Journal of Chromatography, 130 (1977) 65–76
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CHROM. 9483

PROFILES OF PROSTAGLANDINS A, B, E AND F (SERIES I AND II) OBTAINED BY GAS CHROMATOGRAPHY WITH MULTIPLE-ION DETECTION*

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(Received April 13th, 1976)

SUMMARY

A new method is presented for the simultaneous one-step derivatization of prostaglandins A_1, A_2, B_1, B_2, E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ with N,O-bis(trimethylsilyl)trifluoroacetamide–piperidine. The procedure provides stable and reproducible trimethylsilyl derivatives that show good chromatographic properties on OV-17 and OV-225 columns, and precludes any significant degree of interconversion of the prostaglandins. The prostaglandins F, B and E are determined as the corresponding tetrakis(trimethylsilyl), bis(trimethylsilyl) and 9-enol-tetrakis(trimethylsilyl) derivatives, respectively. The enolization of prostaglandins E proceeds quantitatively only if piperidine is added to the acetamide. This mixture also produces the novel derivative 9-piperidyl-tris(trimethylsilyl)prostaglandin A. Evidence is also presented on the quantitative injection losses due to the type of syringe used (1 μl or 10 μl). A modified extraction procedure has been applied to samples of human seminal fluid. The procedure, which involves the centrifugation of the samples, allows the recovery of all of the prostaglandins in one fraction, which is then derivatized and screened by combined gas–liquid chromatography–multiple-ion detection techniques in order to identify the individual compounds.

INTRODUCTION

The wide distribution of prostaglandins (PG) in animal tissues, together with their potent pharmacological effects, suggest that these compounds have a number of physiological roles[1] in different organ and cell systems. The understanding of these roles is based on studies of the biosynthesis and metabolism of this family of closely related lipid acids. From an analytical point of view, these types of study are usually limited to highly sensitive, precise and specific methods of detection, because of the low concentrations of prostaglandins in tissues, body fluids or homogenates[2,3].

* Presented at the 11th International Symposium on Chromatography, Warwickshire, July 5–9, 1976. A summary of the present paper and the majority of the other papers presented at this symposium have been published in *J. Chromatogr.* ,Vol. 122 (1976).

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Spectrophotometric, enzymatic and fluorimetric methods are not sufficiently sensitive and/or reproducible as well as specific[1,4–8] for the determination of these compounds in biological samples. On the other hand, in spite of the high sensitivity and simplicity attributed to the radioimmunoassay techniques for prostaglandins, these methods are not absolutely specific because of the possibilities of immunological cross-reactions or interferences due to unknown immuno-reactive compounds[8].

Although in some cases the fluorimetric and radioimmunoassay techniques may provide sufficient sensitivity and specificity for determination of the prostaglandins as a group, they do not readily differentiate the individual compounds and metabolites because the properties of the compounds are so similar. This is a considerable drawback compared to the possibility of obtaining a qualitative and quantitative determination of characteristic "metabolic profiles", as provided by the gas–liquid chromatographic (GLC)[9–12] and combined GLC–mass spectrometric (MS) methods[13–17]. However, although various derivatization methods have been described for the GLC of prostaglandins[10–13,17], they involve long reaction times[11,13], and in some cases more than one chromatographic peak is formed[13,16]. Some of the methods described rely on an indirect estimation of some of the prostaglandins after their conversion into another member of the same family[13,17]. These structural modifications are often confusing in regard to the actual endogenous levels of the different prostaglandins. Also, extraction methods are still rather elaborate and time-consuming[12,18,19].

This paper describes the kind of results obtained when attempting to develop a simple integrated method for the analysis of prostaglandins. At its present stage of development, the method involves a substantial simplification of the derivatization and extraction procedures, together with sensitive and highly specific GLC–multiple-ion detection (MID) techniques. The derivatization procedure yields novel derivatives for prostaglandins of type A whose MS characteristics are especially suitable for MID.

EXPERIMENTAL

Reagents

Ethyl acetate, methanol, carbon tetrachloride, piperidine, toluene, acetone, hexamethyldisilazane (HMDS) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (chromatography grades) were used as described below. The prostaglandin samples (A, B, E and F; series I and II) were kindly supplied by Dr. J. Pike, Upjohn, Kalamazoo, Mich., U.S.A. and Ono Pharmaceutical Co., Osaka, Japan (A_2, E_1 and E_2).

