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ANALYSIS OF PICOMOLAR CONCENTRATIONS OF 6-OXO-PROSTAGLANDIN $F_{1\alpha}$ IN BIOLOGICAL FLUIDS

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SUMMARY

A highly sensitive and specific assay for the quantitation of 6-oxo-prostaglandin $F_{1\alpha}$, the stable hydrolysis product of prostacyclin, is described. The method involves the addition of [3,3′,4,4′-$^2H_4$]-6-oxo-prostaglandin $F_{1\alpha}$ as internal standard, extraction from biological fluids using μBondapak C_{18} reversed-phase Sep-Paks, and preliminary purification by normal-phase chromatography. Following conversion to the methoxime, tris-trimethylsilyl, pentafluorobenzyl derivative, samples were analysed using combined capillary column gas chromatography negative ion chemical ionisation mass spectrometry. Fragment ions at *m/z* 614 (^1H) and 618 (^2H) [M − $C_7H_2F_5$]$^-$ were monitored for quantitation. This method was used for the measurement of endogenous levels of 6-oxo-prostaglandin $F_{1\alpha}$ in human urine and for the determination of prostacyclin release from rat peritoneal mast cells and from rat aortic rings incubated in human plasma.

INTRODUCTION

Prostacyclin (PGI_2), a product of the cyclooxygenase pathway of fatty acid metabolism, is a potent inhibitor of platelet aggregation and a powerful vasodilator[1]. It has been the subject of numerous investigations designed to define its physiological role more fully. Such studies rely heavily on the availability of an assay capable of quantitating relatively low concentrations contained in a complex biological matrix.

PGI_2 is unstable and is hydrolysed non-enzymically to 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) which is biologically inactive. As a result, physicochemical methods of analysis are dependent upon the measurement of 6-oxo-$PGF_{1\alpha}$ to provide a reflection of PGI_2 concentration. Current techniques for the quantitation of 6-oxo-$PGF_{1\alpha}$ in biological fluids include radioimmunoassay (RIA)[2], electron-capture gas chromatography (GC)[3] and GC–mass spectrometry (MS)[4,5].

It is generally accepted that GC–MS provides the most sensitive and specific methodology available. Nevertheless, extensive mass-spectral fragmentation in the electron impact (EI) mode limits the sensitivity of such assays. In addition, biological fluids tend to contain other endogenous prostanoids which are not easily separated from 6-oxo-$PGF_{1\alpha}$ on packed columns.

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We report a novel assay for 6-oxo-$PGF_{1\alpha}$ based on combined capillary column GC–negative ion chemical ionisation (NICI) MS. This method is capable of providing both the specificity and sensitivity required to measure picomolar concentrations in biological fluids. We have applied this method to a number of studies in our laboratories and in this paper, we describe the determination of endogenous levels of 6-oxo-$PGF_{1\alpha}$ in human urine. This investigation was carried out with a view to using the method in future studies concerning the role of PGI_2 in the kidney[6]. In addition, we have used this method to analyse samples obtained from incubations of rat peritoneal mast cells and rat aortic rings.

EXPERIMENTAL

Reagents

Analytical grade reagents were used at all times and solvents were redistilled immediately before use. μBondapak C_{18} and silica Sep-Pak cartridges are proprietry products manufactured by Waters Assoc. (Northwich, Great Britain). Silica thin-layer chromatography (TLC) plates (Merck) were purchased from BDH (Enfield, Great Britain). Sephadex LH-20 was obtained from Pharmacia (Uppsala, Sweden). Methoxyamine hydrochloride was obtained from Eastman (Rochester, NY, U.S.A.) and was recrystallised from ethanol containing *ca.* 1% concentrated hydrochloric acid before use. Pentafluorobenzyl bromide (PFBB) was purchased from Fluorochem (Glossop, Great Britain) and used without further purification. Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, IL, U.S.A.). N-Methyl-N-nitroso-*p*-toluenesulfonamide (Diazald), for diazomethane generation, was supplied by Aldrich (Milwaukee, WI, U.S.A.). 6-Oxo-$PGF_{1\alpha}$ and [3,3′,4,4′-$^2H_4$]-6-oxo-$PGF_{1\alpha}$ standards were kindly supplied by Dr. J. Pike, Upjohn (Kalamazoo, MI, U.S.A.), and [5,8,9,11,12,14,15(N)^{3}H]-6-oxo-$PGF_{1\alpha}$ (sp.act. = 120 Ci mmole^{-1}) was purchased from New England Nuclear (Southampton, Great Britain). Compound 48/80, a basic histamine releaser (a polymeric condensation product of N-methyl-*p*-methoxyphenethylamine with formaldehyde) was a gift from Dr. A. N. Payne of Wellcome Research Laboratories (Beckenham, Great Britain).

