Gas Chromatographic Determination of Prostaglandins

R. Baranowski* and K. Pacha

Department of Analytical and General Chemistry, Silesian Technical University, 9 Strzody Street , 44-100 Gliwice, Poland

Abstract: Progress in separation and detection of prostaglandins and the other metabolites of arachidonic acid by means of GC-ECD, GC-MS, and GC-MS-MS in the course of the past fifteen years was reviewed. One discussed the problems of sample preparation, selection of proper chromatographic conditions, and detection modes available. Finally, applications of the methods developed to detection and quantification of prostanoids in biological material was presented.

Key Words: Prostaglandins, Sample preparation, Derivatization, Biological samples, Gas chromatography

1. INTRODUCTION

PGs are derived from AA under the influence of two enzymes, i.e. lipoxygenase and cyclooxygenase. They exert a marked impact on various different physiological and patophysiological processes occuring in living organisms (such as, e.g., inflamations, injuries, allergies, intratissue changes accompanying cancer diseases etc.). For this particular reason simultaneous quantification of several different prostaglandins in blood, urine and other body fluids is of great importance.

Due to low concentrations of PGs in biological samples, the development of very sensitive analytical techniques became a challenge and a must. The most suitable technique for quantification of eicosanoids in biological specimens proved the tandem GC-MS. Gradual refinement of the respective GC-MS equipment made possible a routine application of this technique in clinical chemistry. The present-day apparatus allows running the analyses either in the EI, or in the CI mode, positive and negative ions. The GC-MS technique can be used both for identification and quantification of prostaglandins in biological fluids. In fact mass spectrometer has proved to be a very sensitive and selective gas chromatographic detector.

Our present paper aims to review the development of GC equipment and the resulting progress in its application to the prostaglandin analysis.

2. SAMPLE PREPARATION PRIOR TO ANALYSIS BY GC-MS

A simultaneous analysis of several different PGs present in biological specimens proved a difficult task, basically due to their structural diversity and to interactions with a matrix. Thus application of the GC-MS technique to quantification of trace amounts of eicasanoids was negatively affected by interference from endogenous substances. For this particular reason preparation of a sample for the subsequent analysis proved one of the main difficulties which needed to be overcome.

Another important issue was the derivatization of the separated compounds. As PGs undergo decomposition even at ambient temperatures, application of GC to their analysis was only possible after obtaining thermally stable derivatives.

Sample preparation is carried out into two essential step: clean-up and derivatization.

The clean-up procedure varies according to the type of biological samples. For example for various tissues homogenisation incubation and centrifugation prior extraction is necessary, whereas for blood only centrifugation is required and for urine samples even this step prior to extraction can be omitted. For finally isolation of PGs from biological samples two principal methods were used: liquid-liquid and solid-phase extraction. Liquid extraction uses large solvent volumes compared to the sample volumes. This dilution of the sample was too great for direct determination without a preconcentration step. The most widely employed method for sample clean-up is solid-phase extraction since this reduced processing time, substantial solvents savings and simpler processing procedures. As described below researchers employed various single-step or step methods of extraction. Separated compounds then multi-undergo a three-step derivatization which included esterification, methoximation and silylation. Analysed compounds are converted to Me ester by reaction with diazomethane in diethyl ether/methanol or PFB ester by reaction with PFB bromide in acetonitrile. In most instances the carboxyl groups of these compounds are converted into the PFB esters. For oxime formation the most common reagent used is o methoxyamine hydrochloride salt in pyridine. Prostanoids hydroxyl groups were usually converted to silyl ethers by adding of BSTFA or BSA. Excess of derivatizing reagents is removed by using various methods of purification between individual step of derivatization.

*Address correspondence to this author at the Department of Analytical and General Chemistry, Silesian Technical University, 9 Strzody Street , 44-100 Gliwice, Poland; e-mail: baranows@polsl.gliwice.pl