Instrumentation and conditions

Gas–liquid chromatography. The glass columns (2 m × 2.5 mm I.D., and 3 m × 2.5 mm I.D.) were washed with acetone, methanol, water and methanol and silanized for 24 h with a 5% solution of (HMDS) in toluene. After washing with methanol, they were dried and immediately packed with OV-17 (3%) or OV-225 (5%) on Gas Chrom Q (100–120 mesh). These columns were usually conditioned for at least 48 h at 250–300°, and were silanized by repeated injections of BSTFA before use. The separations were achieved on a Perkin-Elmer Model 900 gas chromatograph equipped with dual flame ionization detectors. Samples were injected by use of Hamilton syringes, Models 701 (10-μl capacity) and 7101 (1-μl capacity).

Gas chromatography–mass spectrometry. The mass spectra of authentic prostaglandins, as well as those of their corresponding derivatives, were recorded on a Hitachi RMU-6H mass spectrometer equipped with a standard solids probe and coupled, through a single-stage gold-jet-type separator, to a Perkin-Elmer Model 3920 gas chromatograph. Mass spectra were recorded under the following conditions: chamber voltage, 70 eV; emission, 80 μA; trap current, 55–60 μA, acceleration voltages, 1800 and 1200 V, depending on the mass range covered; ion-source temperature, 180–200°. The GC columns used were the same as those used for the gas chromatographic separations.

Procedure

Preparation of derivatives. All of the prostaglandins (A, B, E and F; series I and II) were derivatized in one step with BSTFA–piperidine (1:1). 50–200 μl of the stock solutions of the prostaglandins in ethanol (500 ng/μl) were evaporated to dryness and allowed to react for 1 h at 60° with 40 μl of the BSTFA–piperidine mixture.

Extraction of prostaglandins from semen. 5–10 ml of pooled human seminal plasma obtained from the Instituto Urologico Puigvert were filtered through a Millipore "Pellicon" membrane (diameter, 25 mm; Type PSAC; mol. wt., 1000). The filtrate was mixed with half its volume of carbon tetrachloride in order to remove the non-polar lipid fraction. After 3 min the mixture was centrifuged at 17,500 *g*, thus separating the aqueous supernatant from the solid pellet which formed on addition of the carbon tetrachloride. The pellet was re-extracted twice and the combined extracts were acidified with HCl to pH 3. The resulting solution was extracted three times with half its volume of ethyl acetate and then centrifuged at 17,500 *g* for 10 min. The ethyl acetate supernatant was mixed with 200 μl of ammonia (1 *N*) and 2 ml of methanol in order to prevent the decomposition of the prostaglandins during subsequent evaporation[12] to dryness on a rotary film evaporator at 40°.

RESULTS AND DISCUSSION

Derivatization kinetics and stability of the derivatives

As shown in Fig. 1, the reaction between $PGF_{1\alpha}$ and the BSTFA–piperidine mixture was very rapid, the maximum yield being obtained in *ca.* 1 h at 60°. This graph illustrates the work carried out to optimize the GC response of each of the fully trimethylsilylated prostaglandins. In fact, the data points for $PGF_{2\alpha}(TMS)_4$ are not shown because they were essentially coincident with those of the $PGF_{1\alpha}(TMS)_4$ derivative (Fig. 1).

The increase in the yields of the derivatives levelled off after the first 60–90 min, the derivatives thus formed being stable for at least 40 h, as shown by the chromatograms in Fig. 2. The upper right-hand profile (A) represents the response obtained by injecting a sample which had been kept for 40 h without any special precautions to preserve it other than storing it in a refrigerator at 4° when it was not being used for a sequential series of injections. In another series of injections the ratio n-C_{24}:$PGF_{2\alpha}$-$(TMS)_4$ was calculated as 0.65:1 after 3 h and 0.64:1 after 6 and 10 h. It has been reported that the methyl TMS derivatives of prostaglandins A and B are stable at 0° for several months[13].

