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DETERMINATION OF PROSTAGLANDINS AND THROMBOXANE AS THEIR PENTAFLUOROBENZYL-TRIMETHYLSILYL DERIVATIVES BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

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SUMMARY

The optimization of the parameters affecting the chromatographic properties and separation of prostaglandin pentafluorobenzyl derivatives by gas chromatography using electron-capture detection is described. The effects of composition and flow-rate of carrier gas, temperatures of detector and column, and nature of stationary phases on the detector response to different pentafluorobenzyl (both oxime and ester) trimethylsilyl ether derivatives of prostaglandins were systematically examined. The stability of some selected prostaglandin derivatives at —20°C was also determined. After standardizing these parameters, prostaglandins and related compounds from biological samples, e.g. semen, rat aorta, dog serum and trout gill were successfully analyzed. Identification of prostaglandins was confirmed by gas chromatography—mass spectrometry.

INTRODUCTION

Progress in the study of factors affecting the concentrations and types of prostaglandins (PGs), thromboxane (TX) and related compounds and in relating different species of PG to specific functions in tissue would be greatly facilitated by the availability of a rapid method for determining the PGs and related compounds occurring in biological tissues. Currently, radioimmunoassay (RIA), a sensitive technique, is most commonly used. However, it requires antisera for each PG, cross-reactivity is a problem and only one PG species is quantified per analysis [1]. Gas chromatography (GC) using a flame ionization detector separates and detects the major PGs and TX but lacks sufficient sensitivity to quantify the low concentrations of PGs found in most biological tissues [2]. However, using the electron-capture detector (ECD) and appropriate derivatization of PGs, a much greater sensitivity can be obtained

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[3—5]. This method is equivalent in sensitivity to GC—mass spectrometry (GC—MS) [1, 2] and should be more feasible and affordable for most research laboratories. However, this method has not yet been widely adopted because the ECD procedure requires several tedious and scrupulous multi-step preparations of sample which make quantitative recovery challenging. Secondly, preparation of a clean sample is essential for quantification from GC peaks [6]. Most importantly there is very limited information concerning the effects of operating parameters, i.e. carrier gas composition, flow-rates, detector temperature, column temperature and chromatographic stationary phases on the resolution capacity and sensitivity of the ECD to the pentafluorobenzyl derivatives of PG. This paper reports the systematic study of these parameters and describes the optimum GC conditions for better resolution and sensitivity of PG determination in biological samples. The use of this technique for profiling the PG and TX from biological tissues as confirmed by GC—MS was demonstrated.

MATERIALS AND METHODS

Methanol, ethanol, chloroform, anhydrous diethyl ether, light petroleum, hexane, sodium sulfate (Mallinckrodt, St. Louis, MO, U.S.A.); pyridine (Fisher Scientific, Pittsburgh, PA, U.S.A.); N-methyl-N-nitroso-*p*-toluenesulfonamide (Sigma, St. Louis, MO, U.S.A.); sodium borohydride ($NaBH_4$) (Alfa, Danvers, MA, U.S.A.); O-methylhydroxylamine hydrochloride, O-pentafluorobenzylhydroxylamine hydrochloride, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *tert.*-butyldimethylchlorosilane imidazole (Applied Science Labs., State College, PA, U.S.A.); pentafluorobenzyl bromide, diisopropylethylamine (Aldrich, Milwaukee, WI, U.S.A.) were used as received. The PGs and TX were gifts from Upjohn (Kalamazoo, MI, U.S.A.). 12-Hydroxyeicosatetraenoic acid (HETE) was provided by Dr. Edward Goetzl, Department of Medicine, Harvard University. The other chemicals used were analytical grade.

Stationary phases 3% SP-2100, 3% SP-2100 OH, 3% SP-2250, 10% SP-2340 (Supelco, Bellefonte, PA, U.S.A.), 15% Silar 10C, 10% EGSS-X (Applied Science Division, State College, PA, U.S.A.), 3% Dexil 300, 3% OV-1, 3% OV-101, 3% SE-30 and 3% OV-351 (Alltech Assoc., Deerfield, IL, U.S.A.) were used to test the effects of coating materials on the chromatographic resolution as well as detector performance.

Extraction of prostaglandins

Several different biological tissues were analyzed for PG. Semen was extracted as described previously [7]. The extraction procedure for the other samples was described in detail elsewhere [8]. Basically the tissue was homogenized in 0.9% saline, the homogenate was adjusted to pH 4.0—4.5 and the PGs were extracted with ethyl acetate [8]. Ten minutes equilibration time was allowed when internal standard procedure was used.

Thin-layer chromatography, using the solvent system described by Goswami and Kinsella [9], was performed to separate and purify the PG samples from tissues.

Derivatization for GC analyses

PGs and TX were esterified, oximated and silylated as described [4, 5]. A scheme of this derivatization is shown in Fig. 1. The recoveries for each step were monitored by tritium-labelled $PGF_{2\alpha}$ and PGE_2 (New England Nuclear, Boston, MA, U.S.A.).