1389-5575/02 $35.00+.00

Table 1. Sample Preparation for GC the Simultaneous Determination of PGs

Extraction	Chromatographic clean-up		Sample	Analyte	Ref.
	Column	Mobile phase			
Adjust pH to 3,2 with formic acid, (1) Sep-Pak clean-up, methylation, (2) Sep-Pak clean-up, (3) preparative HPLC, methoxylation, silylation	(1) Sep-Pak C_{18} (2) Sep-Pak silica (3) Preparative HPLC µ Porasil, silica, 10 µm	Chloroform Chloroform-methanol (98:2) Gradient elution: (A) chloroform (B) chloroform-methanol flow, 1 ml/min	Human urine and plasma	PGE_2 , $PGF_{2\alpha}$	1
Adjust pH to 3,2 with formic acid, (1) Sep-Pak clean-up, evaporate, dissolve in chloroform, (2) separate on HPLC column, evaporate esterification, (3) separate on Sephadex LH-20 column, evaporate, methoximation, (4) apply to Extrelut column, evaporate, silylation	(1) Sep-Pak C_{18} (2) NP-HPLC (3) Sephadex LH-20 (4) Extrelut	Ethyl acetate Chloroform-methanol-formic acid (96,8:2,7:0,5) Flow, 1 mil/min Dichloromethane Chloroform	Human plasma	PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ TXB_2	12
Centrifuge, evaporation, extract with diethyl ether, adjust pH to 3,0, (1) Sep-Pak clean-up, evaporate, esterification, methoximation, extract with diethyl ether, (2) Sep-Pak clean-up, (3) place on Pasteur pipette column, evaporation; silylation	(5) Sep-Pak C_{18} (6) Sep-Pak C_{18} (7) Pasteur pipette column silicic acid	Water-methanol-acetic acid 35:65:0,4 v/v/v) Water-methanol (15:85) Chloroform-methanol (9:1)	Rat blood	PGD_2 PGE_2 $PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$, 6-keto-PGE_1 TXB_2	18
Adjust pH to 3-3,5 with hydrochloric acid, (1) apply on Clin Elut column, extract in Tris buffer, (2) Sep-Pak clean-up, (3) Sep-Pak clean-up, evaporate, convert into PFB-MO derivatives, evaporate, dissolve in dichloromethane, (4) TLC clean-up, extract with ethyl acetate, evaporate, silylation	(1) Clin Elut (2) Sep-Pak C_{18} (3) Sep-Pak silica (4) TLC silica gel	Ethyl acetate Ethyl acetate-dichloromethane (75:25) Methanol-dichloromethane (60:40) Ethyl acetate-acetic acid-hexane-water (54:12:25:60)	Human and rat urine	PGE_2, TXB_2 , dinor-TXB_2 , dinar-6-oxo-$PGF1_{\alpha}$, 6-oxo-$PGF_{l\alpha}$	19
(A) Homogenize in phosphate buffer (pH 7,4), incubate at 37°C, centrifuge, adjust pH to 5 with hydrochloric acid, (B) Centrifuge at 37°C, adjust pH to 3,5 (A) (B)Extract on Sep-Pak cartridges, evaporate to dryness conversion to PFB-MO-TMS derivatives	Sep-Pak C_{18}	Methyl formate	(A) Rat tissue homogenates, (B) Human serum	PGD_2, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{l\alpha}$, TXB_2	22
(A) Adjust pH to 3,0 with hydrochloric acid, (1) Sep-Pak clean-up, (2) Sep-Pak clean-up, (B) Adjust pH to 3,0, (3) Bond-Elut clean-up, evaporate to dryness, dissolve in methanol or adjust pH to 3,0, (1) Sep-Pak clean-up, (2) Sep-Pak clean-up, (A} (B) methoximation, PFB esterification, (4) apply to a short column, evaporate, silylation, evaporate, dissolve in n-dodecane or tetradecane	(1) Sep-Pak C_{18} (2) Sep-Pak silica (3) Bond-Elut (4) Sephadex LH-20	Ethyl acetate Methanol Ethyl acetate Dichloromethane	(C) Human plasma (D) Guinea pig lung perfusion	PGD_2, PGE_2, $PGF_{2\alpha}$, 6-oxo-$PGF_{l\alpha}$, TXB_2 13,14-dihydro-l5-oxo-TXB_2	28
(A) Centrifuge, Sep-Pak clean-up, evaporate, convert into PFB-MO-TMS derivatives, extract twice with hexane, evaporate, dissolve in dodecane (B) Centrifuge, mix with cold methanol, centrifuge, Sep-Pak clean-up, evaporate, convert into PFB-MO-TMS derivatives, extract twice with hexane, evaporate, dissolve in dodecane	Sep-Pak C_{18}	Methanol	Bronchoalveolar fluid	PGD_2, PGE_2, $PGF_{2\alpha}$, 9a, 11b-PGF_2 6-keto-$PGF_{l\alpha}$, TXB_2 , 15-keto-13,14-dihydro-metalmlites of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$	31
Adjust pH to 3,2 with formic acid, (1) Sep-Pak clean-up, evaporate, dissolve in phosphate buffer (pH ó,7), (2) load on the affinity column, incubation, evaporate, esterification, (3) apply to Sephadex LH-20 column, evaporate, methoximation, (4) apply to Extrelut column, silylation	(1) Sep-Pak C_{18} (2) Immunoaffinity (3) Sephadex LH-20 (4) Extrelut	Chloroform-ethyl acetate Acetone-water (95:5) Dichloromethane Chloroform	Human and rat urine	PGE_2, $PGF_{2\alpha}$, 6-oxo-$PGF_{l\alpha}$	42

(Table 1). contd.....