TABLE I

PARTIAL MASS SPECTRA OF PROSTAGLANDIN DERIVATIVES

M = Molecular ion; $M-15$, loss of CH_3; $M-29$, loss of CHO; $M-71$, loss of C_{16}–C_{20}; $M-90$, loss of $HOSi(CH_3)_3$; $M-99$, loss of C_{16}–C_{20} with abstraction of CO; $M-105$, loss of $HOSi(CH_3)_3$ and CH_3; $M-131$, loss of $CH_2COOSi(CH_3)_3$; $M-161$, loss of C_{16}–C_{20} and $HOSi(CH_3)_3$; $M-180$, loss of 2 $HOSi(CH_3)_3$ groups (see refs. 10, 20 and 21). The base peak in the spectra of the derivatives of $F_{1\alpha}$ and $F_{2\alpha}$ appears at m/e 191 and 129 respectively. The base peak of $PGA_2(TMS)_2$ appears at m/e 190.

$PGA_1(TMS)_2$, $PGB_1(TMS)_2$				$PGA_2(TMS)_2$, $PGB_2(TMS)_2$			$PGF_{1\alpha}(TMS)_4$		$PGF_{2\alpha}(TMS)_4$, 9-enol-$PGE_1(TMS)_4$			9-enol-$PGE_2(TMS)_4$		$PGA(PIP)(TMS)_3$				
		%			%					%						%		%
	m/e	A_1*	B_1	m/e	A_2*	B_2	m/e	%	m/e	F_2	E_1	m/e	%		m/e	A_2	m/e	A_1
M	480	17	48	478	16	32	644	0.2	642	<5	5	640	26	M	635	2.4	637	3.2
$M-15$	465	22	34	463	30	39	629	6	627	11	31	625	36	$M-15$	620	3.3	622	3.0
$M-29$	451	–	19	449	–	7	–	–	–	–	–	–	–	$M-71$	564	1.2	566	1.2
$M-71$	409	100	38	407	65	20	573	26	571	11	5	569	<5	$M-85$	550	0.5	552	0.5
$M-90$	390	6	8	388	42	25	554	39	552	32	100	550	86	$M-89$	546	0.6	548	0.5
$M-99$	381	57	100	379	57	100	–	–	–	–	–	–	–	$M-173$	462	100	464	100
$M-105$	375	11	11	373	28	10	539	18	537	17	17	535	41	$M-199$	436	3.3	438	1.5
$M-131$	–	–	–	–	–	–	513	18	511	10	–	–	–					
$M-161$	319	68	4	317	91	17	483	55	481	48	38	479	63					
$M-180$	–	–	–	–	–	–	464	21	462	33	49	460	100					

* Derivatized with BSTFA only (without piperidine) for 3 h at 50° according to ref. 11.

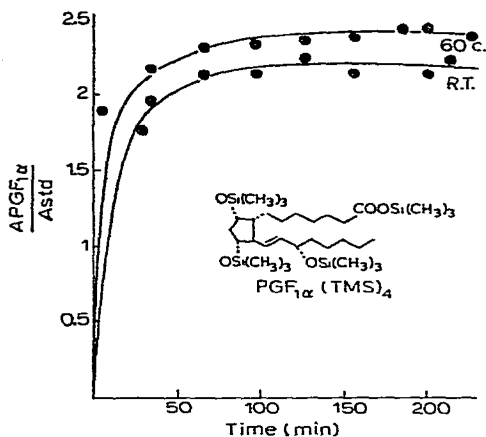

Fig. 1. Kinetic curves representative of the time course of the reaction between $PGF_{1\alpha}$ and a BSTFA–piperidine mixture at room temperature (R.T.) and 60°. (The reaction vials were always kept at constant temperature.) The ordinate represents the ratio between the area under the peak due to $PGF_{1\alpha}(TMS)_4$ and the area under the peak due to the n-C_{24} internal standard.

