Journal of Chromatography, 250 (1982) 100–108
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,193

Note

Separation of prostaglandins and thromboxane B_2 by high-resolution gas chromatography coupled to mass spectrometry or electron-capture detection

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(Received June 10th, 1982)

In recent years much attention has been focused on the identification and measurement of prostaglandins (PGs)[1]. It is generally recognized that mass spectrometry (MS) is the most specific and reliable method so far available for qualitative and quantitative analysis of PGs, especially when coupled to high-resolution gas chromatography (HRGC)[2,3]. The sensitive electron-capture detector (ECD) can also be coupled to HRGC, yielding fairly specific methods of analysis[4]. The use of HRGC is mandatory when the stable cyclooxygenase metabolites of arachidonic acid [$PGF_{2\alpha}$, PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TXB_2)] have to be analyzed as a group, since these compounds cannot be separated by conventional packed columns.

This paper deals with the development of the simultaneous detection of PGs and TXB_2 by HRGC–MS and HRGC–ECD as alternative and integrated methods to be used when different degrees of specificity are required. A derivatization procedure suitable for the gas-phase analysis of all the compounds to be analyzed and for both the detectors to be used (MS and ECD), and a simple procedure for preparing high-resolution glass capillary columns tailored for PG analysis, were necessary.

Quantitative analysis and biological applications will be discussed elsewhere.

EXPERIMENTAL

Standards

$PGF_{2\alpha}$, PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$, TXB_2 and 2a,2b-dihomo-$PGF_{2\alpha}$ were a generous gift from Dr. John Pike of the Upjohn Company, Kalamazoo, MI, U.S.A.

Derivatization

The pentafluorobenzyl ester trimethylsilyl ether (PFB-TMS) derivatives of $PGF_{2\alpha}$ and 2a,2b-dihomo-$PGF_{2\alpha}$ and the pentafluorobenzyl ester methyloxime trimethylsilyl ether (PFB-MO-TMS) derivatives of PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 were prepared as previously described[5].

Mass spectrometry

An LKB 2091-051 gas chromatograph–mass spectrometer equipped with an

0021-9673/82/0000–0000/$02.75

LKB 2130 computer system for data acquisition and calculation was used in the electron impact mode. The gas chromatograph was a DANI 3800.

The instrument was used in the selected ion monitoring (SIM) mode and was tuned on the following ions: m/z 301 for TXB_2, 461 for PGE_2, 544 for PGD_2 and 6-keto-$PGF_{1\alpha}$, 589 for $PGF_{2\alpha}$ and 527 for 2a,2b-dihomo-$PGF_{2\alpha}$ which was used as internal standard for quantitative work. The instrumental conditions were as follows: ion source temperature, 250°C; electron energy, 22.5 eV; trap current, 100 μA; accelerating voltage, 3.5 kV; source slit width, 0.1 mm; collector slit width, 0.3 mm; resolution, 650. The mass spectra (recorded at 2.33 kV, 22.5 eV and resolution 900) are shown in Figs. 1–4, the salient fragments are assigned in Table I and the structures of the derivatives are shown in Fig. 5.

Electron-capture detection

A low dead-volume ^{63}Ni detector (DANI ECD 36/3), especially designed for connection to capillary columns, was used on a DANI 3900 gas chromatograph.

High-resolution gas chromatography

Support-coated open tubular (SCOT) capiliary columns were prepared by a procedure developed in our laboratory as a modification of the method described by German and Horning[6]. Glass capillary columns (0.9 mm O.D., 0.3 mm I.D.) were drawn using a Shimadzu GDM 1 drawing machine. A 150-cm Pyrex glass tube (8 mm I.D., 3 mm I.D.) yields a coil about 90 m long, with a diameter of 12 cm. In this study 30-m columns were employed. The glass column was rinsed with a small amount of acetone. A chloroform plug was then introduced under air pressure (1 atm) in order to wet the column wall; this was followed by a plug (30% of the column internal volume) of the support suspension consisting of 2% LiChrosorb, 0.25% OV-101 and 0.25% OV-17 in chloroform–carbon tetrachloride–methanol (50:49:1 v/v). This suspension was forced through the column at a rate of about 1.5 cm/sec. The column was dried under an air stream for 3 h at room temperature, then coated with additional liquid phase by the same technique used for the support coating. A plug (30% of column internal volume) of a chloroform solution containing 1% of OV-101–OV-17 (8:2 v/v) was moved through the column at a rate of about 3 cm/sec. The column was then dried under an air flow for 12 h at room temperature. In the conditioning step the column was heated by increasing the temperature from 50°C to 260°C at a rate of 0.5°C/min under a helium flow (1 atm head pressure).