Instrumentation

GC–MS. A Finnigan 4000 quadrupole gas chromatograph–mass spectrometer interfaced with a 6110 data system, was used in this study. The system had been modified with a Finnigan PPNICI package to permit monitoring of negative ions in the chemical ionisation mode.

Chromatography was carried out using fused silica capillary columns. An SP 2100 column (25 m × 0.2 mm I.D. × 0.11 μm coating thickness) was obtained from Hewlett-Packard (Wokingham, Great Britain), and an SE-54 column (30 m × 0.3 mm I.D. × 0.3 μm coating thickness) was purchased from GC^2 (Chromatography) (Northwich, Great Britain). Helium was used as a carrier gas at an inlet pressure of 100–140 kPa. Samples were injected using a Grob-type splitless injector set at 270°C and the column temperature programmed from 100°C at 20°C min^{-1}.

For NICI-MS, methane was used as reagent gas with an ion source pressure of 27 Pa. The operating conditions were ioniser temperature, 200°C; electron energy, 100 eV; electron multiplier voltage, 1100–1300 V; conversion dynode voltage ±3 kV; and emission current, 0.33 mA.

In the EI mode, operating conditions were modified to give an ioniser temperature, 210°C; electron impact energy, 25 eV; and ion source pressure <1 Pa.

Selective ion monitoring (SIM) was performed using a four-channel multiple ion monitor operating at a pre-amplifier gain of 10^{-8} AV^{-1}.

Radiochromatogram scanner. A Packard Model 7201 Scanner System was used to monitor standard [^{3}H]6-oxo-$PGF_{1\alpha}$ on TLC plates.

Biological samples

Urine was collected from normal, healthy volunteers and used immediately or frozen and stored at −20°C. Aliquots of 1 ml were taken for analysis.

Rat peritoneal mast cells were obtained as described previously[7] and purified to 88–93% by centrifugation in a bovine serum albumin density gradient by a modification of the method of Sullivan *et al.*[8].

Aliquots of cells ($2.6–3.5 \cdot 10^5$ ml^{-1}) were incubated in Tyrodes buffer at 37°C for 30 min either in the presence of compound 48/80 (1 $\mu g\ ml^{-1}$) or in control buffer. Aliquots (1 ml) of the supernatants were analysed for 6-oxo-$PGF_{1\alpha}$. % Histamine release was determined as described previously[7].

Rat aortic rings were prepared and incubated in human plasma as described previously[9]. Aliquots (200 μl) of the plasma were analysed for 6-oxo-$PGF_{1\alpha}$.

Extraction and purification procedure

Biological samples were made up to 20 ml with distilled water and equilibrated with [3,3′,4,4′-$^2H_4$]6-oxo-$PGF_{1\alpha}$ (10 ng or 2 ng in 10 μl ethanol) at 0°C for 30 min and acidified to pH 3.0–3.5 with 2 *M* HCl. A typical extraction was carried out using a μBondapak C_{18} reversed-phase Sep-Pak which had been preconditioned with methanol (5 ml) followed by distilled water (5 ml). The sample was applied using a polypropylene syringe at a flow-rate of <30 ml min^{-1}. The Sep-Pak was washed with distilled water (10 ml) to remove highly polar components originating from the biological matrix. 6-Oxo-$PGF_{1\alpha}$ was eluted with ethyl acetate (7 ml) and collected into a glass pointed tube. A small amount of water (*ca.* 0.5 ml) was allowed to settle out.

