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QUANTITATIVE PROFILING OF 6-KETOPROSTAGLANDIN $F_{1\alpha}$, 2,3-DINOR-6-KETOPROSTAGLANDIN $F_{1\alpha}$, THROMBOXANE B_2 AND 2,3-DINOR-THROMBOXANE B_2 IN HUMAN AND RAT URINE BY IMMUNOAFFINITY EXTRACTION WITH GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

A rapid and simple method based on immunoaffinity extraction, stable isotope dilution and gas chromatography–mass spectrometry has been developed for profiling urinary metabolites of prostacyclin and thromboxane 6-Ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (2,3-dinor-6-keto-$PGF_{1\alpha}$), thromboxane B_2 (TXB_2) and 2,3-dinor-thromboxane B_2 (2,3-dinor-TXB_2) were quantitatively extracted from human or rat urine spiked with deuterated internal standards using mixed-bed columns containing immobilized anti-6-keto-$PGF_{1\alpha}$ and anti-TXB_2 antibodies (cross-reacting with 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 2,3-dinor-TXB_2, respectively) The extract was directly derivatized to form pentafluorobenzyl ester, methyloxime, trimethylsilyl ether derivatives Quantitation was performed by stable isotope dilution assay and high-resolution gas chromatography–negative ion chemical ionization mass spectrometry, by monitoring the carboxylate anions (M − 181) of the derivatized metabolites The method was applied to evaluate the urinary excretion of 6-keto $PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2 and 2,3-dinor-TXB_2 in humans and rats Results were in accordance with previously reported data obtained by other methods Novel data on the urinary excretion of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in rats under basal conditions are presented This sensitive and selective method represents a significant

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advance in terms of rapidity and simplicity over other immunoaffinity–gas chromatography–mass spectrometry methods for measuring single prostanoids, such as 6-keto-$PGF_{1\alpha}$ or TXB_2, since it allows profiling of a group of metabolites whose balance is important in several physiopathological conditions

INTRODUCTION

Prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) are cyclooxygenase metabolites of arachidonic acid (AA) with opposing, potent effects on platelet aggregation and vascular tone [1] PGI_2 and TXA_2 are the main AA metabolites in endothelial cells and in platelets, respectively A correct balance between the two compounds may be important to maintain cardiovascular homeostasis [2,3] PGI_2 and TXA_2 are also synthesized by other cells and organs, including tumoral and inflammatory cells, and appear to be involved in various biological reactions in health and disease [4,5]

It is commonly accepted that urinary levels of the inactive stable hydrolysis products of PGI_2 and TXA_2 [6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2), respectively] and their β-oxidation metabolites [2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (2,3-dinor-6-keto-$PGF_{1\alpha}$) and 2,3-dinor-thromboxane B_2 (2,3-dinor-TXB_2)] represent a valuable index of the endogenous production of the active but unstable parent compounds In particular, urinary 6-keto-$PGF_{1\alpha}$ and TXB_2 should represent an index of renal biosynthesis, and their dinor metabolites should be indicative of systemic production of PGI_2 and TXB_2, respectively [6]

Efforts have recently been made to develop sensitive and selective chemicophysical methods aimed at monitoring the in vivo biosynthesis of PGI_2 and TXB_2 in physiopathological conditions and during pharmacological manipulation of AA metabolism [7–11] These methods, however, do not include quantitative profiling of urinary metabolites of PGI_2 or TXA_2, an approach that might be useful to monitor any imbalance in the biosynthesis of these metabolites in various clinical and experimental settings A preliminary report dealing with immunoaffinity–gas chromatography–mass spectrometry (GC–MS) profiling of these metabolites, without quantitative applications, has been presented by Vrbanac et al [12]

We have therefore devised a method for the simultaneous measurement of urinary 6-keto-$PGF_{1\alpha}$, TXB_2 and their dinor metabolites using mixed-bed immunoaffinity columns prepared with immobilized anti-TXB_2 and anti-6-keto-$PGF_{1\alpha}$ antibodies (cross-reacting with the dinor metabolites) For this purpose, we have modified our previous immunoaffinity–GC–MS method employing anti-TXB_2 antibodies for profiling urinary thromboxanes [13] Other authors have recently reported on the use of immunoaffinity purification of pre-extracted samples for the MS analysis of single prostanoids, such as 6-keto-$PGF_{1\alpha}$ [14] or TXB_2 [15]