RESULTS AND DISCUSSION

Derivatization

Of the many different derivatives described in the literature for GC analysis of prostaglandins, the PFB-MO-TMS derivatives were chosen since they yielded satisfactory results in terms of GC properties, ECD and MS response and stability. These derivatives show single, well shaped peaks for each compound, except for PGE_2-PFB-MO-TMS, whose *syn-anti* isomers can be seen as well separated peaks. The PFB-MO-TMS derivatives, which are reportedly excellent for ECD analysis[4], also gave satisfactory results when analysed by MS. The mass spectra of the derivatives, shown in Figs. 1–4, give significant intense ions in the high mass range, so that

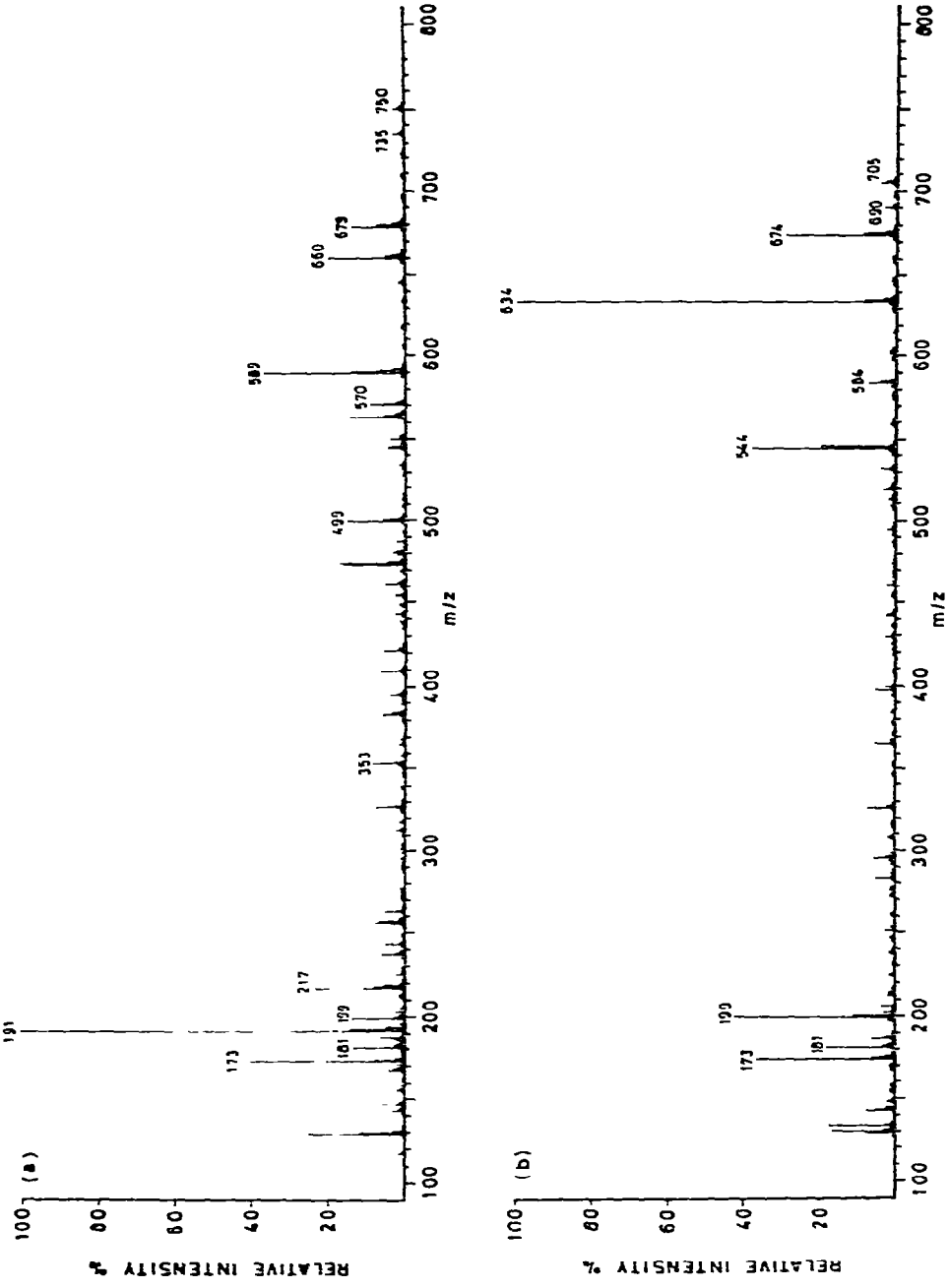

Fig. 1. Mass spectra of the PFB-TMS derivative of $PGF_{2\alpha}$ (a) and the PFB-MO-TMS derivative of PGD_2 (b).