Extraction	Chromatographic clean-up		Sample	Analyte	Ref.
	Column	Mobile phase			
Homogenize, centrifuge at 4°C, evaporate to dryness, dissolve in ethanol, adjust pH to 3 with hydrochloric acid, centrifuge, (1) Clin Elut clean up, (2) Sep-Pak clean-up, evaporate, dissolve in methanol, esterification with diazomet_ane, (3) clean-up on silica gel column , evaporate, methoximation, evaporate, silylation, (4) apply Sephadex LH-20	(1) Clin Elut (2) Sep-Pak C_{18} (3) Silica gel (4) Sephadex LX-20	Ethyl acetate Ethyl acetate n-hexane-ethyl acetate (2:1) for LTB_4 n-hexane-ethyl acetate (l:l) for TXB_4 ethyl acetate-methanol (99:1) for PGs Chloroform-n-hexane-methanol (10:10:1)	Dog and human gall bladders	(A) PGE_2, $PGF_{2\alpha}$, (B) 6-keto-$PGF_{1\alpha}$, TXB_2, LTB_4 ,	47
Adjust pH to 3 with hydrochloric acid, (1) apply to Extube 1003, evaporate, methoximation, esterification, extract with benzene, (2) place on microcolumn, evaporate, silylation, (3), apply to Sephadex LH-20, evaporate, dissolve in n-hexane containing 1% pyridine	(1)Extube 1003 (2) Silica gel (3) Sephadex LH-2O	Benzene-ethyl acetate (90:10) Ethyl acetate-methanol(99:1) Chloroform-n-hexane-methanol (10:10:1)	Urine of spontaneous hypertensive rats	PGE_l, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{l\alpha}$, TXB_2	49
add labelled standards, mix, adjust pH to 3,2 with hydrochloric acid, (1) Bond-Elut clean-up, evaporate, dissolve in methanol, (2) TLC clean-up, extract with 2% acetic acid in methanol, evaporate, dissolve in phosphate-citrate buffer, (1) apply to Bond-Elut column, methoximation, esterifieation, silylation, evaporate, dissohe in hexane	(1) Bond-Elut C_{18} (2) TLC silica gel	Ethyl acetate Ethyl acetate-acetic acid-isooctane-water (11:2:5:10)	Human skin fibroblast cultures, salvia new born and preterm babies	PGE_1,PGE_2, $PGF_{2\alpha}$, TXB_2	72

2.1. Sample Preparation with Derivatization of PGs into Me-MO-TMS Ether

Muller *et ad.* [1] investigated the contents of PGE_2 and $PGF_{2\alpha}$ in human urine and plasma. They employed both reversed-phase C_{18} silica and normal-phase silica cartridges for a selective extraction and purification of this compounds from biological samples.

Proudfoot *et al.* [2] examined the products of LDL oxidation and found substances cross-reacting with both PGE_2 and LTB_4. antibodies. Lipid extracts of copper oxidised LDL were separated by RP-HPLC and the fractions cross-reacting with PGE_2 antibody identified. Fraction were collected and examined as Me-TMS derivatives. 8-epi-PGE_2 was identified as one of the major metabolites eluting in fractions cross-reacting with PGE_2.

Feretti *et al.* established a method for simultaneous quantitative analysis of PGE_2 and PGE_3 [3]. On the basis of some earlier experiments [4] the ability of dietary ω 3 fatty acids to depress biosynthesis of PGE_2 and $PGF_{2\alpha}$ was confirmed. Moreover, it was established that endogenous eicosapentanoic acid of dietary origin is converted to PGE_3 [5]. They studies of essential fatty acid metabolism in animals and humans demand quantification of the PG metabolites, such as arachidonic and 5,8,11,14,17-eicosapentaenoic acids. For this reason the efforts were undertaken aimed at a simultaneous determination of PGE_2 and PGE_3. After a conversion of E series PGs to B series PGs by treatment with methanolic KOH, the resulting compounds was transformed to the Me-TMS derivatives of PGB_2 and PGB_3, respectively. Lipidex-5000 columns, which provided no successful separation of PGE_2 and PGE_3, were used for clean-up of extracts of kidney medullae.

