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QUANTITATIVE ANALYSIS OF 6-KETO-PROSTAGLANDIN $F_{1\alpha}$ USING IMMUNOAFFINITY PURIFICATION AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

This paper describes an immunoaffinity purification technique for 6-keto-prostaglandin $F_{1\alpha}$ ($6KPGF_{1\alpha}$) prior to quantitative analysis by high-resolution gas chromatography–negative-ion chemical ionization mass spectrometry (HRGC–NICIMS). Polyclonal antibodies to $6KPGF_{1\alpha}$ were partially purified using *Staphylococcus aureus* Protein A immobilized on Sepharose CL-4B. This partially purified fraction was covalently bound to silica gel using N-hydroxysuccinimidyl-functionalized silica. Columns constructed using this gel quantitatively bound $6KPGF_{1\alpha}$ which could be eluted quantitatively with acetonitrile–water (19:1). Binding capacity was reconstituted by washing with 0.01 *M* phosphate buffer (pH 7.4). Human urinary and canine plasma $6KPGF_{1\alpha}$ was sufficiently purified using these columns that HRGC–NICIMS analysis of the methoxime–pentafluorobenzyl–tris-trimethylsilyl derivative was interference-free.

INTRODUCTION

Immunoaffinity purification coupled with gas chromatographic–mass spectrometric (GC–MS) analysis is an analytical methodology which shows great promise and most likely will be used with increasing frequency in the quantitative analysis of trace compounds in complex biological milieu. This analytical technique offers a very high degree of specificity due to differences in the physicochemical principles involved in the purification (specific binding based upon stereochemical discrimination), gas chromatography (volatility, polarity, etc.) and mass spectrometry (mass) steps in the analysis [1, 2]. Immunoaffinity purification prior to GC–MS analysis also offers the potential of greatly decreased analysis time [1–4].

Gaskel and Brownsey [1] used immunoaffinity extraction to purify estradiol-17β from plasma prior to GC–high-resolution MS (GC–HRMS) analysis of the

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tris-trimethylsilyl (TMS) derivative. Their study is the first reported use of an immobilized antibody for sample purification prior to GC–MS analyses. In that study anti-estradiol-17β serum was coupled to cyanogen bromide-activated microcellulose. The matrix was not packed in columns but was instead used in repetitive binding, wash and centrifugation cycles. Iloprost®, a stable prostaglandin analogue, has been quantified in plasma with a limit of detection of 5 pg/ml using immunoaffinity extraction followed by HRGC–negative-ion chemical ionization MS (HRGC–NICIMS) analysis of the pentafluorobenzyl–TMS (PFB–TMS) derivative [5]. Cyanogen bromide-activated Sepharose 4B was used to immobilize the antibodies in this study. A chemically stable prostacyclin analogue, CS-570, has been quantified in plasma using immunoaffinity chromatography (antisera was coupled to Sepharose 4B) and NICIMS of the PFB ester derivative [6]. This technique has also been successfully used in quantifying 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine [7]. We recently reported an immunoaffinity extraction HRGC–NICIMS method for urinary thromboxane B_2 (TXB_2) monitoring $M^- - 181$ of the methoxime–PFB ester–tris-*tert.*-butyldimethylsilyl (MO–PFB-tBDMS) derivative [3, 4]. In this study polyclonal TXB_2 antibody was partially purified using immobilized *Staphylococcus aureus* Protein A and then immobilized using an N-hydroxysuccinimidyl silica gel. Urinary TXB_2 and dinor-TXB_2 have recently been reported to be sufficiently purified by immobilized antibody to TXB_2 for subsequent GC–MS analysis [8].