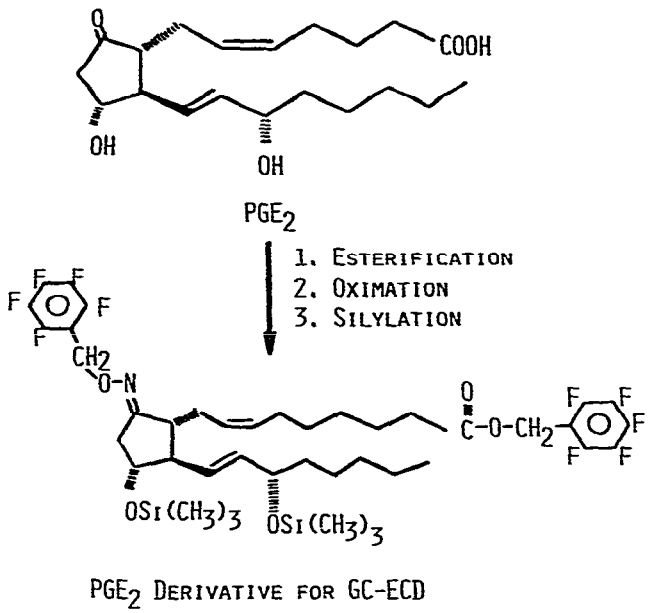

Fig. 1. Schematic illustration of the procedure for derivatization of prostaglandins and thromboxane for gas chromatography—electron-capture detection quantitation.

Gas chromatography—electron-capture detection

A Hewlett-Packard 5830A automated gas chromatograph equipped with a HP-18803A ^{63}Ni electron-capture detector was used. The retention times were automatically measured with an 18850A GC terminal. Silanized pyrex glass columns of 2.8 m × 0.32 cm and 4.7 m × 0.32 cm were used. The columns were packed with 3% OV-101 (100—120 mesh) and/or 1% SE-30 (100—120 meh) and conditioned for 24—48 h before use.

The conditions employed for the separation of the PG pentaflurobenzyl ester (PFBE) derivatives were: injector temperature 270°C, detector temperature 320°C and column temperature, unless otherwise specified, was maintained at 270°C for 10 min and then programmed at 5°C/min until 285°C. The carrier gas was set at a flow-rate of 17—20 ml/min.

Argon—methane carrier gas at either 95:5 or 90:10 ratio and nitrogen was used to test if the composition of carrier gas affected the response of the ECD.

Gas chromatography—mass spectrometry—selective ion monitoring

A Hewlett-Packard 5995A GC—MS system was used to conduct GC—MS—selective ion monitoring (SIM) analysis for the identification of PGs and hydroxy fatty acid from biological tissues. A fused silica capillary column (12

m × 0.2 mm) coated with methyl silicone (Hewlett-Packard) was used to separate the PG derivatives.

RESULTS AND DISCUSSION

Because stationary phases, column temperature, carrier gas composition, gas flow-rate and detector temperature are important parameters in determining the sensitivity and resolution of PGs for GC—ECD, we examined these parameters using a few PFBE derivatives of PG as model compounds.

GC stationary phases

The type and properties of stationary phases are critical for successful analyses of PG. Thus a series of stationary phases was tested and their suitability for PG analysis is summarized (Table I). The 3% SP-2100, 3% SP-2100 OH,

TABLE I

SUITABILITY OF STATIONARY PHASES TESTED FOR THE GC—ECD ANALYSIS OF PENTAFLUOROBENZYL DERIVATIVES OF PROSTAGLANDINS

+ = good performance; ± = intermediate; — = unsuitable.

Stationary phase	Selectivity	Stability	Temperature limit (°C)
10% SP-2340	+	±	275
3% SP-2250	—	—	250
3% SP-2100	+	+	350
3% SP-2100 OH	+	+	350
3% OV-101	—	—	275
3% OV-1	+	+	350
3% SE-30	+	+	300
3% Dexil 300	±	+	400
15% Silar 10C	+	—	275
10% EGSS-X	—	—	230

3% SE-30, 3% OV-1 stationary phases were most satisfactory for the general separations of all the PG pentafluorobenzyl derivatives. The 3% SP-2340 column was extremely good for the separation of PGF isomers. The 15% Silar 10C column, although selective for the PGF isomers, presented problems because of bleeding and contamination. Frequently, after a few runs on this column, the ECD becomes contaminated to the point where column bleeding interferes with the signal peaks. The stationary phases suitable for high-temperature runs are generally very non-polar. In order to separate derivatives differing slightly in functional structure or degree of unsaturation, capillary columns are necessary. There is a need for stationary phases which are more polar but cause less contamination of detector at high temperature.

Column temperature

To study the effect of column temperature on ketonic and non-ketonic PGs,

a 2.8 m × 0.32 cm 3% OV-101 glass column was used. The responses of the ECD to $PGF_{2\alpha}$-pentafluorobenzyl ester-trimethylsilyl (PFBE-TMS) and PGE_2-PFBE-TMS at different temperatures are shown in Fig. 2. As the column temperature was increased, the response to $PGF_{2\alpha}$-PFBE-TMS decreased slightly. The PGE_2-pentafluorobenzyl ester-pentafluorobenzyl oxime-trimethylsilyl (PFBE-PFBO-TMS) was not eluted below a column temperature of 260°C, probably because of the presence of the high-molecular-weight pentafluorobenzoxime group. From 270—285°C the ECD response to PGE_2-PFBO-PFBE-TMS increased due to the increase of volatility of the derivative. After several trials, we found that holding the column at 270°C then programming to 285°C at 5°C/min gave satisfactory resolution and a reasonably short running time of ca. 25 min.