Structures of the derivatives

The structures of these derivatives were verified by combined GLC–MS. Table I summarizes the relative abundances of the most characteristic high-mass ions observed in each spectrum. The mass spectral patterns agree with those predicted from the electron-impact fragmentation of these compounds, with two significant exceptions. On one hand the spectra of the PGE_1 and PGE_2 derivatives did not show their respective molecular ions at m/e 570 and 568 corresponding to the expected $PGE_1(TMS)_3$ and $PGE_2(TMS)_3$ derivatives. Instead, as shown in Table I, these PGE

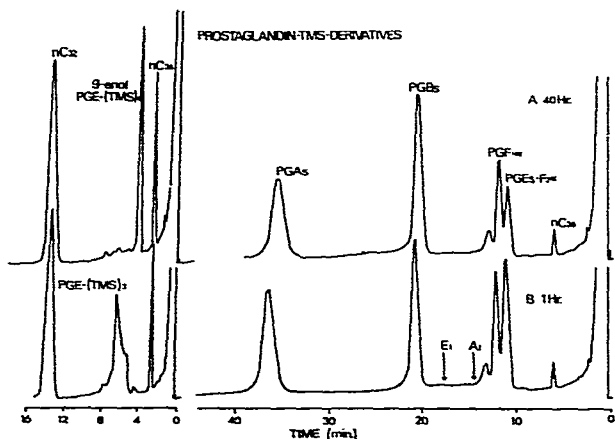

Fig. 2. Upper and lower right: GC profiles of TMS derivatives of prostaglandins obtained on 3% OV-17 (2-m glass column) at 230°; helium flow-rate, 45 ml/min; injector temperature, 280°; detector (FID) temperature, 290°. A = profile after 40 h, B = profile after 1 h. Upper and lower left: GC profiles of PGE_1 derivatives obtained on 3% OV-17 (2-m glass column) at 250°; helium flow-rate, 45 ml/min; upper trace, 9-enol-$PGE_1(TMS)_4$ derivative; lower trace, $PGE_1(TMS)_3$ derivative; n-C_{26} and n-C_{32} are the reference peaks.

TABLE II

RETENTION INDICES FOR PROSTAGLANDINS (ALL AS TMS DERIVATIVES)

Injector temperature, 250°; detector temperature, 300°; column temperature, 250°. $PGA(TMS)_2$ and $PGE(TMS)_3$ were obtained by derivatization with BSTFA (without piperidine) for 3 h at 50° as described in ref. 11. For comparison, the values given in parentheses are the figures reported in ref. 11.

Prostaglandin	*Series I*		*Series II*	
	OV-17	*OV-225*	*OV-17*	*OV-225*
9-enol-$PGE(TMS)_4$	2774*	2888	2774	2888
$PGF(TMS)_4$	2807	2936	2780	2924
$PGA(TMS)_2$	2853 (2870)			
$PGE(TMS)_3$	2924 (2960)			
$PGB(TMS)_3$	3006	3536	3006	3536
$PGA(PIP)(TMS)_3$	3185	3350	3185	3350

* See Fig. 2.

derivatives gave molecular ions at *m/e* 640 and 642, corresponding to the 9-enol-$PGE_2(TMS)_4$ and 9-enol-$PGE_1(TMS)_4$ derivatives, respectively. These structures are supported by the remaining *m/e* values (Table I). Prostaglandins B_1 and B_2 did not undergo a similar enol reaction, possibly because of the higher stability of the 9-oxo group which has two doubly conjugated double bonds in positions 8,12 and 13,14.

On the other hand, the derivatization of prostaglandins A with BSFTA–piperidine yielded a single peak which, on an OV-17 column, was eluted after the peak of the PGB derivatives (see Fig. 2), a fact evidently at variance with the retention indices reported in the literature[11,20] which indicate that the fully trimethylsilylated prostaglandins A should occur between $PGF_{1\alpha}$ and prostaglandins B (Table II). The reason for these differences was found in the mass spectra of our PGA_1 and PGA_2 derivatives which showed that we were dealing with a totally new type of derivative structure as indicated by the molecular ions appearing at *m/e* 637 and 635 respectively and the predominant ions at *m/e* 464 and 462 (Table I).

One of the most interesting features of these mass spectra are the odd-numbered molecular ions which indicate the incorporation of a nitrogen group from the piperidine of the reaction mixture. Also, the lack of a $M-99$ ion (Table I) indicates that the carbonyl group is lost in the derivatization. This observation, together with a detailed evaluation of the rest of the mass spectral pattern, leads us to propose that the structures of these novel derivatives correspond to the TMS esters of 9-piperidyl-9,15-bis(trimethylsiloxy)-10,13-*trans*-prostadienoic acid, $PGA_1(PIP)(TMS)_3$, and 9-piperidyl-9,15-bis(trimethylsiloxy)-5-*cis*-10,13-*trans*-prostatrienoic acid, $PGA_2(PIP)(TMS)_3$, respectively. A full account of these derivatives will be published elsewhere. It is noteworthy that the exclusion of piperidine from the reaction mixture leads to the expected PGA_1 and PGA_2 $(TMS)_2$ derivatives[11,20,21] as indicated by their mass spectral patterns (Table I) and retention indices (Table II). Piperidine also plays a decisive role in the enolization of prostaglandins E in high yields as shown in Fig. 2.

