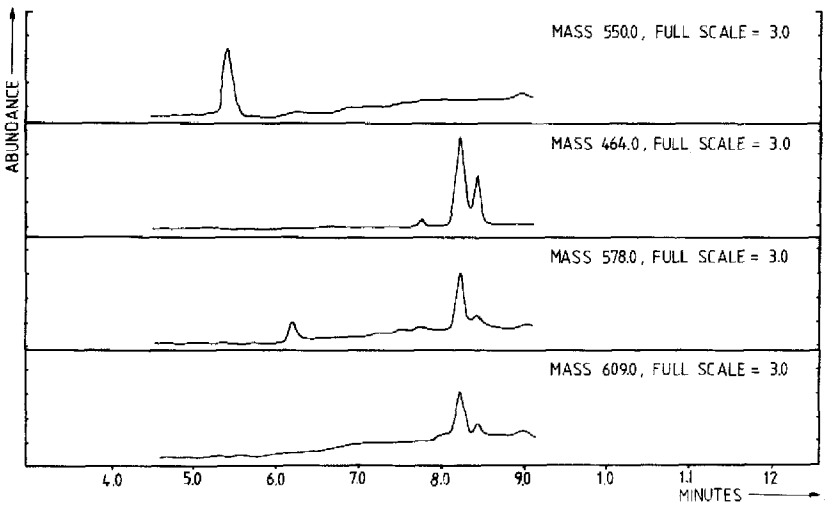

Fig. 4. Selected ion monitoring of the derivatives of 6-oxo-$PGF_{1\alpha}$ and nor-$PGF_{2\alpha}$, obtained from an aorta extract, chromatographed on an OV-101 glass capillary column. For experimental conditions see system C.

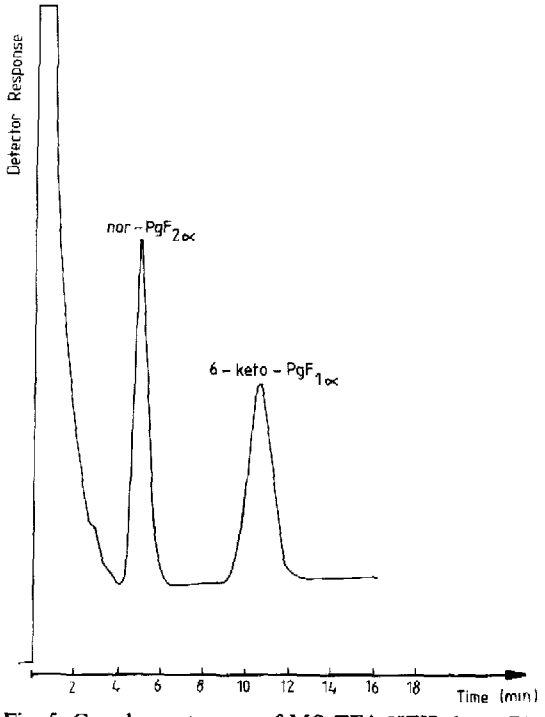

Fig. 5. Gas chromatogram of MO-TFA-HFIP-6-oxo-PGF$_{1\alpha}$, with the use of TFA-HFIP-nor-PGF$_{2\alpha}$ as internal standard on a 10% OV-1 column. Conditions as in system A.