One of the research efforts in this laboratory is the development of simplified, rapid yet highly specific MS procedures for the analysis of arachidonic acid metabolites (AAM) in urine and plasma. Prostacyclin (PGI_2) is of great interest to medical scientists because of its potent effects to inhibit blood platelet aggregation and relax vascular smooth muscle, thus decreasing the tendency for thrombosis to occur and decreasing total peripheral resistance. In vivo, PGI_2, which is produced by endothelial cells, is believed to act in balance with thromboxane A_2 (produced by platelets), an AAM that aggregates platelets and constricts vascular smooth muscle. Since PGI_2 is rapidly hydrolyzed to 6-ketoprostaglandin $F_{1\alpha}$ ($6KPGF_{1\alpha}$) there is interest in measuring this AAM as an index of PGI_2 formation. Urinary levels of $6KPGF_{1\alpha}$ are of particular interest because of difficulties in measuring plasma levels. Plasma $6KPGF_{1\alpha}$ levels are very low, approximately 1 pg/ml [9] and $6KPGF_{2\alpha}$ is generated during the sampling process. Also, urine collection is non-invasive.

This paper reports on an immunoaffinity purification GC–MS analytical technique for $6KPGP_{1\alpha}$ in human urine and canine plasma using an N-hydroxysuccinimidyl-functionalized silica coupling gel and HRGC–NICIMS.

EXPERIMENTAL

Materials

Analytical-grade solvents were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Methoxyamine hydrochloride was purchased from Regis (Morton Grove, IL, U.S.A.). Polypropylene syringes and pentafluorobenzyl bromide (PFBB) were purchased from Aldrich (Milwaukee, WI, U.S.A.). Sep-

Pak® octadecylsilyl (ODS) cartridges were obtained from Waters (Milford, MA, U.S.A.). ODS cartridges were preconditioned with 3 ml methanol followed by 5 ml glass-distilled water prior to use. Acetonitrile was used as the solvent for stock solutions. Glassware was silanized using dimethyldichlorosilane. $6KPGF_{1\alpha}$ was purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). $[^2H_4]6KPGF_{1\alpha}$ was a gift of Dr. John Pike, Upjohn Company (Kalamazoo, MI, U.S.A.). Polyvinylpyrrolidone (PVP) was purchased from Sigma (St. Louis, MO, U.S.A.). PVP buffer, pH 7.4, consisted of 8.76 g sodium chloride, 6 g Trizma, 1 g sodium azide and 1 g PVP per liter of water. Phosphate-buffered saline (PBS) consisted of 0.05 *M* potassium dihydrogenphosphate and 0.15 *M* sodium chloride adjusted to pH 7.4. Porous polypropylene sheets were obtained from Porex Plastics (Fairburn, GA, U.S.A.). Hydroxysuccinimidyl SP500 silica was purchased from Serva Feinbiochemica (Heidelberg, F.R.G.). Immobilized Protein A on Sepharose CL-4B and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Pierce (Rockford, IL, U.S.A.). $[^3H]6KPGF_{1\alpha}$ was obtained from NEN Research Products (Boston, MA, U.S.A.). Keyhole limpet hemocyanin was purchased from Calbiochem (San Diego, CA, U.S.A.).

Preparation of rabbit $6KPGF_{1\alpha}$ antiserum

$6KPGF_{1\alpha}$ (10.6 mg) was dissolved in 500 μl of anhydrous dioxane containing 20 μl of dry triethylamine and the mixture was cooled to 8–10°C. A solution of 4.1 mg of isobutyl chloroformate in 50 μl of dry dioxane was added to the cold mixture and the reaction was incubated at 8–10°C for 30 min. After this interval, the reaction mixture was frozen by reducing the temperature to 0°C, and a cold solution of keyhole limpet hemocyanin (50 mg) in 580 μl of 50% aqueous glycerol diluted with 1.5 ml distilled water was added. The mixture was maintained at 0°C for 1 h with stirring after the frozen layer had redissolved, then allowed to gradually warm to room temperature over another hour. The reaction mixture was then transferred to a dialysis bag and dialyzed against 4 l of distilled water at 4°C overnight. The water was changed three times at 24 h intervals and the dialysate was subsequently lyophilized to yield 26 mg of antigen.