In accordance with the data in Table I and disregarding the *cis* or *trans* conformation of the 1–8 chain, the structures obtained can be summarized as follows:

PG (series I):

F_1 $R_1 = Si(CH_3)_3$; $R_9 = R_{11} = CH\text{–}OSi(CH_3)_3$

E_1 $R_1 = Si(CH_3)_3$; $R_9 = COSi(CH_3)_3$; $R_{11} = CH\text{–}OSi(CH_3)_3$; $\Delta^{9,10}$

B_1 $R_1 = Si(CH_3)_3$; $R_9 = CO$; $R_{11} = CH_2$; $\Delta^{8,12}$

A_1 (derivatized with BSTFA)

$R_1 = Si(CH_3)_3$; $R_9 = CO$; $R_{11} = CH_2$; $\Delta^{10,11}$

(derivatized with BSTFA–piperidine)

$R_1 = Si(CH_3)_3$; $R_9 = COSi(CH_3)_3\text{–}C_5H_{10}N$; $R_{11} = CH$; $\Delta^{10,11}$

PG (series II):

as series I plus a $\Delta^{5,6}$ bond in each case

Fig. 3. Response curves obtained for $PGF_{2\alpha}(TMS)_4$ and 9-enol-$PGE_2(TMS)_3$. The chromatographic conditions were as indicated in Fig. 2, except for the temperature, which was 230°.

Detection limits

Response curves for $PGF_{2\alpha}(TMS)_4$ and $PGE_2(TMS)_4$ (9-enol form) are given in Fig. 3. The data show good linear responses in the 50–1000 ng range for both compounds. However, in spite of the constant conditioning and silanization of the columns with BSTFA, there were still some residual losses as indicated by the fact that both curves do not pass exactly through the origin. The lowest response obtained under these conditions corresponds to 12 ng of $PGF_{2\alpha}(TMS)_4$ and 24 ng of 9-enol-$PGE_2(TMS)_4$. It is interesting that, while investigating ways of achieving lower detection limits, it was noticed that the quantitative data appeared to be dependent to a relatively large extent on the type of syringe used to inject the derivatized samples in the gas chromatograph (Table III). The variation coefficients ($s/\bar{x}$) corresponding to 1-μl injections of a n-C_{24} alkane indicate that, as expected, the precision obtained with the 1-μl syringe (9.1% compared with 11.66% for the 10-μl syringe) was greater. However, it is striking that the variation coefficients corresponding to the $PGF_{1\alpha}$ derivative were 23% lower when using the 10-μl syringe. This difference could be interpreted by taking account of the different modes of operation of these syringes.

TABLE III

RELATIVE RESPONSE DIFFERENCES AS A FUNCTION OF SYRINGE TYPE

S = Standard deviation, $S/\bar{x}$ = variation coefficient. The number of injections is given in parentheses. The plus and minus signs in the final column indicate the deviations relative to the values obtained with the 1-μl syringe. In each case, 1 μl of the $PGF_{1\alpha}(TMS)_4$ derivative was injected.

	Hamilton 1 µl		*Hamilton 10 µl*		*Percentage difference*
	S	$S/\bar{x}$	S	$S/\bar{x}$	*in* $S/\bar{x}$
n-C_{24}	0.264 (4)	0.091	0.295 (4)	0.1166	+22
PGF_1/n-C_{24}	0.174 (5)	0.2148	0.174 (5)	0.1657	−23

In the 10-μl syringe the sample is measured and contained in the borosilicate glass barrel, while in the 1-μl syringe the sample volume is read from the glass barrel but it is contained in the metal needle. This could give rise to different kinds of interactions at the moment of introduction of the needle into the hot injection block and the subsequent pressing of the plunger to discharge the syringe contents into the injector glass liner. One series of injections was made by alternately using the 1-μl and the 10-μl syringes. In another series of injections the 1-μl syringe was used to inject samples of 0.5 μl of a $PGF_{1\alpha}(TMS)_4/n$-C_{24} mixture with and without 0.1 μl of BSTFA, average area ratios of 1.25 and 0.70:1, respectively, being obtained. A comparison of these values with that (1.36) obtained by injecting a 1-μl sample, illustrates the greater relative importance to smaller samples of the losses due to adsorption, an effect which can be partially compensated for by the presence of BSTFA.