Initial sample clean up was achieved by application of the upper organic layer to a normal-phase silica Sep-Pak which had been prewashed with methanol (5 ml) and ethyl acetate (5 ml). The Sep-Pak was washed with ethyl acetate (5 ml) to remove components of relatively low polarity and 6-oxo-$PGF_{1\alpha}$ was eluted with methanol (5 ml).

Solvent was removed at ambient temperature under a stream of nitrogen and the residue transferred with methanol to a small vial. This furnished a crude prostanoid extract.

Preparation of derivatives

Methoximation. The methoxime derivative of 6-oxo-$PGF_{1\alpha}$ was prepared by treating the residue with 100 μl methoxyamine hydrochloride in anhydrous pyridine (5 mg ml^{-1}). The sample was allowed to stand overnight at ambient temperature and the pyridine was then removed *in vacuo*.

Esterification. The 6-oxo-$PGF_{1\alpha}$–methoxime derivative was converted to a pentafluorobenzyl (PFB) ester by adding acetonitrile (30 μl), 35% PFBB in acetonitrile (10 μl) and diisopropylethylamine (10 μl) and heating at 40°C for 30 min. Alter-

natively, the methyl ester was prepared using an alcoholic ethereal solution of diazomethane prepared according to the method of Fales *et al.*[10]. The residue was first dissolved in methanol (200 μl) and the diazomethane solution (0.5 ml) added and allowed to stand for 5 min. In both cases, samples were evaporated to dryness under a stream of nitrogen at ambient temperature.

Sephadex LH-20. Electron-capturing impurities and excess derivatising reagents were removed using a short column of pre-swollen Sephadex LH-20 (30 × 5 mm I.D.). The ester residue was taken up in dichloromethane (200 μl), applied to the column and eluted with dichloromethane (2 ml). The sample was taken to dryness under a stream of nitrogen.

Trimethylsilylation. All samples were converted to tris-trimethylsilyl (tris-TMS) derivatives by adding BSTFA (125 μl) and allowing to stand overnight at ambient temperature. The samples were transferred to small vials, evaporated under dry nitrogen and immediately reconstituted in BSTFA (25 μl). Generally, aliquots (2.5 μl) were analysed by GC–MS immediately but it was possible to store samples for up to 3 weeks in a desiccator at −20°C.

TLC Purification

It was necessary to include an additional purification step for the urine extracts. Preparative TLC was carried out on the residue obtained after the methoximation step. Typically, a sample was taken up in methanol (60 μl) and applied to the preconcentration zone of a precoated silica gel 60 TLC plate (200 × 50 × 0.25 mm thickness). The sample was developed using the organic phase obtained from a mixture of ethyl acetate–acetic acid–hexane–water (54:12:25:60). The band corresponding to the methoxime derivative of 6-oxo-$PGF_{1\alpha}$ was located by comparison with a tritiated standard. A sample of the methoxime derivative (2 ng) containing its ^3H analogue (*ca.* $4 \cdot 10^5$ dpm) was applied to a separate TLC plate. After development alongside the biological extracts, the appropriate band was located by radiochromatogram scanning. Under these conditions, the R_F value was 0.19.

The corresponding zone for 6-oxo-$PGF_{1\alpha}$–methoxime, in the urinary extracts, was scraped off the TLC plate and eluted with methanol (2 × 2 ml). The combined eluates were evaporated to dryness under a stream of nitrogen and the residue transferred to a small reagent vial. Esterification and trimethylsilylation was carried out as described above.