Antigen (2 mg) was ground up in 1.9 ml of distilled water and emulsified in 2.0 ml of complete Freund's adjuvant. The emulsion (~2 ml) was injected intramuscularly (i.m.) into each of two New Zealand White rabbits at multiple sites in the flanks. One week later, each animal received the same dose of antigen administered i.m. in the flanks. This dosage was repeated at monthly intervals thereafter. Blood samples were taken one week after the third and subsequent injections, allowed to clot overnight at 5°C and centrifuged at 4 *g* to obtain the serum. The cross-reactivities of the antibody were 0.06% for TXB_2, 3.2% for $PGF_{2\alpha}$, 0.76% for PGE_2 and 50% for dinor-$6KPGF_{1\alpha}$.

Isolation of γ-globulin fraction from rabbit $6KPGF_{1\alpha}$ antiserum

A 1-ml volume of rabbit anti-$6KPGF_{1\alpha}$ serum, which was observed to bind ~50% of available $[^3H]6KPGF_{1\alpha}$ at a dilution of 1:36000 in a 400-μl volume, was diluted with 2.0 ml of isotonic phosphate buffer (0.1 *M*, pH 7.4) and added to a 10-ml bed of immobilized Protein A on Sepharose CL-4B. The solution was

allowed to remain on the column for 15 min at ambient temperature and the bed was subsequently washed with 50 ml of PBS buffer and 10 ml of 0.15 *M* sodium chloride. The bound immunoglobulin G (IgG) was then eluted with 15 ml of 1% glacial acetic acid in 0.15 *M* sodium chloride, and this fraction was immediately neutralized to pH 7 with 1 *M* sodium hydroxide and lyophilized.

Immobilization of 6KPGF$_{1\alpha}$ antiserum on hydroxysuccinimidyl silica gel

The lyophilized IgG was redissolved in 15 ml of distilled water and 250 mg of sodium bicarbonate added to bring the pH to 8. A 2.5-g portion of hydroxysuccinimidyl SP500 silica gel was added to a 50-ml pear-shaped flask and the IgG solution introduced to form a suspension with the silica. The flask was placed on a rotary evaporator under house vacuum (about 350 mmHg) and rotated 2 h at room temperature. Unreacted functional groups on the silica were subsequently capped by addition of 1 ml of 10% aqueous ethanolamine adjusted to pH 8.5 and rotation for 45 min more. After this interval, the gel was pipetted into three syringe columns such that each contained about 2 ml of silica. The columns were washed with 50 ml each of PBS, then 25 ml of 95% acetonitrile followed by 30 ml of PBS. The columns were stored under the same buffer saturated with nitrogen containing 0.1% sodium azide as a bacteriostat at 4°C.

Recovery of [^{3}H]6KPGF$_{1\alpha}$ and binding capacity of the anti-6KPGF$_{1\alpha}$ silica

The three columns prepared above using the IgG fraction from 1 ml of anti-6KPGF$_{1\alpha}$ serum bound to 2.5 g of SP500 silica were washed with 10 ml each of PBS buffer (50 m*M*, pH 7.4) and a solution of approximately 0.1 μCi of [^{3}H]6KPGF$_{1\alpha}$ (specific activity 150 Ci/m*M*) dissolved in 0.5 ml of PVP buffer was added to each column and allowed to incubate 15 min at room temperature. The labelled 6KPGF$_{1\alpha}$ had been previously purified by affinity chromatography prior to this experiment to remove all of the radioactive but immunologically unreactive impurities which constituted about 6% of the total radioactivity. Following incubation, each column was washed with 20 ml of PBS buffer and then 5 ml of distilled water. The liquid in the column void volume was pushed off with gentle positive pressure and the bound 6KPGF$_{1\alpha}$ was subsequently eluted with 20 ml of acetonitrile–water (19:1, v/v). The columns were immediately washed with PBS to remove the last traces of solvent. The three flasks containing the eluate from each column were taken to dryness on the rotary evaporator, and the residue was redissolved in 10 ml of counting cocktail. The individual solutions were transferred to scintillation vials for counting and the observed counts compared to a control consisting of 0.5 ml of the original tracer solution pipetted directly into a vial for counting.