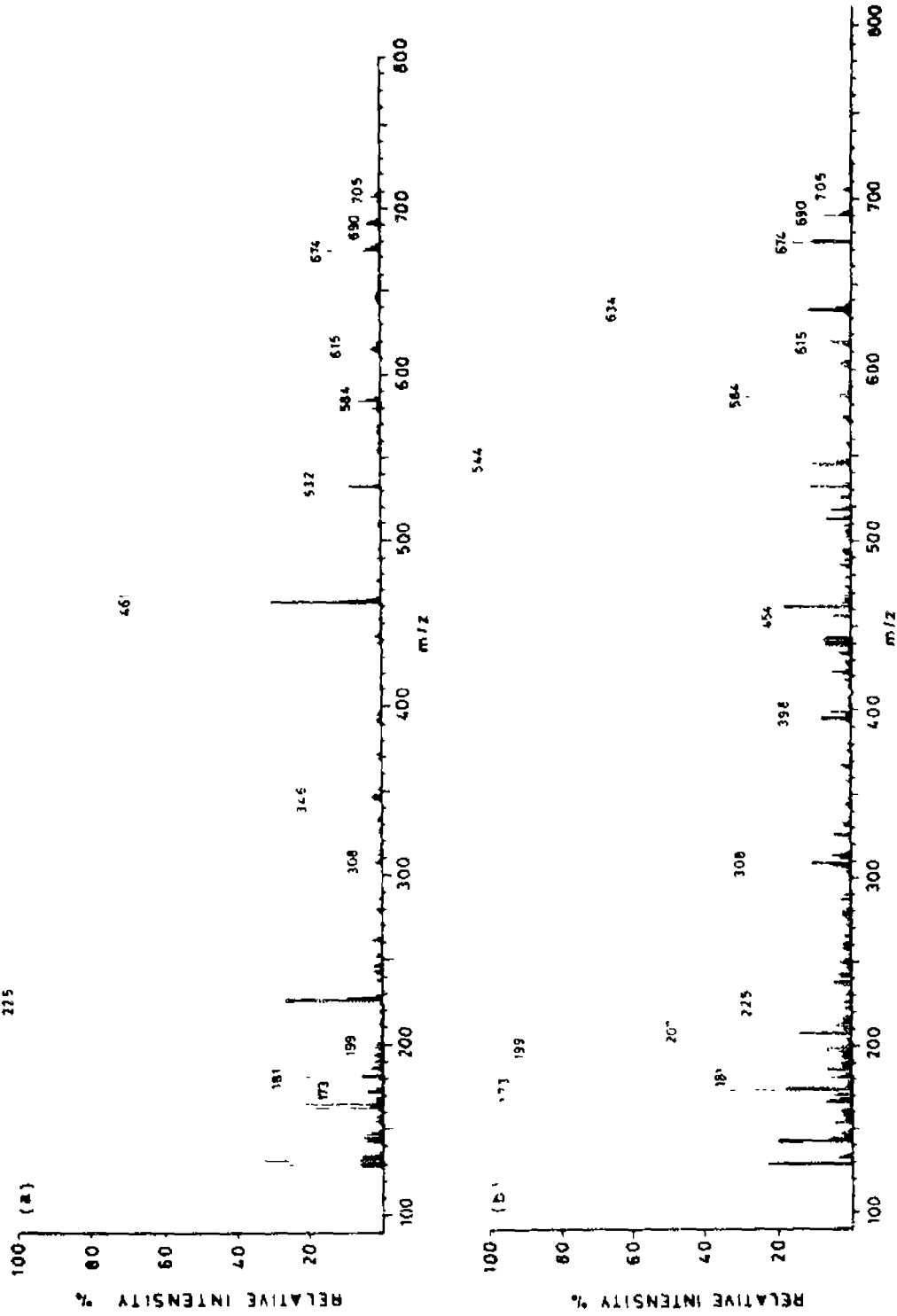

Fig. 2. Mass spectra of the PFB-MO-TMS derivative of PGF_2: major (a) and minor (b) isomers.