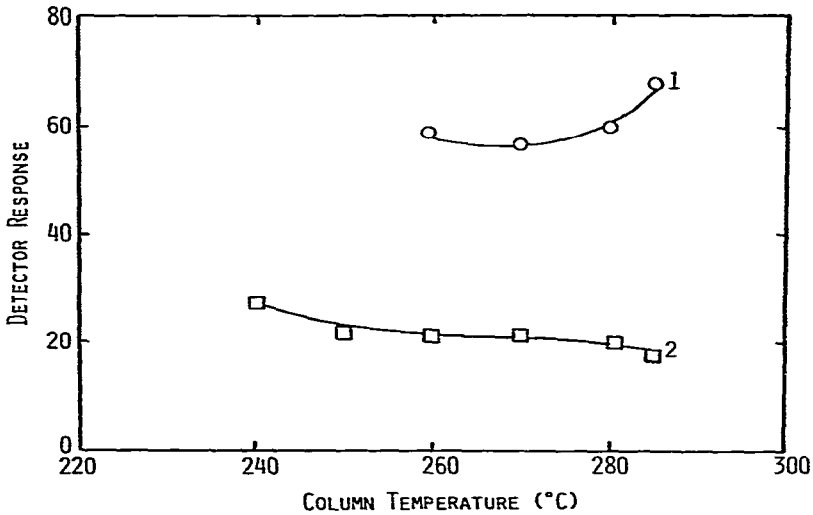

Fig. 2. The effects of column temperatures on the electron-capture detector response to PGE_2-PFBO-PFBE-TMS (1) and $PGF_{2\alpha}$-PFBE-TMS (2).

Detector temperature

The temperature dependence of ECD response to PG derivatives is represented by plotting $\ln A/n \times T^{3/2}$ versus $1/T$, where A is the peak area in arbitrary units, n the number of moles injected, and T the temperature in °K [10]. All the derivatives tested gave the same response patterns, i.e. as the detector temperature increased, the responses also increased (Fig. 3). The observed change in response is probably due to the variations of the electron absorption coefficient, k, with the temperature for each PG or TX derivative [11]. For the PFB derivatives of PG, it seems probable that the electron is initially captured by the resonance system, which has a high cross-sectional area for collisions and offers the possibility of interaction with other free electrons [10—12]. High detector temperature seems to facilitate this reaction. For highest sensitivity it is recommended that the detector temperature be set at 350°C. The maximum temperature for the Hewlett-Packard ^{63}Ni detector is 370°C [12].

From the above studies, the running conditions for optimum quantification

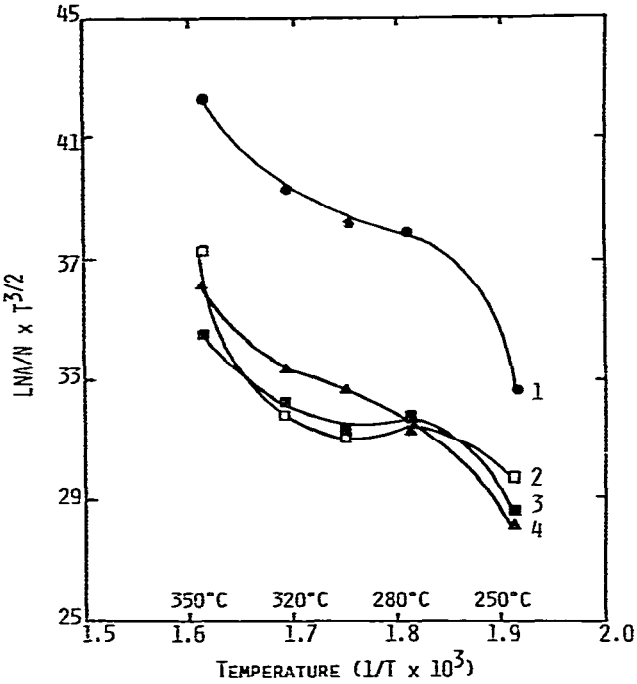

Fig. 3. The effects of detector temperatures on the ECD response to prostaglandin derivatives. (1) Cholanic acid-PFBE, (2) PGE_2-PFBO-PFBE-TMS, (3) $PGF_{2\alpha}$-PFBE-TMS, (4) PGE_1-PFBO-PFBE-TMS.

of the PG derivatives were detector temperature 350°C, flow-rate of carrier gas 16 ml/min and column temperature holding at 270°C for 10 min and then programmed at 5°C/min until 285°C when the high temperature stable non-polar stationary phases were used.

Effect of composition of carrier gas on ECD response

The effect of composition of carrier gas on ECD response to PG derivatives showed that argon—methane (95:5) resulted in slightly higher responses than did argon—methane (90:10). This is expected since the higher proportion of scavenger methane gas in the carrier gas trapped more thermal electrons [13]. Generally, the electronically excited species are deactivated by hydrocarbons and because electrons are generated in this reaction, a negative peak would result if the hydrocarbons were in the column effluent. By adding methane to the carrier gas the deactivation process in the ECD cell is continuous, and a constant number of electrons are added to the cell.