The binding capacity of the silica was determined in a similar manner by incubating a solution of about 0.1 μCi of freshly purified [^{3}H]6KPGF$_{1\alpha}$ and 4 μg of 6KPGF$_{1\alpha}$ dissolved in 1.0 ml of PBS buffer on one of the columns (about 2 ml of silica). After 15 min at room temperature the column was washed with 25 ml of PBS and 5 ml of distilled water. The last of the distilled water in the column void volume was pushed off with gentle pressure and the bound 6KPGF$_{1\alpha}$ eluted with 20 ml of acetonitrile–water (19:1). This fraction was collected in a 50-ml

pear-shaped flask and evaporated to dryness under vacuum. The eluted $6KPGF_{1\alpha}$ was dissolved in 10 ml of counting solution, and the observed counts were compared to the total counts originally added.

Extraction of samples

Urine samples were collected in silanized glass flasks from seven non-smoking male subjects who were not taking medication known to influence prostaglandin synthesis. Venous and arterial blood samples from anesthetized dogs were collected in silanized glass flasks containing heparin. Samples were centrifuged and plasma aliquots (5 ml) removed. All samples were stored at −20°C. The $[^2H_4]6KPGF_{1\alpha}$ internal standard was added to 5-ml aliquots of each sample. Following adjustment of the pH to 3.0 using 10 *M* formic acid, samples were passed through ODS cartridges using a silanized glass syringe. ODS cartridges were rinsed with 5 ml glass-distilled water followed by 5 ml hexane. $6KPGF_{1\alpha}$ and co-eluting material were eluted with 10 ml ethyl acetate. The eluate was dried under a stream of nitrogen in a 40°C water bath. PBS, 1.0 ml, was added to the dried residue and samples were then sonicated to ensure total dissolution. The immunoaffinity columns were rinsed with 5 ml PBS before use. Each sample was added carefully to the column so as not to disturb the bed. Columns were equilibrated for 15 min and then washed with three 5-ml volumes of PBS and ten 5-ml volumes of distilled water. Bound $6KPGF_{1\alpha}$ was eluted with 95% acetonitrile (10 ml) into a silanized 15-ml glass tube. The eluate was then dried under nitrogen at 40°C. The residue was dissolved in three 0.35-ml volumes of methanol and transferred to a 1-ml conical-shaped reaction vial and dried under nitrogen. Columns were washed with 20 ml of 95% acetonitrile to remove any trace $6KPGF_{1\alpha}$ remaining. Columns were then washed with two 5-ml volumes of distilled water followed by two 5-ml volumes of PBS and stored refrigerated in PBS.

Derivatization

The MO–PFB–TMS derivative of $6KPGF_{1\alpha}$ was prepared as follows. All steps were carried out using 1-ml conical-shaped-bottom glass reaction vials. The dried eluate from the immunoaffinity column was dissolved in 100 μl of a solution of recrystallized methoxyamine hydrochloride in dry pyridine (3 mg/ml). The solution was heated at 60°C for 1 h or allowed to stand at room temperature overnight, then evaporated under nitrogen. The residue was dissolved in 100 μl of 1 *M* formic acid and extracted twice with 0.6 ml of ethyl acetate. The organic extracts were combined and evaporated under nitrogen. The residue was dissolved in 30 μl of acetonitrile, 20 μl of 20% PFBB in acetonitrile and 10 μl of 10% diisopropylethylamine in acetonitrile. The solution was vortex-mixed and allowed to stand at room temperature. After 30 min the sample was evaporated under nitrogen at ambient temperature. The residue was dissolved in 1 ml of 50% (v/v) hexane–methylene chloride and washed with 0.5 ml of glass-distilled water. The organic layer was transferred to a clean vial and the aqueous layer extracted with a further 0.5 ml of 50% hexane–methylene chloride. The organic extracts were combined and evaporated to dryness under nitrogen. The residue was dissolved in 25 μl of BSTFA and heated at 80°C for 1 h or allowed to stand overnight at

ambient temperature. The reaction mixture was evaporated under nitrogen until 1–2 μl of BSTFA remained and 20 μl of *n*-dodecane were added. The sample was vortexed and stored dessicated until GC–MS analysis.