The data points shown in Fig. 3 were obtained with the 1-μl syringe, which, according to the results given in Table III, should cause the responses of the prostaglandins to be reduced by 23%. Thus these values could be adjusted to compensate for this syringe effect. This would bring both curves closer to the origin, leaving only a small difference related to the true adsorption effects, operating at the low nanogram level[13].

Prostaglandin profiles

Table II gives the retention indices calculated on OV-17 and OV-225 columns. These values indicate that the 9-enol forms of the $PGE_1(TMS)_4$ and $PGE_2(TMS)_4$ derivatives are co-eluted in both these stationary phases. The same conclusion applies to the derivatives of prostaglandins A and B. Other stationary phases also tried include, in order of increasing polarity, SE-30, Poly I-110 and EGSS-X, which did not separate the two members of each prostaglandin group. However, the fully silylated prostaglandins F were separated on OV-17 (Fig. 2) with a ΔI value of 27, in agreement with the value of 25 reported in the literature[11]. The ΔI value on OV-225 is 32.

As shown in Fig. 2, two of the most significant features of the prostaglandin profiles are the determination of the E compounds as their 9-enol $(TMS)_4$ derivatives and the determination of the A compounds as the piperidyl TMS derivatives, both of which offer a remarkable advantage in terms of the reproducibility, stability and chromatographic behaviour of these prostaglandins.

In the lower left-hand trace in Fig. 2 appear the results obtained by derivatization of PGE_1 with BSTFA alone, as described by Middleditch and Desiderio[11].

This can be compared with the sharply defined peak of 9-enol-$PGE_1(TMS)_4$, shown in the upper left-hand trace, obtained with the BSTFA–piperidine mixture. The response shown corresponds to half the amount of PGE_1 used for the derivatization with BSTFA alone.

It has also been reported[13] that with BSTFA the methyl ester of PGE_2 gives rise to the derivatives of 9-enol-$MPGE_2$ plus MPGA as a minor component, and that with bis(trimethylsilyl)acetamide (BSA), $MPGE_2$ is transformed into $MPGB_2$ plus 10–15% of 9-enol-$MPGE_2$. However, these workers favour the determination of the methyl esters of prostaglandins E either as MPGA compounds by reaction with pyridine–BSA, in order to obtain the MPGA(TMS) derivative plus some of the corresponding 9-enol-$MPGE(TMS)_3$ derivative, or as MPGB(TMS) compounds by base-catalyzed dehydration of MPGE and reaction with BSA. In both these cases, especially in the latter, the procedures are rather time-consuming. On the other hand, Nicosia and Galli[17] described the use of piperidine in conjunction with trimethylsilyl-imidazole, reporting an instantaneous transformation of MPGE compounds into MPGB(TMS) derivatives.

We have found that it is more convenient to use piperidine with BSTFA as the reaction mixture since this produces the enol of prostaglandins E almost in quantitative yields, thus avoiding the transformation of these compounds into either prostaglandins A or B. This method also provides a novel derivative which is exclusively selective for PGA_1 and PGA_2. Apart from the interesting reaction mechanism which will be reported in detail elsewhere, the $PGA(PIP)(TMS)_3$ derivative extends the range of retention index values of prostaglandins A from 2853 to 3185 (Table II). This may be very convenient in avoiding interferences when analyzing biological extracts.