Determination of E series PGs after conversion to B series PGs has found application in forensic sciences, e.g., to investigation of sexual offences [6-8]. On the basis of some earlier investigations [9-11] the presence of PGE_1, PGE_2, PGE_3, 19-hydroxy-PGE_1 and 19-hydroxy-PGE_2 in human semen was established. It was also found out that the contents of these prostaglandins in semen are considerably higher than in cervical mucus [11]. Douse elaborated a method of tracing the low picogram levels of the E series PGs (i.e. of PGE_1, PGE_2, PGE_3, 19-hydroxy-PGE_1, and 19-hydroxy-PGE_2) in human semen after alkaline conversion to the B series PGs, esterification with diazomethane and silylation with BSA [7]. This method was utilised for detection of traces of semen on post-coital vaginal swabs and on rectal, oral and skin swabs after simulated sexual acts.

2.2.Sample Preparation with Derivatization of PGs into PFB-MO-TMS Ether

2.2.1. Several-step Separation of Prostaglandins Prior to Derivatization

Schweer *et al.* established a method for a simultaneous determination of $PGF_{2\alpha}$., PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 in human plasma [12]. They changed formerly applied methylation to formation of the corresponding PFB derivatives. The analysed compounds were extracted by C_{18}

Sep-Pak cartridges, and separated on NP-HPLC. Recoveries after the extraction and purification steps ranged from 45 to 55%.

Investigation of the differenced products of the arachidonic acid transformation was carried out with using liquid-liquid extraction and a preliminary separation of the analysed compounds by column chromatography, too. A packings silicic acid [13] or XAD-7 resin were usually employed [14]. This preparatory step prior to the analysis proper was time-consuming and in the most cases demanded evaporation of the considerable volumes of organic solvents.

Table 2. Conditions for GC Determination of PGs

Compound	Column	Stationary phase	Column temperature	Detection	Derivative	Ref.
1	2	3	4	.5	6	7
PGE_2 , $PGF_{2\alpha}$	glass capillary, 20 m.	SE-30	100°C to 3V0°C	MS	Me-MO-TMS	1
PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{l\alpha}$, TXB_2	silica capillary column, 30 m x 0.259 mm	DB-1	140°C, 25°C/min to 290°C, 7,5°C/min to 320°C	MS, PICI, NICI, SIM	PFB-MO-TMS	12
PGD_2, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, 6-keto-PGE_1, TXB_2	silica capillary column, 6 m.	DB-1	100°C (l min.), 30°C/min to 270°C	MS, NICI, SIM ECD	PFB-MO-TMS	18
PGE_2, TXB_2 , dinor-TXB_2 dinor-6-oxo-$PGF_{1\alpha}$, 6-oxo-PGFia,	(A) packed column 1,2 m x 2mm (B) capillary column 12 m x 0,2mm	SP-2100	(A) 270°C(l min.), 10°Clmin to 300°C (B) 100°C(l min.), 15°C/min to 290°C	MS, NICI, SIM	PFB-MO-TMS	19
PGD_2, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2	glass column, 20 m x 0,35mm	OV-1, 5E-54	150°C to 280°C at 20°C/min	MS, SIM	PFB-MO-TMS	22
$PGD_{2\alpha}$ $PGE_{2\alpha}$ $PGF_{2\alpha}$, 6-ogo-$PGF_{1\alpha}$, 13,14-dihydro-15-oxo-TXB_2	Silica capillary column, 23 m x 0,23mm 25m x 0,23mm	Sil-5	(A)195°C to 240°C at 20°C/min, 40°C/min to 300°C (B) 195°C to 240°C at 20°C/min, 30°C/min to 300°C	MS, NICI, SIM	PFB-MO-TMS	28
PGD_2, PGE_2, $PGF_{2\alpha}$, 9α ,11β-PGF_2, 6-keto-$PGF_{1\alpha}$ TXB_2, 15-keto-13,14-dihydro-metabolites of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$	silica capillary column, 30 m x 0,25mm	SPB-5	205°C(l min.), 40°C/min to 240°C, 1,5°C/min to 287°C	MS, NICI	PFB-MO-TMS	31
PGE_2, $PGF_{2\alpha}$, G-oto-$PGF_{1\alpha}$	capillary column, 20 m x 0,25mm	DB-1701	100^oC (2 min), 30^oC/min to 280°C 5°C/min to 310^oC	MS-MS, NICI	PFB-MO-TMS	42
(A) PGE_2, $PGF_{2\alpha}$, (B) 6-keto-$PGF_{1\alpha}$, TXB_2, LTB_4	silica capillary column, 25 m x 0,32 mm	Ultral	(A) 280°C (B) 200^oC to 280^oC at 5°C/min	MS, SIM	Me-MO-DMiPS	47
PGE_1, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{l\alpha}$, TXB_2	(A) silica capillary column, 2S m. x 0,25 mm (B) packed column 2 m x 2,Smm	OV-101	(A) 270°C to 280°C (B) 250°C to 270°C	(A) EDC (B) MS	PFB-MO-DMES PFB-MO-DMnPS PFB-MO-DMiPS	49
$PGE_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, TXB_2	silica column, 30 m x 0,25mm	DB-5	100^oC (l min.), 40^oC/min to 320°C	SID, MS, EI, NICI, PICI	Me-MO-TMS PFB-MO-TMS	72