Gas chromatography–mass spectrometry

A Nermag R30-10 triple quadrupole mass spectrometer–mass spectrometer system interfaced to a Varian 3400 gas chromatograph and a Digital Equipment Corporation 11/73 processor with SIDAR software was used in these analysis. A cross-linked methylsilicone fused-silica capillary column (Hewlett-Packard Ultra 1, 25 m×0.2 mm I.D., 0.11 μm film thickness) was directly interfaced with the ion source of the mass spectrometer. The following conditions of analysis were used: linear velocity was set at 40 cm/s at 290°C oven temperature, on-column injection (J&W Scientific) at 190°C oven temperature, ramping GC oven temperature up to 250°C at 40°C/min, then up to 305°C at 4°C/min, ammonia reagent gas, source pressure of $8 \cdot 10^{-2}$ Torr and operating in the single-stage mode using the third quadrupole as the mass analyzer.

RESULTS

Recovery of [^{3}H]6KPGF$_{1\alpha}$ and binding capacity of the anti-6KPGF$_{1\alpha}$ silica

Recoveries of [^{3}H]6KPGF$_{1\alpha}$ were greater than 95% for all determinations. A small percentage of the 6KPGF$_{1\alpha}$ was retained by each column. Therefore, when using the same columns repeatedly for a series of extractions, we found it necessary to follow each elution step with an additional acetonitrile wash of about 20 ml to completely remove all traces of 6KPGF$_{1\alpha}$ before reconstituting the column with PBS buffer. The antibody gel was observed to have a binding capacity of approximately 1 μg/ml. Such a capacity is far in excess of the levels found in practical sample volumes of urine or plasma.

GC–MS analysis

Fig. 1 shows the *m/z* 614 (endogenous 6KPGF$_{1\alpha}$) and *m/z* 618 ([^{2}H$_4$]6KPGF$_{1\alpha}$) SIM traces obtained for a representative canine plasma sample. Ions monitored are the carboxylate anions, formed by dissociative electron capture, of the MO–PFB–TMS derivative of 6KPGF$_{1\alpha}$ ($M^- - 181$). The NICI mass spectrum of this compound has been published previously [9]. No interferences at these ion currents were noted in the region where plasma (*m/z* 614) and labeled (*m/z* 618) 6KPGF$_{1\alpha}$ elute. The concentrations of 6KPGF$_{1\alpha}$ in canine plasma were higher than anticipated (approximately 100–400 pg/ml of plasma) presumably due to vascular trauma. [^{2}H$_4$]6KPGF$_{1\alpha}$ was present at 50 pg/ml, thus the greater response seen for *m/z* 614 in Fig. 1.

Reproducibility of the 6KPGF$_{1\alpha}$ immunoaffinity purification technique was determined by repeated purification of the same pooled urine sample (pooled from seven subjects) following addition of the [^{2}H$_4$]6KPGF$_{1\alpha}$ internal standard. Concentration of 6KPGF$_{1\alpha}$ in the pooled urine sample was found to be 146 ± 5.7 pg/ml (mean ± S.D., $n=5$). Urinary 6KPGF$_{1\alpha}$ levels in a population of healthy non-smoking males free of medications known to influence PGI$_2$ production were

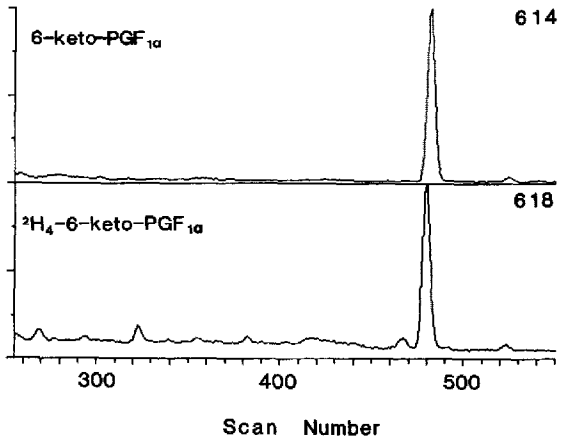

Fig. 1. SIM plots of m/z 614 ($M^- - 181$ for MO-PFB-TMS-6KPGF$_{1\alpha}$) and m/z 618 ($M^- - 181$ for MO-PFB-TMS-[$^2H_4$]6KPGF$_{1\alpha}$). A 20-ml aliquot of canine plasma was purified using immunoaffinity chromatography. Relative sensitivities (m/z 614/618) are 339 000/74 500. Conditions of analysis are given in the text.