GC–MS Quantitation

Standard curves were prepared for 6-oxo-$PGF_{1\alpha}$ in the range 0–20 ng. Appropriate concentrations of 6-oxo-$PGF_{1\alpha}$ and either 10 ng or 2 ng of [$^2H_4$]6-oxo-$PGF_{1\alpha}$ were added to Krebs buffer (pH 7.4). These standard solutions were then processed through the relevant assay procedure. Quantitative SIM analyses were performed in the NICI mode using the fragment ions at m/z 614 (^1H) and 618 (^2H). Quantitation was based on peak area ratios ($^1H/^2H$) which for the standard mixtures were plotted against the known weights of 6-oxo-$PGF_{1\alpha}$.

Curves were obtained using an unweighted least squares linear-regression analysis. Using the parameters obtained, unknown levels of 6-oxo-$PGF_{1\alpha}$ could be determined.

RESULTS

The recovery through the entire assay procedure, including TLC purification, was determined using 5 urine samples spiked with [^{3}H]6-oxo-$PGF_{1\alpha}$ ($4 \cdot 10^5$ dpm) and [$^2H_4$]6-oxo-$PGF_{1\alpha}$ (10 ng). The recovery was 51.4 ± 7.4% (mean ± S.D.). The recovery through the procedure where no TLC step was included was determined using 5 aqueous samples spiked as above. In this case, the recovery was 90.5 ± 6.9% (mean ± S.D.).

Retention data for the capillary-column GC separation of the methoxime, methyl ester, tris-trimethylsilyl (MO–Me–TMS) and the methoxime, PFB ester, tris-trimethylsilyl (MO–PFB–TMS) derivatives of 6-oxo-$PGF_{1\alpha}$ are given in Table I.

TABLE I

CAPILLARY COLUMN GC RETENTION DATA FOR THE DERIVATIVES OF 6-OXO-$PGF_{1\alpha}$

Column	*Temperature programme*	*Inlet pressure (kPa)*	*Retention times (min)*	
			MO–Me–TMS derivative	*MO–PFB–TMS derivative*
SE-54	100–290°C (20°C min^{-1})	100	8.4	12.0
SP-2100	100–280°C (20°C min^{-1})	140	10.9	16.5

Analyses were carried out on an SP-2100 or an SE-54 capillary column. The latter column was more convenient as shorter run times could be employed with little loss of resolution. No separation of *syn* and *anti* isomers was observed on either column.

The EI mass spectrum of the MO–Me–TMS derivative of 6-oxo-$PGF_{1\alpha}$ is shown in Fig. 1. Extensive fragmentation of the molecule occurs but SIM quantitation could be carried out using the fragment ion at *m/z* 508 $[M–TMSOH–MeO]^+$ for the ^{1}H form and *m/z* 512 for the ^{2}H form. The limit of detection of a standard sample was set at 500 pg injected on column. This corresponded to a signal-to-noise ratio of 3:1. It was not possible to use the fragment ions at *m/z* 378 (^{1}H) and *m/z* 382 (^{2}H) for quantitative analysis because of substantial interference in this lower mass range.

The NICI mass spectrum of the MO–PFB–TMS derivative of 6-oxo-$PGF_{1\alpha}$ is shown in Fig. 2. The inclusion of the PFB ester group enhanced the electron-capturing ability of the molecule and allowed efficient negative ion chemical ionisation. The base peak at *m/z* 614 $[M - C_7H_2F_5]^-$ for the ^{1}H form and *m/z* 618 for the ^{2}H analogue were used for quantitation. A limit of detection when employing a 10 ng internal standard was set at 2 pg injected on column. This amount could be detected with a signal-to-noise ratio of 10:1 and provided a peak area ratio which was twice the value obtained for the intercept on the standard curve. The enhanced sensitivity and specificity provided by NICI–MS, was employed in subsequent analyses.