Nitrogen gas failed to give an acceptable baseline for GC—ECD analysis of PG. Theoretically the use of nitrogen as carrier gas results in an increase in the absolute sensitivity of the ECD but the noise increases proportionately so the minimum detectable level is not improved.

Effect of flow-rate of carrier gas

The gas flow-rate affected responses of ECD to different PG derivatives.

PGE_1-PFBO-PFBE-TMS, PGE_2-PFBO-PFBE-TMS and TXB_2-PFBO-PFBE-TMS all showed slight decreases in response as the flow-rate increased (Fig. 4A). However, the PGF-PFBE-TMS showed a sigmoidal type of response, i.e. at the higher and the lower flow-rates the response became drastically low and high, respectively, while at the intermediate flow-rates (10—16 ml/min) the responses of all the PGFs tested were rather constant. This phenomenon was not observed for all the keto-containing derivatives. Since the only difference between the PGEs and PGFs is the PFBO group, it is conceivable that the pentafluorobenzoxime may have stabilized the response of the ECD to change of flow-rates but the actual mechanism for the sigmoidal response of the PGF

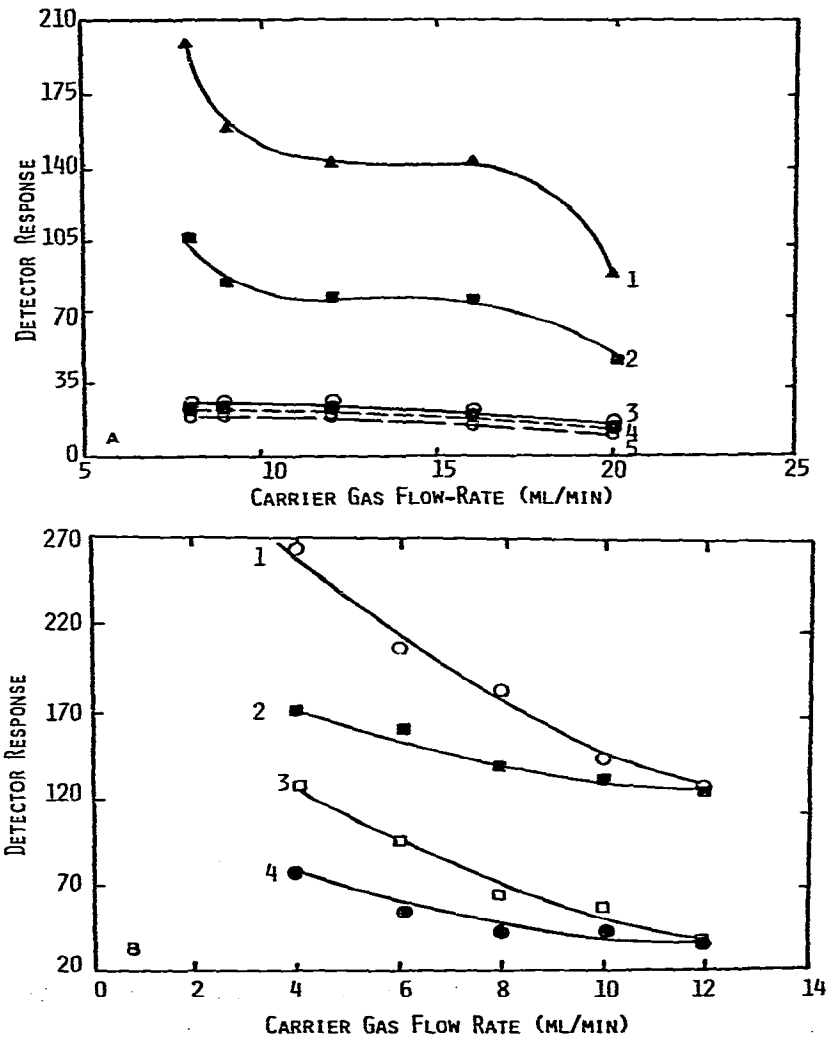

Fig. 4. The effects of carrier gas flow-rates on the ECD responses to different prostaglandin derivatives. (A) At flow-rates between 8 and 20 ml/min: (1) $PGF_{1\alpha}$-PFBE-TMS, (2) $PGF_{2\alpha}$-PFBE-TMS, (3) TXB_2-PFBO-PFBE-TMS, (4) PGE_2-PFBO-PFBE-TMS, (5) PGE_1-PFBO-PFBE-TMS. (B) At flow-rates between 4 and 12 ml/min: (1) PGE_1-PFBO-PFBE-TMS, (2) PGE_1-MO-PFBE-TMS, (3) $PGF_{2\alpha}$-PFBE-TMS, (4) cholanic acid-PFBE.

derivatives as the flow-rate changed is not understood. In general, at lower flow-rates there was a tendency for the ECD responses to increase. The ECD responses to other PG derivatives at slower flow-rates were plotted in Fig. 4B. These data show that as the flow-rates decreased, the ECD responses to all the derivatives consistently increased. Comparing responses at 4 ml/min to those at 12 ml/min, the ECD responses of $PGF_{2\alpha}$-PFBE-TMS and cholanic acid-PFBE were more than doubled.