Mayer *et al*. focused their attention on development of a fast and convenient method for the separation of free AA, HETEs, and PGs on a special silica gel [15]. The analysed compounds produced by incubated bovine aortic endothelial cells were extracted with ethyl acetate and then separated on a column made of Silicar-4 in a Pasteur pipette. In order to lower delectability limits, they had to change the earlier described methods of derivatization [16]. The formerly applied methylation as a derivatization procedure was presently changed to formation of the corresponding PFB derivatives [15,17]. The modified method was then applied to determination of cyclooxygenase products in other biological fluids, including supernantants of cultured cells, sputum, plasma, tissue homogenates and inflammatory exudate.

In their investigations Pace-.Asciak and Micaleff made use of a purification procedure of whole blood employing the diethyl ether extraction and C_{18} Sep-Pak chromatography [18]. Separated compounds underwent a three-step derivatization with the purification procedure taking place between step two and three. The recovery of this procedure was equal ta ca. 73%.

This procedure was also applied by Martineau *et al.* [19]. However, the authors changed the method of sample preparation. For the purpose of extracting and purifying urinary PGs an approach was established, based on the sequential use of small columns with distinct properties combined with a TLC step. The overall final yields ranged only between 20 and 25%.

Multi-step methods of clean-up was also employed for determination of trace amounts of individual PGs for example for quantification of PGE_2 in plasma of schizophrenics and of healthy controls [20]. The PG of interest was examined as PFB-MO-TMS derivative after a preliminary separation and purification on a Bond Elut C_{18} and a Bond Elut Si cartridge column, and on a silica gel TLC plate.

Gopaul *et al.* reported plasma levels of specific nonenzymatic peroxidation product of AA, esterified 8-epi-$PGF_{2\alpha}$, from healthy- and non-insulin dependent diabetes mellitus individuals as an index of oxidative stress in vivo [21]. Plasma 8-epi-$PGF_{2\alpha}$, was isolated by solid-phase extraction on a C_{18} cartridge followed by an NH_2 cartridge.

2.2.2. Single-step Extraction of Prostaglandins Prior to Derivatization

Chiabrando *et al.* employed the method based on a single-step extraction of PGs from biological samples on the C_{18} cartridges [22,23]. After purification the investigated prostaglandins were then derivatized into the PFB-TMS and the PFB-MO-TMS derivatives as previously described [22]. The method described enables quantification of PGs in rat lung, kidney and seminal vesides.

The above method used for clean-up of the compounds discussed was, e.g., reported in the articles of Waddell *et al.* [25-28]. The considered prostanoids were extracted from biological fluids by liquid chromatography and then converted to PFB-TMS and PFB-MO-TMS derivatives, with the purification procedure after esterification. Some efforts were also undertaken to apply the Bond-Elut cartridges for the extraction of biological fluids, but the yields observed were considerably lower than in the case of the Sep-Pak cartridges. Some other studies were also carried out, aimed at establishing of the mechanism which control a release of prostanoids during anaphylaxis [29].

Bersen *et al.* reported PG quantification (PGE_1, PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{l\alpha}$ by making use of the respective PFB-TMS derivatives and employing RP extraction [30], too. The method was successfully applied to the analysis of PG production in cell cultures derived from cell of cancer patients.

For quantification of PGs in the aliquots of dilute solutions of airway and alveolar lining fluid obtained by BAL, Liu [31] employed modifications of two methods of sample preparation, proposed earlier [32,33]. The first method consisted in a direct extraction on C_{18} cartridges. In the second method fluid samples were first mixed with methanol, then centrifuged and after dilution with water the compounds of interest were separated on the Sep-Pak C_{18} cartridges. In both cases the recoveries ranged from 70 to 95%.