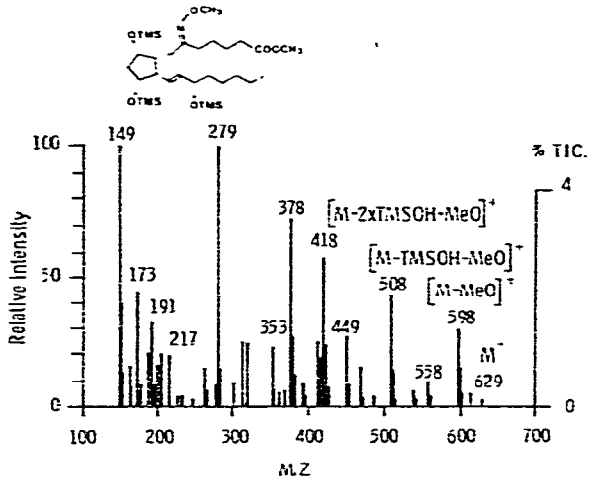

Fig. 1. EI mass spectrum of 6-oxo-$PGF_{1\alpha}$ as MO–Me–TMS derivative.

The standard curve for the range 0–10 ng using 10 ng internal standard gave a regression line of $y = 0.0103 + 0.1347x$; $r^2 = 0.997$. For the range 0–20 ng when 2 ng internal standard was used, a regression line of $y = 0.0090 + 0.6877x$; $r^2 = 0.994$ was obtained.

Endogenous levels of 6-oxo-$PGF_{1\alpha}$ in human urine were detected using this assay. A typical limited mass chromatogram is illustrated in Fig. 3. In this example, the concentration of 6-oxo-$PGF_{1\alpha}$ was found to be 46 pg ml^{-1}. This assay has also been used to investigate the generation of prostaglandins by mast cells in response to an immunological stimulus. Fig. 4 shows an SIM analysis of the medium in which purified rat peritoneal mast cells had been stimulated using compound 48/80. In this case, no TLC purification of the fluid was necessary and thromboxane B_2 (TxB_2) in addition to 6-oxo-$PGF_{1\alpha}$ was identified on the chromatogram. This compound has

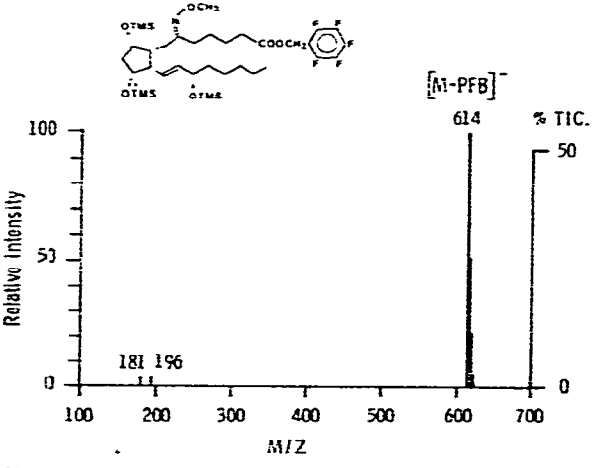

Fig. 2. NICI mass spectrum of 6-oxo-$PGF_{1\alpha}$ as MO–PFB–TMS derivative.