The sensitivity of the ECD depends upon the instantaneous concentration of the sample molecules in the active sensing region of the detector cell because it is a concentration-dependent detector. Thus, the highest sensitivity is obtained at lower gas flow-rates [14]. However, at low flow-rates (<10 ml/min) increased noise levels and poor resolution are problems which affected both quantification and separation of the peaks. The best practical flow-rate was 16 ml/min.

Stability of the PG derivatives

The stability of PG derivatives is an important criterion in deciding which derivatives to use for GC analysis of PG from biological samples. Most of the PG derivatives tested were fairly stable (Fig. 5). The estimated half-lives ($t_{1/2}$) for $PGF_{2\alpha}$-PFBE-TMS, PGE_2-PFBO-PFBE-TMS and PGA_2-PFBO-PFBE-TMS were 30, 20 and 14 days, respectively. In general, the PGF-PFBE-TMSs were more stable than the keto-containing PG-PFBO-PFBE-TMSs.

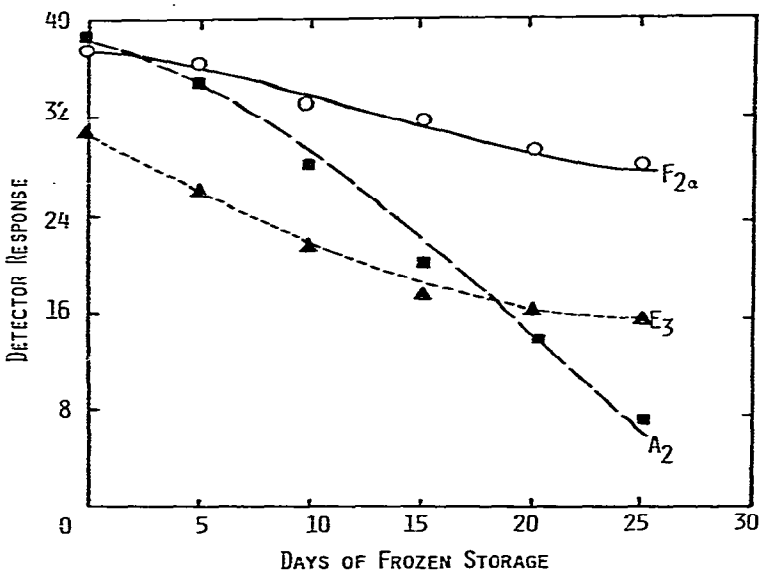

Fig. 5. The relative stabilities of PG-PFBE-TMS or PG-PFBO-PFBE-TMS derivatives during storage at −20°C.

Stability of the PG derivatives for GC detection has rarely been determined. Miyazaki et al. [15] reported that the stability of the dimethylethylsilyl ether of the $PGF_{2\alpha}$ methyl ester was better than the commonly used trimethylsilyl ether of $PGF_{2\alpha}$ methyl ester. The other advantage of dimethylethylsilyl ether

derivatives as claimed by the authors was that they give more characteristic fragmentation peaks upon mass spectroscopic analysis than the trimethylsilyl derivatives. However, the dimethylethyl silylating reagent is not commercially available.

Recently we have tried a more stable *tert.*-butyldimethylsilyl (tBDMS) ether derivative (1000 times more stable than TMS ether) for GC—ECD determination. While these were much more stable to hydrolysis the PFBE-(PFBO)-tBDMS ethers required much higher column temperature for successful GC—ECD separation.

The linearity of the detector response

The detector responses to $PGF_{2\alpha}$-PFBE-TMS at concentrations from 50 pg to 1000 pg were linear. This mass range encompasses the concentration of PGs and TX commonly found in most biological samples.

Standard curves for different PG derivatives

For quantification of PG from biological tissues, standard curves for known amounts of various PG derivatives were constructed. The area responses were plotted against the amounts used for derivatization, i.e. 0.2—10 ng. Except for the $PGF_{2\alpha}$-PFBE-TMS which gave lower response, the responses to other derivatives showed the same trend as reported earlier by Fitzpatrick et al. [16] using a glass capillary column coupled to the ECD. The lower response of $PGF_{2\alpha}$-PFBE-TMS may be related to the temperature used in the study which was 270°C instead of 250°C as used by Fitzpatrick et al. [16]. The relatively low response to TXB_2-PFBO-PFBE-TMS was expected because alkaline conditions (which favor the quantitative formation of PFBE) tend to open the acetal ring on the TXB_2 [5]. The responses of PGB-PFBO-PFBE-TMSs were also relatively low. Prostaglandin Bs have inherent electron-capturing properties and have been used for electron-capture detection after being derivatized to their methoxime methyl ester TMS ethers [17]. A derivatization of a compound having an inherent electron-capturing property with pentafluorobenzyl bromide may reduce rather than enhance the response to the ECD [18]. This was also observed by Fitzpatrick et al. [5], i.e. >400 pg of PGB_1-PFBO-PFBE-TMS and PGB_2-PFBO-PFBE-TMS are required for electron-capture detection. For some other PG derivatives, concentrations as low as 30 pg can be detected, e.g. PGA_1, PGD_2, PGE_1 and 15-keto-$PGF_{2\alpha}$.

A tabulation of response factors for quantitative work using internal standard procedure is included (Table II). Both 1a,1b-dihomo-$PGF_{2\alpha}$ and cholanic acid are appropriate internal standards to use.