The work up procedure proposed by Powell [32] was also applied by Shindo *et al.* for a simultaneous quantification of 11 prostanoids in the normal human embryonic lung fibroblast WI38 [32]. In order to retain the stability of prostanoids containing a carbonyl group, such as TXB_2, during the purification and derivatization steps of biological materials, methyl acetate was used in place of methyl formate as an eluent for Sep-Pak C_{18} purification.

Weber *et al.* undertook quantification of PGE_2, PGD_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2, and 2,3-dinor TXB_2 in the rat brain [35] and human urine samples [36]. For tracing these compounds in brian, analytical samples were prepared via lyophilization, pulverization, extraction and derivatization to MO-PFB-TMS derivatives. Two extraction procedures, modifications of those elaborated earlier by Powell [32] and Mayer were employed [15]. In the case of the first extraction method, recovery was about 75%, while with the second method it was 85%. When tracing the discussed PGs in human urine, the method developed consisted in methoximation in urine samples followed by separation and purification of the methoximized prostanoids by means of phenylboronic acid cartridges, RP cartridges and TLC. The pretreated compounds were ultimately derivatized to PFB-TMS derivatives.

The method of purification of the methoximized prostanoids by TLC applicated also Schweer *et al.* [37]. After extraction on a RP cartridge, compounds (PGE_1, 15-keto-PGEO and PGE_O) were converted into corresponding PFB-MO-TMS derivatives. After prior purification by TLC the PFB-MO derivatives were extracted on a short Extrelut column. This method was applied to determination of PGs in plasma.

2.2.3. Separation of Prostaglandins by Immunoaffinity Technique

In the analysis of prostanoids the immunoaffinity technique were first applied for extraction of the prostacyclin (PGI_2) analogue iloprost from plasma [38J. Ilprost antiserum was coupled to cyanogen bromide-actived Sepharose 4B. A similar method was used for purification of urine samples, using a polyclonal anti-TXB_2 antibody immobilized on N-hydroxysuccinimidyl-silica gel [39]. An antibody-mediated extraction was also employed for TXB_2, and 2,3-dinor-TXB_2, in human and rat urine [40], and for 6-oxo-$PGF_{1\alpha}$ in human urine and canine plasma [41]. The analysed compounds were quantified as MO-PFB-TMS derivatives. Mackert *et al.* developed a method of simultaneous determination of PGE_2, $PGF_{2\alpha}$, and 6-oxo-$PGF_{1\alpha}$ using an immunoaffinity column with monoclonal antibodies[42].

2.2.4. Direct Derivatization of PGs

A method based on the derivatization without a prior extraction and/or chromatography was used by Hubbard *et al.* for tracing of the $PGF_{2\alpha}$, PGE_2, PGD_2, 6-keto$PGF_{1\alpha}$ and TXB_2 biosynthesis in human lung carcinomas and in a normal human lung [43]. Aliquots of incubates of human lung tissue fragments were derivatized into PFB-TMS and MO-PFB-TMS derivatives. The derivatized analytes were then extracted with three volumes of dry hexane.

Schweer *et al.* determined prostanoids in urine [44]. The prostanoids were derivatized to their methoximes and extracted with ethyl acetate-hexane. The sample was further derivatized to the PFB esters and purified by TLC. The prastanoid derivatives were finally converted to their TMS ethers. In each run, two or three prostanoids were determined.

2.3. Other Silylating Reagents in Method of the Derivatization

New reagents which are homologs or analogs of the trimethylsilylating reagents were developed to improve hydrolytic stability or detectability of the silyl ether derivatives. The most important are the alkyldimethylsilyl reagents containing a propyl, isopropyl or *tert*.-butyl substituent.

Miyazaki *et al.* was particularly concerned with proper derivatization of PGs studied [45-54]. They were separated as DMES, DMnPS and DMiPS derivatives.

The best results were obtained when applying DMnPS derivatives of PGs and TXB_2 PFB esters [51,54]. This method was used for determination of PGD_2, PGE_1, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{l\alpha}$. Finally the method established was applied to detection and quantification of $PGF_{2\alpha}$ in an extract from the urine of spontaneous hypertensive rats and in an extract from plasma of a lung-heart preparation from a dog [52].

Work research was also carried out on the determination of 13,14-dihydro-l5-keto $PGF_{2\alpha}$ in form of Me-MO-DMiPS ether [52, 53]. However, this method proved insufficient for tracing of 13,14-dihydro-15-keto $PGF_{2\alpha}$ in the presence of PGD_2 and PGE_2, due to their mutual structural similarity. 13,14-dihydro-15-keto $PGF_{2\alpha}$ was analysed as the Me-nBO-DMiPS or the ME-nBO-DES derivatives [45]. The method developed was applied to quantification of the compound in human blood, following a prior purification on a Chem. Elut.