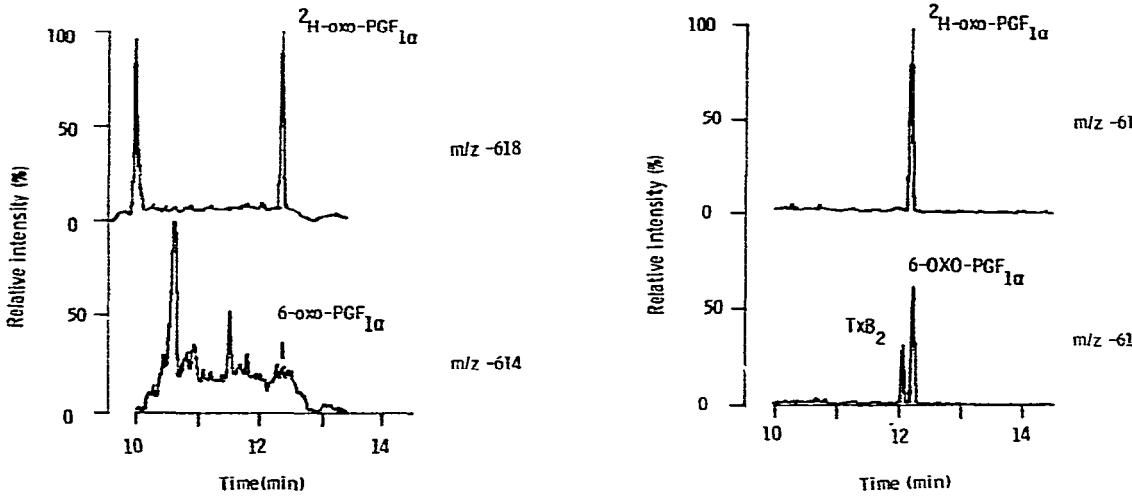

Fig. 3. Limited mass chromatogram of 6-oxo-PGF$_{1\alpha}$ and [3,3',4,4'-^{2}H$_4$]6-oxo-PGF$_{1\alpha}$ extracted from human urine.

Fig. 4. Limited mass chromatogram of 6-oxo-PGF$_{1\alpha}$ extracted from mast cell incubation medium after stimulation with compound 48/80.

the same molecular weight as 6-oxo-PGF$_{1\alpha}$ and similar gas chromatographic properties. Fig. 5 illustrates the NICI mass spectrum of the MO–PFB–TMS derivative of TxB_2 which can be only distinguished from that of 6-oxo-PGF$_{1\alpha}$ by the additional fragment ion at *m/z* 582 $[M - C_7H_2F_5 - MeOH]^-$. Concentrations of 6-oxo-PGF$_{1\alpha}$ determined in this example for cells incubated in the presence of compound 48/80 and in control buffer were 12.5 ng per 10^6 mast cells and 11.6 ng per 10^6 mast cells respectively. Values of 63% and 6% of total histamine release were obtained for these incubates.

A limited mass chromatogram of 6-oxo-PGF$_{1\alpha}$ obtained from an incubation of rat aortic rings in human plasma is shown in Fig. 6. In the example illustrated an

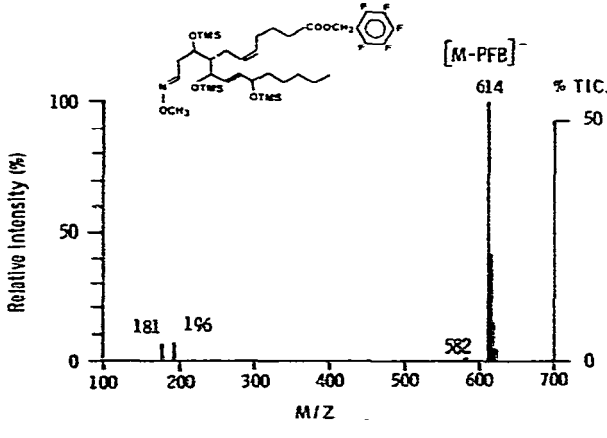

Fig. 5. NICI mass spectrum of TxB_2 as MO–PFB–TMS derivative.

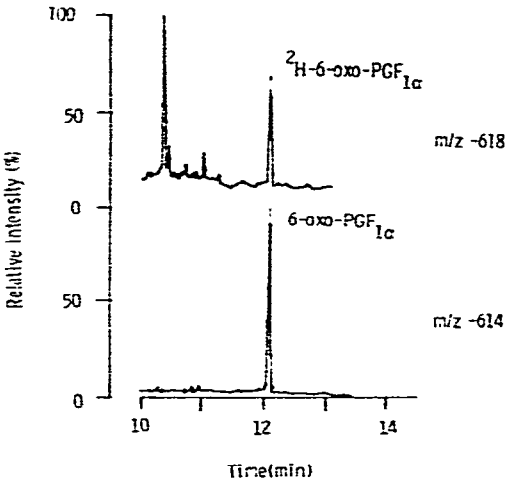

Fig. 6. Limited mass chromatogram of 6-oxo-PGF$_{1\alpha}$ and [3,3',4,4'-^{2}H$_4$]6-oxo-PGF$_{1\alpha}$ extracted from human plasma after incubation with rat aortic rings. The internal standard chromatogram (−618) has been multiplied by a factor of five.

internal standard of 2 ng rather than 10 ng was used and the concentration of 6-oxo-PGF$_{1\alpha}$ in the plasma incubate was determined by GC–MS as 6.37 ng/100 μl.