Extraction of prostaglandins

A detailed review and evaluation of several of the multistep extraction procedures described in the literature[6,12,17–19,22,23], together with a consideration of our results of the experimental application of two of the procedures reported for plasma and rat renal papilla homogenates[12,23], induced us to explore the possibilities of simplifying the method of extraction in order to reduce the amount of sample manipulation to a minimum. We verified that most of the currently applied methods make use of various solvent extraction steps combined with column chromatographic fractionations on silica gel or Sephadex LH-20. The method that appeared best suited to our aims was that of Sugiura and Hirano[12]. However, we found that the initial deproteinization and filtration steps were rather cumbersome and required our constant attention. In contrast, the centrifugation of the sample, which is may be left unattended, provides a very convenient and trouble-free way of obtaining a clear filtrate. The filtrate can then be directly extracted with carbon tetrachloride, thus eliminating the usual lengthy filtration of precipitates and evaporation to dryness before the carbon tetrachloride extraction (see Experimental Section).

Fig. 4 shows the profile of an extract of human seminal plasma, obtained according to our method. The chromatographic positions corresponding to the various prostaglandin peaks are identified in the biological profile (Fig. 4C) by comparison with a synthetic profile (Fig. 4A) and by co-injection of the authentic prostaglandins (Fig. 4B).

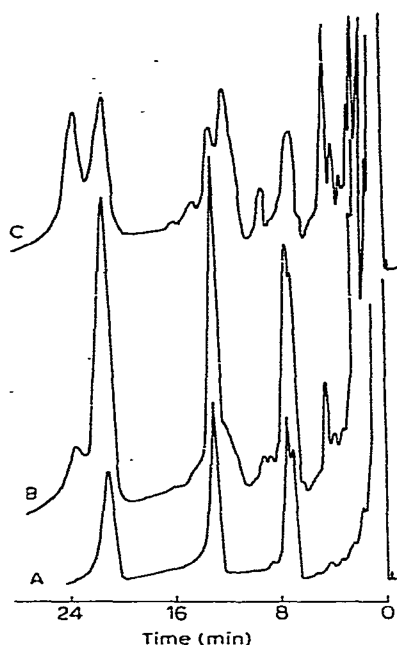

Fig. 4. GC profiles of (A) a synthetic mixture of prostaglandins A, B, E and F (series I and II), (B) a human seminal plasma extract co-injected with the synthetic mixture (A) and (C) a human seminal plasma extract. Conditions: 3% OV-17 on Gas Chrom. Q (100–120 mesh); glass column (2 m × 2.5 mm I.D.) at 230°; helium flow-rate, 45 ml/min; FID detection.

Multiple-ion detection

The simplification of the extraction and derivatization procedures reported may require, depending on the complexity of the samples analyzed, a high level of specificity of the detection system in o: :r to ensure the correct identification and quantification of the substances of interest. This can be best achieved through the technique of multiple-ion detection (MID)[16] which allows for the simultaneous recording of selected ion-current profiles that characterize specifically the metabolite to be identified. By use of the MID unit designed in our laboratory[24], we can monitor up to four ions simultaneously as shown in Fig. 5. In this case the four channels of the MID (I to IV) were focused on the ions having *m/e* values of 483, 481, 469 and 467. The ion-current profile of the peak at *m/e* 483 combined with its retention time is specific for $PGF_{1\alpha}(TMS)_4$ (see Table I). There was also a small response for *m/e* 483 at the retention time of the 9-enol of PGE_1, which is to be expected since the mass spectrum of its TMS derivative shows a prominent ion at *m/e* 481 (38%) and this small leading peak would represent the isotopic contribution of the peak at *m/e* 481 to the profile at *m/e* 483. At the retention time corresponding to the peaks of prostaglandins B, the channel monitoring the ion at *m/e* 483 (I) also gave a small response which arises from the abundance of one of the ions in the isotopic cluster of the molecular ion of $PGB_1(TMS)_2$ (*m/e* 480 + 3). Thus one would expect that the response of channel II (Fig. 5), which monitors the peak at *m/e* 481, would be higher at this same point as observed, because of the isotopic (*m/e* 480 + 1) ion of PGB_1-