Prostanoids in the form of DMiPS derivatives were also traced and quantified by Ohyama *et al.* [55]. They developed a method of separation of $PGF_{2\alpha}$ and $PGF_{3\alpha}$. This method was applied to the analysis of prastanoids in biological samples after administration of eicosapentaenoic acid.

Derivatization with dimethylisopropylsilyl imidazole has been utilised, among others, by Obata *et al.* [56]. The compounds investigated were extracted from human urine and plasma with use of the Kieselgel 60 Chem-tube column and Sep-Pak C_{18} cartridge. They were then derivatized in a two-step procedure with etheral diazomethane and dimethylisopropylsilyl imidazole , and cleaned-up on the Sephadex LH-20 column and silica gel column.

Hishinuma *et al.* established a method for the determination of 2,3-dinor-6-keto$PGF_{1\alpha}$ and 11-dehydro-TXB_2 in urine of diabetic and healthy volunteers [57]. These samples were extracted by chromatography using Sep-Pak C_{18} and silica gel. The compounds were then analysed by means of the lactone ring opening reaction and dimethylisopropylsilylation. The 2,3-dinor-6-keto- $PGF_{l\alpha}$ was determined in the form of Me-propylamide-DMiPS derivative and 11-dehydro-TXB_2 was determined in the form of Me-MO-DMiPS derivative.

Steffenrud *et al.* proposed determination of PGE_2 and $PGF_{2\alpha}$, LTB_4 and 5β-pregane-3α, 20α,-diol as their allyldimethylsilyl ether derivatives [58]. The respective derivatives were prepared with the navel reagent BASTFA. This method proved valid only for the compounds without sterically hindered hydroxyl groups, limiting the use of the reagent to derivatization of unhindered hydroxyl groups only.

For use with the ECD detection PFBO derivatives have been used [59-63]. Optimised GC-ECD conditions for the analysis of PGs and TXB_2 as their PFBO-PFB-TMS derivatives were described by Mai *et al.* [60]. Christ-Hazelhof and Nugteren applied this reagent for quantitation of PGI_2 in human whole blood [61]. Leffler *et al.* proposed a method for isolating climax products of the AA cascade, which allows a simultaneous measurement of among others, PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$, TXB_2, 6-keto-PGE_1, and 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ in blond, urine, or in artificial organ perfusates [62]. The method described was applied to the determination of PGE_2 in biological incubate-10% fetal calf serum, human lung fibroblast and human plasma.

Rosello *et al.* used of GC-MS for the determination of PGs from mouse peritoneal macrophage cells using Me-PFBO-butylboronate-TMS derivatives of TXB_2 and 6-keto-$PGF_{1\alpha}$ [64,65]. This involved a four-step derivatization,

beginning with esterification followed by oximation with PFBHA, boronation and silylation.

PFBO-TMS-Me derivatives of PGE_1, PGE_2, PGE_3, 6-oxo-$PGF_{1\alpha}$, 1-carboxyrioprostil and rioprostil were also prepared and analysed by GC-MS-MS by Schweer [66].

3. DETERMINATION OF PROSTAGLANDINS BY GC

There are several techniques for the determination of PGs by GC in urine , plasma and other biological materials including GC-ECD, GC-MS and GC-MS-MS.

Some efforts were have been made in determination of PGs by GC, mainly with ECD [6-11,54,59-63]. The pioneer studies in the field demonstrated that the accuracy obtained is comparable with those of biochemical methods. Detection limits for PG derivatives by GC-ECD result from the presence of contaminants and not from the instrument sensitivity. Thus for this important reason elaboration of the effective procedures for removing sample contaminants became a really crucial problem.

The ECD has been coupled with a high-resolution capillary GC (HRGC). This method was applied to determination of $PGF_{2\alpha}$, PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 [22] and PGE_1, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ in cell cultures derived from cell of cancer patients [30].

In order to lower detectable limits of PGs the GC-MS has been used. This method was considered to be the most specific and reliable technique and was employed by the numerous researchers [1-5, I2, 15-23, 25-29, 31, 34-36, 48-51, 56, 67]. The analysis were carried out by GC-MS in the EI, or in the CI mode with positive and negative ions.