DISCUSSION

The extraction, purification and quantitation of 6-oxo-PGF$_{1\alpha}$ from biological fluids, outlined in this paper, offers several advantages over methods described previously. μBondapak Sep-Pak cartridges provided a very quick and simple extraction procedure using small volumes of organic solvents. Silica Sep-Pak cartridges were incorporated into the method to provide a simple clean-up step. For fluids obtained from mast cell and aortic ring incubations, no additional sample purification was necessary prior to derivatisation and GC–MS analysis. Recovery through the assay was virtually quantitative.

It was necessary to include an additional clean up step in the assay of urine samples. In this case, contaminants can overshadow the relatively low concentrations of 6-oxo-PGF$_{1\alpha}$ present. If these contaminants are not removed, or at least minimised, suppression of ionisation in the source of the mass spectrometer can result in a dramatic decrease in sensitivity.

In our initial studies, preparative TLC was carried out on samples prior to methoximation. However, we found that no 6-oxo-PGF$_{1\alpha}$ could be detected by GC–MS following derivatisation unless a borate back extraction was included. TLC residues were dissolved in borate buffer at pH 8.5 which was extracted with ethyl acetate (discarded); the aqueous layer was acidified to pH 3.0 and 6-oxo-PGF$_{1\alpha}$ recovered by a second extraction with ethyl acetate. Even using this method, recoveries were rather variable. As an alternative, we investigated the TLC properties of the methoxime derivative of 6-oxo-PGF$_{1\alpha}$ and found that this compound gave a sharp band on TLC which could be eluted with consistently good recovery. It was not

necessary to include any additional extraction procedure following TLC and residual silica in the samples did not interfere with subsequent derivative formation.

We have speculated that 6-oxo-$PGF_{1\alpha}$ may be converted to a ketal or hemiketal[11] on TLC plates and such structures are not then accessible to methoximation. It is possible that by including a borate back extraction[4], or by just partitioning the TLC residue between an aqueous and organic phase[5], the ketal formation is reversed so that methoximation proceeds smoothly. In the method described in this paper, the possibility of ketal formation is avoided by trapping the ketone functionality as a methoxime prior to TLC purification.

The specificity of this assay was aided by the use of capillary column GC. This is illustrated by the baseline separation of the MO–PFB–TMS derivatives of TxB_2 and 6-oxo-$PGF_{1\alpha}$ derived from incubations of rat peritoneal mast cells. These compounds are not fully resolved on packed columns. Previous investigators have resorted to a TLC separation prior to GC–MS analysis when both these compounds have been present in a biological fluid. Using capillary columns this was not necessary.

Other GC–MS methods used to quantitate 6-oxo-$PGF_{1\alpha}$ have relied on EI-MS. In this mode, considerable fragmentation of the molecule leaves few ions suitable for sensitive SIM. We reasoned that a softer form of ionisation might reduce such fragmentation and provide an ion of sufficiently high intensity and high mass to give increased sensitivity and selectivity over EI-MS. Positive ion chemical ionisation (PICI) gives minimal fragmentation but poor efficiency of ionisation. For NICI-MS, it was necessary to enhance the electron capturing ability of the 6-oxo-$PGF_{1\alpha}$ molecule. This was achieved by esterification with PFBB[15]. Using this derivative and monitoring the fragment ion at m/z 614, it was possible to improve the limit of sensitivity from 500 pg (EI-MS) to 2 pg (NICI-MS) injected on column. This method of quantitation should be applicable to the full range of prostaglandins where in each case the carboxylic acid functionality can be converted to an electron-capturing ester.