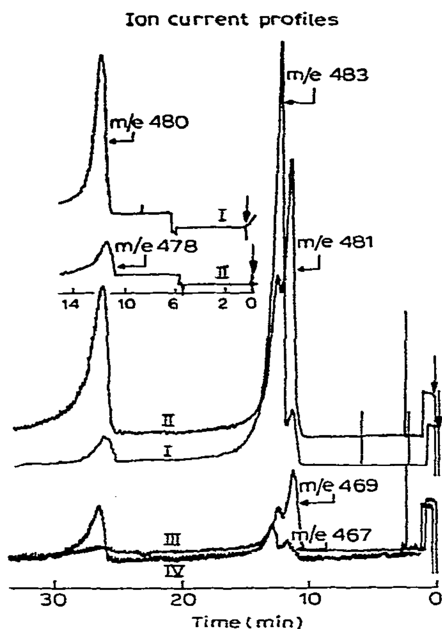

Fig. 5. Selected ion-current profiles obtained by monitoring with the MID unit of the mass spectrometer the masses 467, 469, 481 and 483 in the eluate from a glass column (3 m × 2.5 mm I.D.) packed with 3% OV-17 on Ga· Chrom. Q (100–120 mesh) at 250°. The traces were recorded on two Hitachi 56 dual-pen recorders. The vertical arrows point to the time of injection of the sample. Both pens were slightly offset in each recorder. The profiles of the ions at *m/e* 480 and 478 are shown on a different time scale because they were obtained with a shorter column (2 m × 2.5 mm I.D.).

$(TMS)_2$. As can be seen from Table I, the profile for *m/e* 481 is specific for $PGF_{2\alpha}$-$(TMS)_4$ and, as indicated above, the 9-enol of $PGE_1(TMS)_4$ (48 and 38% respectively), which explains the double peak obtained. The first peak of the current profile of *m/e* 481 corresponds to the retention time of prostaglandins E, while the second peak, only partially resolved, corresponds to the retention time of $PGF_{2\alpha}(TMS)_4$. These assignments can be confirmed by other ions such as those of *m/e* 469 and 467 (channels III and IV in Fig. 5; not included in Table I) which could be used to monitor the presence of prostaglandins E, $PGF_{1\alpha}$ and PGB_1.

Thus, identifications can be based on: (1) the simultaneous detection of four specific ions per analysis (Table I); (2) the characteristic retention times of these ions (Table II) and (3) the relative abundances of the selected ions. In this way this highly specific method of detection may compensate for any lack of chromatographic resolution, allowing the detection and quantitation of co-eluting or partially resolved components. For instance, although the derivatives of PGB_1 and PGB_2 were not separated (Table III), these two compounds can be determined simultaneously by monitoring the ion currents corresponding to *m/e* 478 and 480, *i.e.*, the molecular ions of $PGB_2(TMS)_2$ and $PGB_1(TMS)_2$, respectively, as shown on Fig. 5. For quantitative purposes, the only consideration that needs to be taken into account is the isotopic abundance of the $M + 2$ ion of $PGB_2(TMS)_2$ at *m/e* 480. The abundance of this ion (21% of the *m/e* 478 molecular ion) is then subtracted from the response

obtained in the channel monitoring the ion-current profile at *m/e* 480. For an accurate quantitation, the height of the peak at *m/e* 480 registered in channel I in Fig. 5 would have to be reduced by an amount equivalent to 21 % of the height obtained for the peak at *m/e* 478 registered in channel II (Fig. 5). Fig. 5 also shows that if more specificity is needed a second injection can be made in order to monitor other selected ions, as in the case of prostaglandins B, identified here by the appearance and relative abundances of six ions (*m/e* 476, 469, 478, 480, 481 and 483) at the expected retention times.

Finally, the new PGA(PIP)$(TMS)_3$ derivatives are ideal for MID in the sense that they offer two high-intensity ions (*m/e* 462 and 464, Table I) at an optimum GLC retention time for biological applications, thus providing a remarkable advantage over other types of derivatives.

ACKNOWLEDGEMENTS

We are grateful to Dr. J. Pike of the Upjohn Company for providing all of the prostaglandins used in this work. Receipt of samples of PGA_2, PGE_1 and PGE_2 from Mr. S. Matsumoto of the Ono Pharmaceutical Co. is also gratefully acknowledged. We also thank Dr. R. Castellet of the Instituto Urologico Puigvert, Barcelona, for supplying samples of human seminal fluid and Mrs. C. Suñol for her valuable technical assistance. This work was supported in part by a Research Grant from the Comision Asesora de Investigación Cientifica y Tecnica (100.01.721/74). One of us (J. R.) was the recipient of a C.S.I.C. Grant.

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