Using negative-ion chemical ionization (NICI) with participation of the reaction gas and a stream of electrons enabled a marked lowering of detectability limits of the analysed prostaglandins, as compared with electron ionization. Optimized GC-NICI-MS conditions for the analysis of the prostaglandins were described by Shindo [34]. In order to establish the optimal chemical ionization conditions ammonia, methane or izobutane as reagent gases were applied. Shindo studied the influence of the column packing and length on separation of the analytes discussed as well. For this reason banded and unbonded OV-1 capillary columns 3,6 and 29 m long were applied. The best results were obtained when using ammonia as a reagent gas and a six meters long capillary column for separation of 11 prostanoids.

However, the disadvantage of these methods was the time consuming purification prior to quantification.

The introduction of GC-MS-MS techniques [37,42,44,6ó,68,69] allowed some reduction in the clean-up procedure, but this problem is current as important as before. Using NICI-GC-MS-MS allowed measurement the investigated compounds at trace levels [58-60]. The lower detectability limit of the analysed PGs ranged from 1 to 5 pg.

For determination of the PGs the SID GCMS has been used. For example, methods for measurement of the metabolites of AA (PGs included), using SID GC-MS were described by Picked and Murphy [71] and Gleispach *et al.* [72-75]. The method enables quantification of PGE_1, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2 and different HETEs. The analysis was carried out by GC coupled to mass spectrometer with positive-ion EI and pulsed PNICI.

A SID HRGC-MS method applied to determination of PGE_2 and 6-keto-$PGF_{1\alpha}$ in urine was described by David A. Herold *et ad.* [76-78] and by [79-81].

GC-MS and SID techniques have been utilized frequently in clinical chemistry [82-84] for examply for determination of hydroxyeicosatetraenoic acids, hydroxyheptadecatrienoic acids, TXB_2 and $PGF_{2\alpha}$ formed during platelet aggregation after stimulation of gel-filtered platelets with thrombin or collagen [85].

5. CONCLUSION

The present review focuses on methods of samples preparation prior to analysis by GC, which are necessary for the determination of prostaglandins in real samples. Although many procedures are available for the separation of the prostaglandins, sample preparation causes a variety of problems , as before.

In described methods are required complex pretreatments, including extraction and several-step derivatization. Analysis time, sample size and quality of the analytical results are much more influenced by sample preparation than by the separation part of the analytical method. Any sample work up operation (separation, purification and derivatization) may involve impurities, formation of artifacts, a decrease in analytical precision and increase in analysis time. The different steps of sample preparation should be as few and as simple as applied means of detection and the analytical aims permint.

Consequently work must continue on the development of rapid and selective procedures of extraction and precise method of purification and simple and yields a quantitative reaction of derivatization. The new method must allow to increase the number of analyses for a day and simultaneous determination of greater number of metabolites of arachidonic acid. The lowering of limit of detection of these compounds must be also obtain.

LIST OF ABBREVIATIONS

AA	=	Arachidonic acid
BAL	=	Bronchoalveolar lavage
BASTFA	=	N,O-bis (allyldimethylsilyl)-trifluoroacetamide

BSA = Bis (trimethylsilyl) acetamide

BSTFA = Bis (trimethylsilyl) trifluoroacetamide

CI = Chemical ionization

DES = Cyclic diethylsilylene

DMES = Dimethylsilyl

DMiPS = Dimethylisoproylsilyl

DMnPS = Dimethyl-n-propylsilyl

ECD = Electron-capture detector

EI = Electron impact

GC = Gas chromatography

GC-MS = Gas chromatography -mass spectrometry

HETEs = Monohydroxyeicosatetraenoic acid

HFAs = Hydroxy fatty acids

HPLC = High-performance liquid chromatography

HRGC = High-resolutian capillary gas chromatography

LDL = Low-density lipoproteins

LT = Leukotriene

Me = Methyl esters

MO = Methoxime

MS-MS = Tandem mass spectrometry

n-BO = N-butyloxime

NICI = Negative-ion chemical ionization

NP = Normal-phase

PFB = Pentofluorobenzyl

PFBHA = O- (2,3,4,5,6-pentofluorobenzyl) hydroxylamine hydrochloride

PFB-MO -TMS = Pentofluorobenzyl ester-methoxime-trimethylsilyl ether

PFBO = O- (2,3,4,5,6-pentofluorobenzyl) oxime

PG = Prostaglandin

PGs = Prostaglandins

RP = Reversed-phase

SID = Stable isotopes dilution

SIM = Selected-ion monitoring

TLC = Thin-layer chromatography

TMS = Trimethylsilyl

TXs = Thromboxanes

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