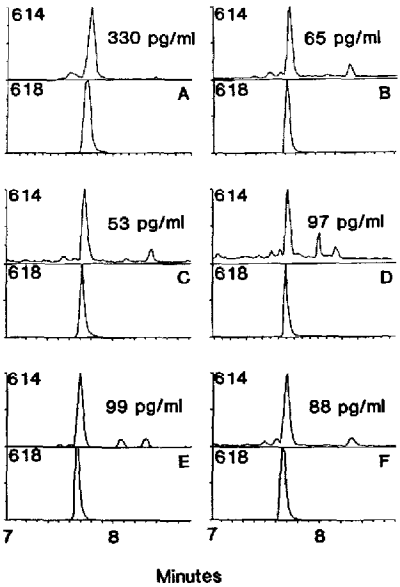

Fig. 2. SIM of m/z 614 ($M^- - 181$ for MO-PFB-TMS-6KPGF$_{1\alpha}$) and m/z 618 ($M^- - 181$ for MO-PFB-TMS-[$^2H_4$]6KPGF$_{1\alpha}$) obtained for six different urine samples. Subjects were non-smoking males not taking non-steroidal antiinflammatory drugs. Relative sensitivities (m/z 614/618) for each trace are: (A) 97 100/315 000; (B) 41 700/634 000; (C) 10 100/171 000; (D) 41 000/454 000; (E) 18 200/95 000; (F) 77 100/80 900. Urinary concentrations of 6KPGF$_{1\alpha}$ calculated for each sample are also shown.

determined in the usual manner by addition of a fixed amount of [$^2H_4$]6KPGF$_{1\alpha}$ to an aliquot (10 ml) of each urine sample and constructing a standard curve by varying the amount of unlabeled 6KPGF$_{1\alpha}$ standard. The SIM traces for each urine sample are shown in Fig. 2. Individual values are also indicated. The

mean ± S.D. value obtained for urinary $6KPGF_{1\alpha}$ was 122 ± 94.5 pg/ml of urine ($n=6$).

DISCUSSION

The utility of GC–MS in the quantitative analysis of arachidonic acid metabolites in urine and other biological samples has been well established. Quantitative analysis of AAM using stable-isotope dilution GC–MS in highly complex biological milieu, such as urine, can be divided into discrete steps. In general, these include the addition of a stable isotope-labeled internal standard, crude extraction of the analyte(s) using solvent or solid-phase methods (e.g. extraction from acidified aqueous sample into ethyl acetate or adsorption from acidified aqueous sample into ODS silica and elution with ethyl acetate), one or more chromatogaphic purification steps (e.g. thin-layer chromatography, high-performance liquid chromatography), chemical derivatization to compounds with appropriate GC (volatility) and MS (formation of an intense, characteristic ion) properties and finally the GC–MS analysis. Chromatographic purification procedures for urinary AAM necessary for obtaining interference-free chromatograms when using GC–MS can be particularly troublesome. In general, these procedures can result in significant loss of analyte and are relatively time-consuming. In view of the problems associated with the purification step(s) and our interest in the biology of PGI_2, we have developed immunoaffinity columns for the purification of $6KPGF_{1\alpha}$. This overall analytical approach to the measurement of urinary $6KPGF_{1\alpha}$ is attractive in that the biological matrix is very complex and the method offers a high degree of specificity due to the difference in the physiochemical principles involved in each step of the analysis. Using this technique human urinary and canine plasma $6KPGF_{1\alpha}$ was sufficiently purified that interference-free chromatograms were obtained in subsequent MS analysis. This approach allows for relatively rapid purification and represents an improvement over other chromatographic procedures. Indeed the purification step is less time-consuming than the extraction, derivatization or GC–MS analysis steps individually. The values we obtained for urinary $6KPGF_{1\alpha}$ in a population of "normal" volunteers using immunoaffinity purification MS are not different from previously reported literature values [10–12].