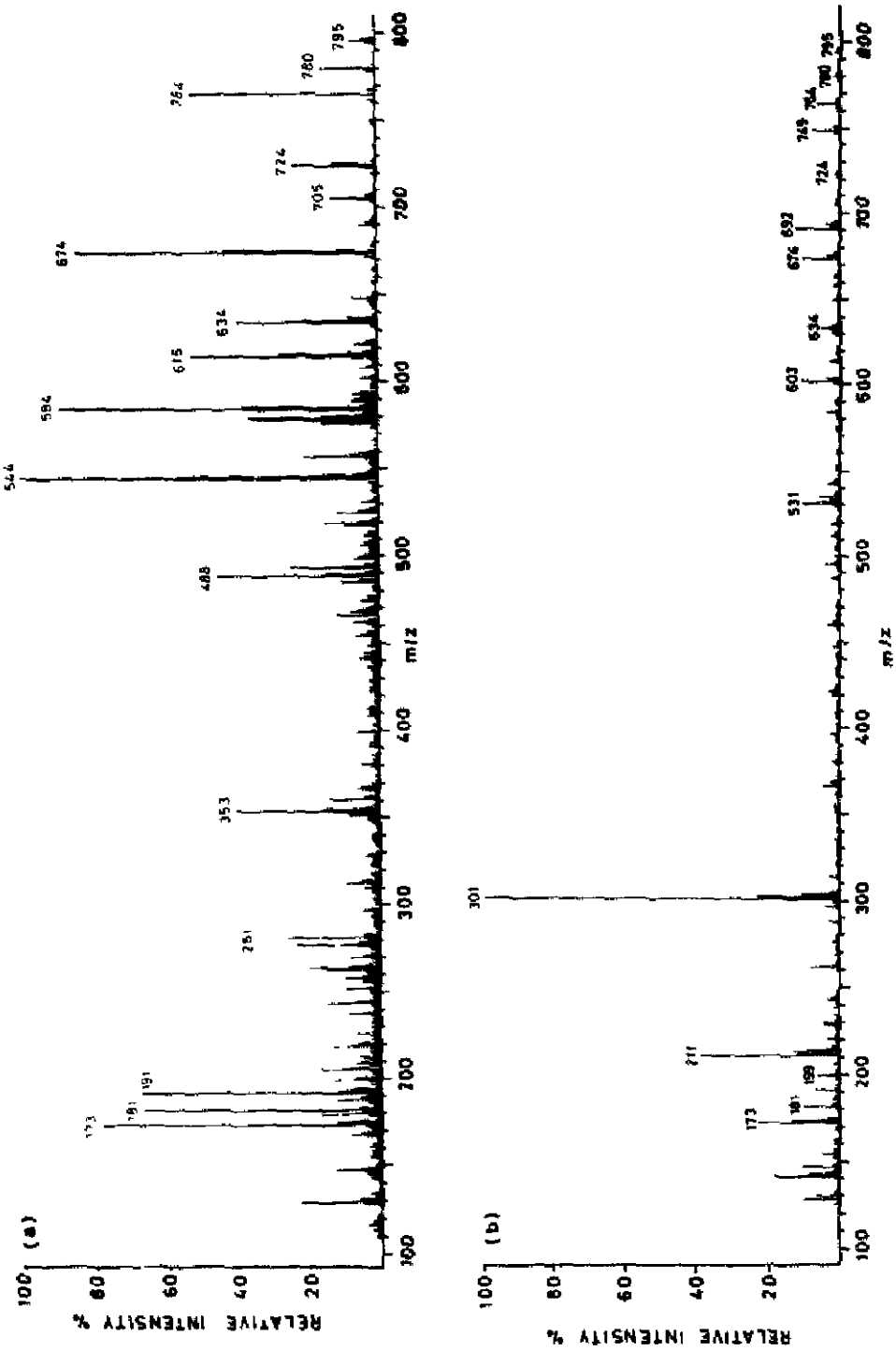

Fig. 3 Mass spectra of the PFB-MO-TMS derivative of 6-keto-$PGF_{1\alpha}$ (a) and TXB_2 (b)

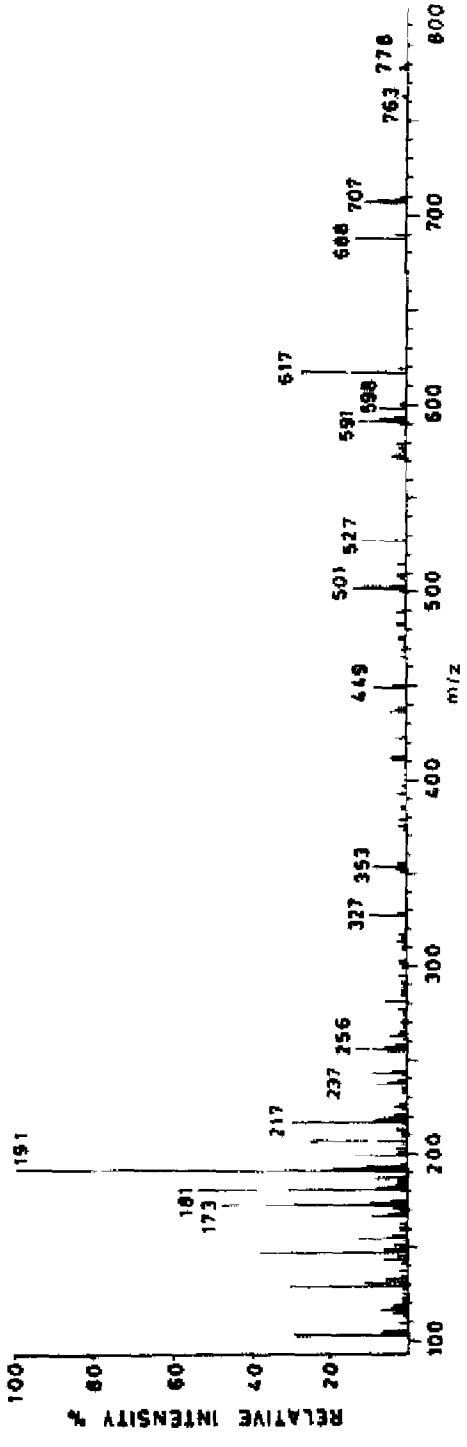

Fig. 4 Mass spectrum of the PFB-TMS derivative of 2a 2b-dihomo-$PGF_{2\alpha}$

TABLE I

PARTIAL MASS SPECTRAL DATA OF PROSTAGLANDINS AND THROMBOXANE B_2 AS PFB-TMS AND PFB-MO-TMS DERIVATIVES

Fragment	*$PGF_{2\alpha}$*		*PGE_2 minor isomer*		*PGE_2 major isomer*		*PGD_2*		*TXB_2*		*6-keto-$PGF_{1\alpha}$*		*2a,2b-dihomo-$PGF_{2\alpha}$*	
	m/z	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*
$[M]^+$	750	2	705	4	705	3	705	4	795	< 1	795	5	778	2
$[M - 15]^{+*}$	735	2	690	11	690	4	690	3	780	1	780	10	763	1
$[M - 31]^{+**}$			674	15	674	15	674	29	764	6	764	44		
$[M - 71]^{+***}$	679	14	634	63	634	< 1	634	100	724	1	724	20	707	11
$[M - 90]^{+\S}$	660	16	615	11	615	9	615	< 1	705	< 1	705	8	688	14
$[M - (31 + 90)]^+$			584	29	584	6	584	7	674	10	674	52		
$[M - (71 + 90)]^+$	589	33	544	100			544	38	634	2	634	24	617	28
$[M - (2 \times 90)]^+$	570	10	525	4					615	3	615	22	598	8
$[M - (71 + 173)]^{+\S\S}$					461	67								
$[M - (71 + 2 \times 90)]^+$	499	12	454	20			454	1	544	2	544	100	527	12
$[M - (173 - 307)]^{+\S\S\S}$					225	100								
									301†	100				