The *syn*- and *anti*-isomers of all the PFBO-PFBE-TMS derivatives were resolved on the chromatogram except for PGBs and 6-keto-$PGF_{1\alpha}$ where the two isomers emerged as a single peak. The ratios of the first isomer to the second isomer are given in Table III. Except for the PGA derivatives, the ratios were very reproducible between the OV-101 and SE-30 columns over a range of 200 pg to 7.5 ng of sample injected. The PGE_3 derivatives gave three peaks and in this case the ratio was obtained by arbitrarily assigning the first peak to be one unit.

Since no GC method, so far, can satisfactorily separate all the PG derivatives

TABLE II

RELATIVE RESPONSE FACTORS OF SOME PENTAFLUORO DERIVATIVES OF PG COMPARED TO THE DERIVATIVES OF 1a,1b-DIHOMO-PGF$_{2\alpha}$ ON TWO STATIONARY PHASES, 3% OV-101 AND 1% SE-30, RESPECTIVELY

Columns used: 3% OV-101, 100—120 mesh, 2.8 m × 0.32 cm glass column; 1% SE-30, 100—120 mesh, 4.7 m × 0.32 cm glass column. GC conditions: 285°C isothermal, argon—methane (95:5) at 20 ml/min, detector temperature, 300°C. Amounts injected: 500 pg for each PG.

PG compounds	OV-101	SE-30
A_1	1.17, 1.40	0.74, 0.88
A_2	1.10, 1.30	0.70, 0.82
B_1	1.75	1.10
B_2	1.70	1.07
D_2	1.32, 1.51	0.85, 0.95
E_1	1.38, 1.64	0.88, 1.03
E_2	1.81, 1.54	0.82, 0.96
E_3	1.09, 1.30, 1.52	0.74, 0.83, 0.96
$F_{1\alpha}$	0.67	0.45
$F_{2\alpha}$	0.61	0.41
6-Keto-$F_{1\alpha}$	1.62	1.00
15-Keto-$F_{2\alpha}$	1.35, 1.44	0.86, 0.91
13,14-Dihydro-15-keto-$F_{2\alpha}$	1.38, 1.45	0.88, 0.92
TXB_2	1.79, 1.93	1.11, 1.18

TABLE III

RATIO OF *syn*- AND *anti*-ISOMERS OF SOME PENTAFLUORO DERIVATIVES OF PG SEPARATED BY GAS CHROMATOGRAPHY ON COLUMNS CONTAINING 1% SE-30 AND 3% OV-101

PG derivatives*	Ratio of first peak vs. second peak	
	1% SE-30	3% OV-101
A_1	1.30 ± 0.23	3.67 ± 0.38
A_2	1.65 ± 0.30	2.70 ± 0.47
D_2	0.25 ± 0.01***	0.26 ± 0.01
E_1	0.40 ± 0.03	0.42 ± 0.05
E_2	0.42 ± 0.00	0.47 ± 0.03
E_3**	1:3.33:6.55 ± 0.10 ± 0.12	1:3.41:6.02 ± 0.60 ± 0.78
15-Keto-$F_{2\alpha}$	0.45 ± 0.03	0.44 ± 0.02
13,14-Dihydro-15-keto-$F_{2\alpha}$	0.97 ± 0.08	0.97 ± 0.08
TXB_2	0.56 ± 0.03	0.40 ± 0.10

*Pentafluorobenzyl oxime pentafluorobenzyl ester trimethylsilyl ether derivatives.
**Three instead of two isomers were observed. The exact structure of each isomer has not yet been identified.
***Mean ± S.D. from four samples of different concentration ranged from 7.5 ng to 950 pg.

and their isomers, knowing the ratio of the *syn*- and *anti*-isomers can help in calculating the individual amount of PG in a merged peak. A typical application of this calculation can be demonstrated clearly in Fig. 6. In Fig. 6A the complete separation of the $PGF_{1\alpha}$,$PGF_{2\alpha}$-PFBE-TMS and PGE_1,PGE_2-PFBO-PFBE-TMS and TXB_2-PFBO-PFBE-TMS is shown. On the upper right corner of Fig. 6A, the retention time of 6-keto-$PGF_{1\alpha}$-PFBO-PFBE-TMS is given as 20.13 min. This peak eluted after the second isomer of PGE_2 and before the second isomer of PGE_1. However, this peak of 6-keto-$PGF_{1\alpha}$ emerged coincidentally with the second isomer of PGE_1 as shown in Fig. 6B. With the information given in Table II, one can calculate the quantity of 6-keto-$PGF_{1\alpha}$ in the merged peaks by subtracting the amount of the second isomer of PGE_1 as calculated from the first PGE_1 isomer.