Agarose was used as the support matrix in the early phase of the development of this method. We subsequently noted interferences due to non-specific adsorption when using agarose as the supporting matrix and evaluated silica as the support matrix. A commercially available coupling gel (hydroxysuccinimidyl SP500, Serva) has given favorable results. We have had some success synthesizing our own silica coupling gels. We have recently noted differences in the performance (amount of non-specific adsorption) of different batches of silica coupling gel and believe that deactivation of the silica is critical to the successful use of these immunoaffinity gels, at least in the present application. These immobilized antibody gels can be used repeatedly with little or no loss in binding capacity. One column was cycled 50 times and retained greater than 95% of the original total capacity. However, use of 100% acetonitrile will decrease binding

capacity. The total binding capacity for different batches of immunoaffinity gels is variable. However, this is not a problem since the total capacity is far in excess of the amount of AAM in a typical urine aliquot (5–20 ml) and 1 ml of antiserum will typically have enough specific binding activity for 20 columns with 100–200 ng total capacity per column. Supply of columns is not a problem since the columns can be used repeatedly and a large number of columns can be constructed using one rabbit (up to 20 columns per ml antiserum × up to 25 ml antiserum per bleed × up to 50 bleeds per rabbit = up to 25 000 columns per rabbit).

In summary, we have developed silica-based immunoaffinity gels for $6KPGF_{1\alpha}$ which can be used repeatedly. We have previously described a similar immunoaffinity GC–MS analytical technique for the measurement of urinary TXB_2 and using MO–PFB–tBDMS derivatives [4]. The gels can be used repeatedly and interference-free chromatograms were obtained using HRGC–NICIMS and monitoring the carboxylate anion ($M^- - 181$). By using columns constructed from these gels the purification step became the least time-consuming step in the overall analytical scheme.

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REFERENCES

1 S.J. Gaskell and B.G. Brownsey, Clin. Chem., 29 (1983) 677.
2 J.J. Vrbanac, T.D. Eller, H.L. Hubbard, R.H. Baker, P.V. Halushka and D.R. Knapp, 33rd Annual Conference on Mass Spectrometry and Allied Topics, San Diego, CA, 1985, p. 692.
3 J.J. Vrbanac, T.D. Eller, H.L. Hubbard, R.H. Baker, P.V. Halushka, I.A. Blair and D.R. Knapp, 34th Annual Conference on Mass Spectrometry and Allied Topics, Cincinnati, OH, 1986, p. 110.
4 H.L. Hubbard, T.D. Eller, D.E. Mais, P.V. Halushka, R.H. Baker, I.A. Blair, J.J. Vrbanac and D.R. Knapp, Prostaglandins, 33 (1987) 149.
5 W. Krause, U. Jakobs, P.E. Schulze, B. Nieuweboer and M. Hümpel, Prostaglandins Leukotrienes Med., 17 (1985) 167.
6 A. Nakagawa, Y. Tanishima, T. Hirota, H. Takahagi, M. Horiguchi and Y. Kawahara, Bunseki Kagaku, 35 (1986) 298.
7 U. Lemm, J. Tenczer, H. Baudisch and W. Krause, J. Chromatogr., 342 (1985) 393.
8 C. Chiabrando, A. Benigni, A. Piccinelli, C. Carmmati, E. Cozzi, G. Remazzi and R. Fanelli, Anal. Biochem., 163 (1987) 255.
9 I.A. Blair, S.E. Barrow, K.A. Waddell, P.J. Lewis and C.T. Dollery, Prostaglandins, 23 (1982) 579.
10 S. Fischer, B. Scherer and P.L. Weber, Biochim. Biophys. Acta, 750 (1983) 127.
11 H. Gleispach, B. Mayer, L. Rauter and E. Wurz, J. Chromatogr., 273 (1983) 161.
12 C. Fischer and C.O. Meese, Biomed. Mass Spectrom., 12 (1985) 399.