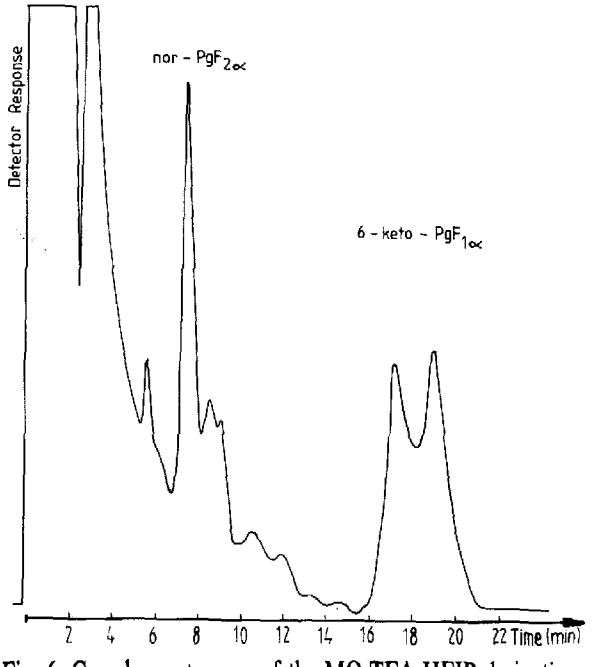

Fig. 6. Gas chromatogram of the MO-TFA-HFIP derivative of 6-oxo-PGF$_{1\alpha}$ on a 5% OV-17 column, with the TFA-HFIP derivative of nor-PGF$_{2\alpha}$ as internal standard. The sample is an extract of rat aorta. For experimental conditions see system A.

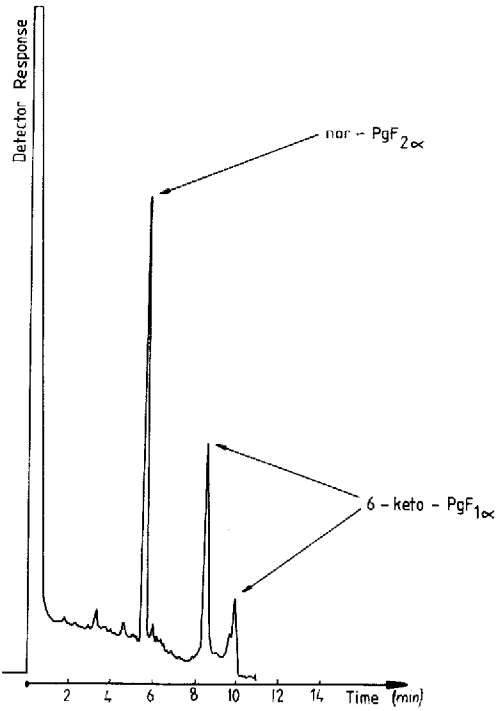

Fig. 7. Gas chromatogram of MO-TFA-HFIP-6-oxo-$PGF_{1\alpha}$ on an OV-101 capillary column. The internal standard was TFA-HFIP-nor-$PGF_{2\alpha}$. For experimental conditions see system B.

TFA-nor-$PGF_{2\alpha}$ (monitoring the ion at m/z 550) was 200 pg and for MO-HFIP-TFA-6-oxo-$PGF_{1\alpha}$ at m/z 464 was 500 pg. The detection limit for endogenous 6-oxo-$PGF_{1\alpha}$ (after extraction and derivatization) was 1 ng by GC–MS mass fragmentography.

The SIM profiles for 6-oxo-$PGF_{1\alpha}$ and nor-$PGF_{2\alpha}$, obtained from an extract of aorta, chromatographed on an OV-1 packed column and an OV-101 glass capillary column, are illustrated in Figs. 3 and 4. Each mass signal is detected by focusing the mass spectrometer on the ions in Table I.

The GC characteristics of MO-TFA-HFIP derivatives of 6-oxo-$PGF_{1\alpha}$ were also examined on stationary phases with different selectivities. Fig. 5 shows the gas chromatogram of the MO-TFA-HFIP derivative of 6-oxo-$PGF_{1\alpha}$ and the TFA-HFIP derivative of nor-$PGF_{2\alpha}$ on a 10% OV-1 packed column. The peaks obtained were quite symmetrical. The chromatogram on an OV-17 column of the new derivatives of compounds I and II, obtained from an aorta extract, is shown in Fig. 6. Peak I was eluted as a double peak, indicating the resolution of the *syn-* and *anti*-isomers. using a capillary column and ECD, two peaks were obtained for the derivatives of compound I (Fig. 7), showing also the presence of *syn-* and *anti*-isomers.

The experiments were carried out on columns with different polarities under different GC conditions. The aim of these experiments was to ascertain the most suitable experimental conditions for the quantitation of 6-oxo-$PGF_{1\alpha}$. Since decomposition on the columns was not significant, the new derivative was suitable for the quantitative analysis of nanogram amounts of 6-oxo-$PGF_{1\alpha}$ in the aorta of rats.

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