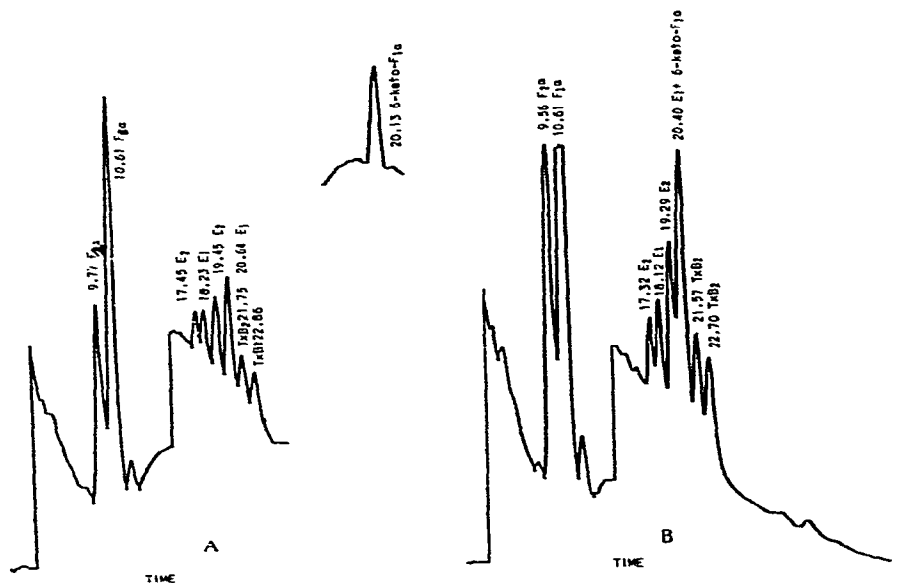

Fig. 6. Examples of separation of some major prostaglandins and thromboxane standards. Column: 2.8 m × 0.32 cm, 3% OV-101 (100—120 mesh) column. (A) $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_1, PGE_2 and TXB_2 were well resolved. (B) Merging of 6-keto-$PGF_{1\alpha}$ with the second isomer of PGE_1.

Analyses of PGs and TX from biological samples

After we developed and standardized the GC—ECD procedures, we analyzed the PGs, HETE and TX extracted from human semen, dog serum, trout gill and rat aorta. Each of the organs contain different levels and types of PGs so they were useful in evaluating the applicability of the GC—ECD system to biological samples generally. Preliminary identification of PGs was done by comparing the retention times of the suspected PGs with those of the authentic PGs. Confirmation of the identity of specific PGs was achieved by GC—MS—SIM.