The observation that PGI_2 is the major prostanoid produced by the renal cortex[6] prompted us to attempt quantitation of 6-oxo-$PGF_{1\alpha}$ in urine. Infused PGI_2 and 6-oxo-$PGF_{1\alpha}$ can be recovered as 6-oxo-$PGF_{1\alpha}$ in the urine of human subjects[12]. There is only one report of quantitation of endogenous 6-oxo-$PGF_{1\alpha}$ in human urine and no methodological detals or concentrations were given[13]. Modification of the present assay to include a chromatographic separation step permits routine determinations of urinary 6-oxo-$PGF_{1\alpha}$. The assay is currently being employed to investigate a possible renal role for PGI_2 in man.

This assay was also used in a preliminary study to measure levels of 6-oxo-$PGF_{1\alpha}$ released from mast cells. We found that both stimulated and unstimulated rat peritoneal mast cells were capable of synthesizing PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$). However, no significant increase in the concentration of 6-oxo-$PGF_{1\alpha}$ was observed after stimulation with compound 48/80. This contrasts with the work of Roberts *et al.*[14] who reported very variable elevations of 6-oxo-$PGF_{1\alpha}$ levels after stimulation with the ionophore A 23187. This may reflect differences in the mode of mast cell activation by these agents. Work is now in progress to compare the release of prostaglandins by a range of different releasors. The chromatographic resolution of 6-oxo-$PGF_{1\alpha}$ from TxB_2 will allow quantitation of the latter prostanoid in future studies.

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REFERENCES

1 S. Moncada, R. Gryglewski, S. Bunting and J. R. Vane, *Nature (London)*, 263 (1976) 663.
2 J. A. Salmon, *Prostaglandins*, 15 (1978) 383.
3 F. A. Fitzpatrick, D. A. Stringfellow, J. Maclouf and M. Rigaud, *J. Chromatogr.*, 177 (1979) 51.
4 C. N. Hensby, G. A. Fitzgerald, L. A. Friedman, P. J. Lewis and C. T. Dollery, *Prostaglandins*, 18 (1979) 731.
5 M. Claeys, C. van Hove, A. Duchateau and G. A. Herman, *Biomed. Mass Spectrom.*, 7 (1980) 544.
6 A. R. Whorton, M. Smigel, J. A. Oates and J. C. Frolich, *Biochim. Biophys. Acta*, 529 (1978) 176.
7 G. Atkinson, M. Ennis and F. L. Pearce, *Brit. J. Pharmacol.*, 65 (1979) 395.
8 T. J. Sullivan, K. L. Parker, W. Stenson and C. W. Parker, *J. Immunol.*, 114 (1975) 1473.
9 J. M. Ritter, M. A. Orchard, I. A. Blair and P. J. Lewis, *Biochem. Pharmacol.*, in press.
10 H. M. Fales, T. M. Jaouni and J. F. Babashak, *Anal. Chem.*, 45 (1973) 2302.
11 K. C. Nicolaou, W. F. Barnette and R. L. Magolda, *J. Chem. Res. (M)*, (1979) 2444.
12 B. Rosenkranz, C. Fischer, K. E. Weimer and J. C. Frolich, *J. Biol. Chem.*, 255 (1980) 10194.
13 J. C. Frolich, B. Rosenkranz, W. Kitajuna, C. Fischer and L. Walker, in P. J. Lewis and J. O'Grady (Editors), *Clinical Pharmacology of Prostacyclin*, Raven Press, New York, 1981, p. 53.
14 L. J. Roberts II, R. A. Lewis, J. A. Oates and K. F. Austen, *Biochim. Biophys. Acta*, 575 (1979) 185.
15 B. H. Min, J. Pao, W. A. Garland, J. A. F. de Silva and M. Parsonnet, *J. Chromatogr.*, 183 (1980) 411.