* Loss of CH_3 group

** Loss of CH_3O

*** Loss of C_5H_{11}

§ Loss of TMS–OH

§§ Mass of 173 is equivalent to $CH_3ON = C\text{–}CH_2\text{–}CH\text{–}OTMS\urcorner^+$

§§§ Mass of 307 is equivalent to top chain

† Equivalent to $TMSO^+ = CH\text{–}CH = CH\text{–}CHOTMS\text{–}C_5H_{11}$

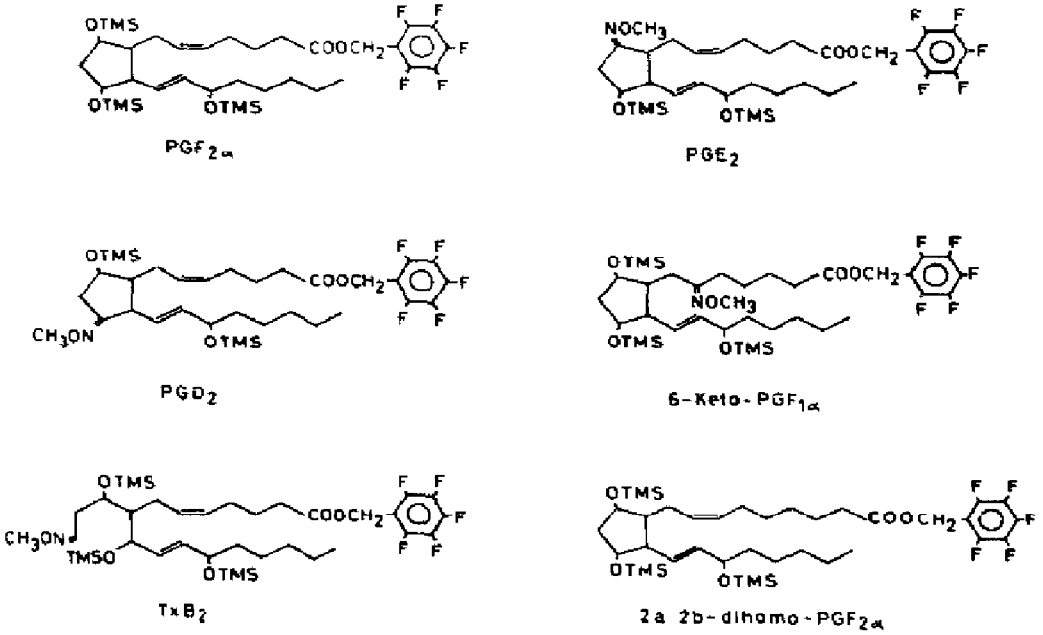

Fig 5 Structures of PFB-MO-TMS and PFB-TMS derivatives of prostaglandins and TXB_2

specific and sensitive responses are obtained when the SIM technique is used

A typical SIM analysis of authentic PGs and TXB is shown in Fig 6

Finally, as previously pointed out for 6-keto-$PGF_{1\alpha}$[5], these derivatives are stable for months when kept in bis(trimethylsilyl)trifluoroacetamide (BSTFA) solution,

Capillary column properties

Columns were characterized with respect to the isothermal separation of tetracosane at 180°C, revealing a typical theoretical plate efficiency of about 1900 plates per metre A TZ parameter of 7 3 was obtained by injecting tetradecane and pentadecane No significant drop in column efficiency was noted after months of daily use

These columns proved particularly suitable for prostaglandin analysis, since complete separation of all the major metabolites of arachidonic acid via the cyclooxygenase pathway can be obtained in a relatively short time (Fig 7) The composition of the stationary phase mixture used for column coating seems to be critical for the separation of all the compounds, and columns prepared with less polar phase mixtures did not completely separate $PGF_{2\alpha}$-PFB-TMS from the PGE_2-PFB-MO-TMS minor isomer