Identification of PG by GC—MS—SIM

The PGs present in the biological samples were derivatized into either methyl

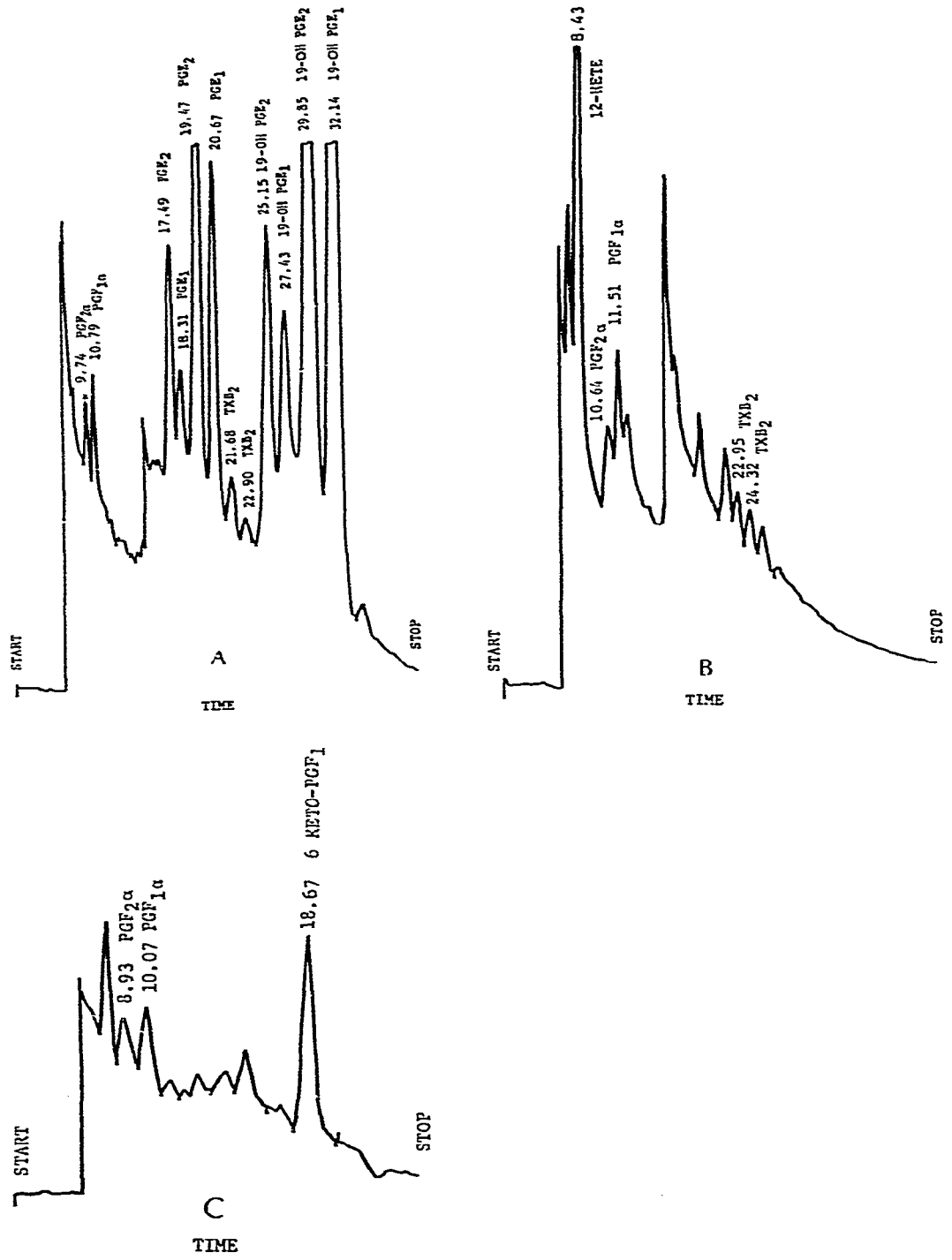

Fig. 7. (A) Separation of derivatives of human semen prostaglandins and thromboxane by GC—ECD. (B) GC—ECD profile of prostaglandin derivatives prepared from dog serum. (C) Prostaglandins from extract of rat aorta incubation. 2.8 m × 0.32 cm, 3% OV-101 (100—120 mesh) glass columns. Conditions are detailed in the text.

ester (methoxime) trimethylsilyl ether or methyl ester (methoxime) *tert.*-butyldimethyl ethers and identified by GC—MS—SIM technique. The details of this GC—MS—SIM technique are described elsewhere [19,20].

Semen prostaglandins. Seven species of PGs and TX are found in human semen (Fig. 7A). These PGs are $PGF_{2\alpha}$, $PGF_{1\alpha}$, PGE_1, TXB_2, 19-OH-PGE_2 and 19-OH-PGE_1. The ratios of PGE_2 to PGE_1 and 19-OH-PGE_2 to 19-OH-PGE_1 were 1.7 and 1.0, respectively. PGFs and TXB_2 were comparatively minor components in human semen.

Dog serum prostaglandins. PGs were extracted from dog serum, derivatized and resolved by GC—ECD (Fig. 7B). The most abundant peak was 12-HETE tentatively identified from an authentic standard. Also identified were $PGF_{2\alpha}$, $PGF_{1\alpha}$ and TXB_2. No significant PGE_2 peaks were observed. Several unknown peaks were observed.

Rat aorta prostaglandins. A significant amount of 6-keto-$PGF_{1\alpha}$ was extracted from rat abdominal aorta after 15 min incubation in 0.05 *M* phosphate buffer (pH 7.4) (Fig. 7C). $PGF_{2\alpha}$ and $PGF_{1\alpha}$ were also identified.

Trout gill. $PGF_{3\alpha}$ was the major PG found in trout gill tissue followed by PGE_3 (Fig. 8A). A chromatogram for authentic standard is also provided for comparison (Fig. 8B). We also found an unknown peak (retention time 14.25 min) which eluted between $PGF_{3\alpha}$ and PGE_3. Using the GC—MS procedure, we further determined the structure of this compound as a PG containing four

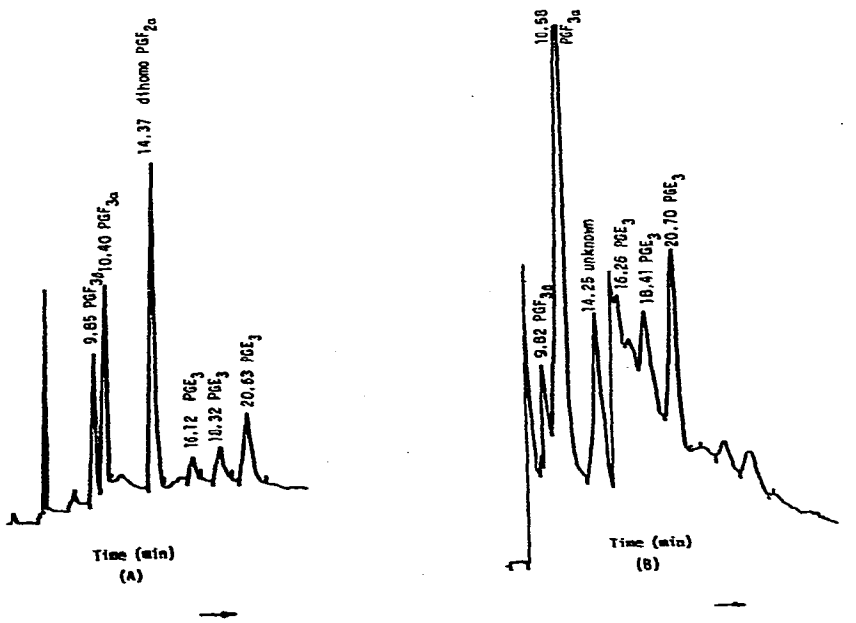

Fig. 8. Separation of prostaglandin derivatives from trout gill. (A) Authentic PG standards; (B) trout gill PG extract.

double bonds tentatively identified as C_{22}-$PGF_{4\alpha}$ [21], the first report of a PG with four double bonds.

This paper demonstrates the systematic calibration of GC—ECD and the successful use of this method for determining PGs from biological samples containing different concentrations and types of PGs. The technique should greatly facilitate the routine quantification of PG from biological materials and is being used in our laboratory for studying the effects of dietary *trans*-linoleic acid on the production of PGs and TX in different organs of rats.

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