Interesting features of the method described are the constant and reproducible chromatographic properties of columns and the short time required for column preparation in that no time-consuming deactivation steps are required

Electron-capture detection

As we previously demonstrated for 6-keto-$PGF_{1\alpha}$, HRGC–ECD can be suc-

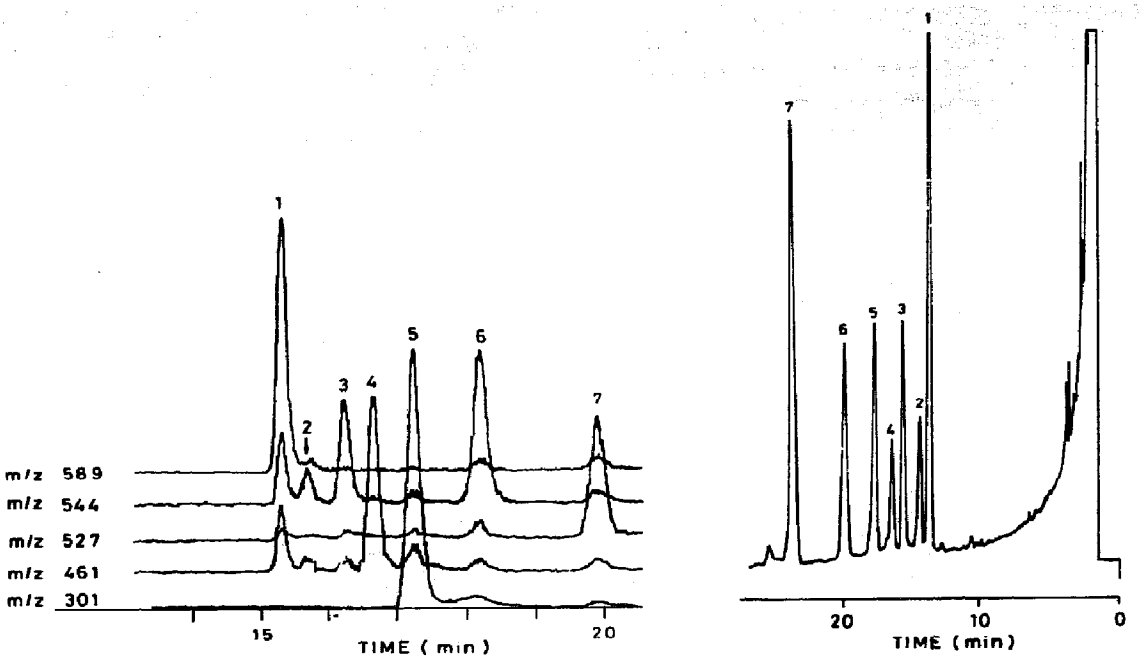

Fig. 6. Selected ion monitoring of (1) $PGF_{2\alpha}$, (2) PGE_2 minor isomer, (3) PGD_2, (4) PGE_2 major isomer, (5) TXB_2, (6) 6-keto-$PGF_{1\alpha}$, (7) 2a,2b-dihomo-$PGF_{1\alpha}$. Column: 30 m OV-101–OV-17 (8:2), 220°C isothermal. Carrier gas: helium, 25 cm/sec.

Fig. 7. HRGC–ECD separation. Compounds and conditions as in Fig. 6.

cessfully used for PG analysis. A typical ECD chromatogram of authentic PGs is shown in Fig. 7 HRGC–ECD is a simple, sensitive and fairly specific alternative method for PG determination in selected experimental models previously characterized by mass spectrometry. If numerous samples have to be analyzed, the combined use of HRGC–MS and HRGC–ECD may be convenient using the first technique mainly for identification work and the second for routine quantitation.

Studies in progress on the applicability of this technique to complex biological matrices show that a critical aspect of HRGC–ECD is that it requires greater purification of biological samples than HRGC–MS; this question will be discussed in detail in a subsequent paper.

ACKNOWLEDGEMENTS

This project was supported by grants from the National Research Council (CNR CT 79.01868.04 and 81.01966.04).

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