EXPERIMENTAL

Materials

6-Keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2, 2,3-dinor-TXB_2, [3,3′,4,4′-$^2H_4$]6-keto-$PGF_{1\alpha}$ (6-keto-$PGF_{1\alpha}$-d_4) were kind gifts from Dr J Pike of Upjohn (Kalamazoo, MI, U.S A). [17,17′,18,18′-$^2H_4$]2,3-Dinor-6-keto-$PGF_{1\alpha}$(2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4) was obtained through the courtesy of Dr. G A. FitzGerald of Vanderbilt University (Nashville, TN, U.S A) [5,6,8,9,11,12,14,15-$^2H_8$]TXB_2 (TXB_2-d_8) was kindly provided by Dr. G. Galli of the University of Milan (Milan, Italy). Pentafluorobenzyl (PFB) bromide, N,N-diisopropylethylamine and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Fluka (Buchs, Switzerland); methoxyamine hydrochloride (MOX) was obtained from Pierce (Rockford, IL, U.S A), bis([$^2H_9$]trimethylsilyl)acetamide was obtained from ICN Biomedicals (Cambridge, MA, U.S A) Cyanogen bromide (CNBr)-activated Sepharose 4B was obtained from Pharmacia (Uppsala, Sweden)

Antisera

Antisera against TXB_2 (35% cross-reacting with 2,3-dinor-TXB_2) or 6-keto-$PGF_{1\alpha}$ (36% cross-reacting with 2,3-dinor-6-keto-$PGF_{1\alpha}$) were obtained in rabbits as described previously [16] Complete cross-reactivity data for both antisera are reported elsewhere [13,16]

The immunoglobulin G (IgG) fraction was isolated from the antisera and coupled to CNBr-activated Sepharose-4B as previously described for anti-TXB_2 antiserum [13], according to the procedure of Krause et al [17]. 16 mg of IgG from the anti-TXB_2 antiserum were coupled to 1.75 g of Sepharose and 9 mg of IgG from the anti-6-keto-$PGF_{1\alpha}$ antiserum were coupled to 0 92 g of Sepharose The immobilized antibodies were suspended in 0.1 *M* phosphate buffer containing 0.02% merthiolate and stored at 4 °C in the dark until used.

Immunoaffinity extraction

The extraction procedure, described in detail elsewhere [13], was slightly modified as follows

Different urine volumes were used, corresponding to ca. 4 min diuresis for human urine (1–10 ml) and ca 5 h diuresis for rat urine (2–5 ml). Filtered urine was diluted to a final volume of 20 ml with phosphate buffer (50 m*M*, pH 7 4), containing deuterated analogues of the analytes.

Samples were then acidified to pH 2 0 with 1 *M* hydrochloric acid, and after 1 h at room temperature neutralized to pH 7.4. This procedure was necessary to equilibrate rapidly the deuterated form of 2,3-dinor-6-keto-$PGF_{1\alpha}$ with the endogenous compound [7]. Urinary samples were then percolated at a flow-rate of ca 0 5 ml/min through the immunoaffinity column (empty Bond-Elut 3-ml reservoir) containing the immobilized anti-6-keto-$PGF_{1\alpha}$ antibodies (0 25

ml of gel) mixed with the immobilized anti-TXB_2 antibodies (0 3 ml of gel) Some experiments were performed using columns containing only the anti-6-keto-$PGF_{1\alpha}$ antibodies

The column was then washed with 20 ml of water and eluted with acetone–water (95 5, v/v), the columns could then be reused after appropriate washing [13] The eluate was dried under a stream of air and derivatized to form the PFB ester methyloxime (MO), trimethylsilyl (TMS) ether derivatives of the extracted compounds The reaction was carried out as follows 30 μl of PFB bromide–acetonitrile (1 20, v/v) and 5 μl of diisopropylethylamine were added to the residue, heated for 5 min at 40 °C and dried under a stream of air This procedure was repeated, and 50 μl of MOX-acetonitrile (2 3, v/v) were added (60 °C, 1 h) The sample was then dried, and the residue was dissolved in 50 μl of BSTFA After 15 min at 60 °C the sample was dried, then redissolved in appropriate volumes (down to 20 μl) of BSFTA and directly injected into the gas chromatograph–mass spectrometer

High-resolution gas chromatography–negative ion chemical ionization mass spectrometry

A Finnigan 4000 EI/CI quadrupole mass spectrometer, equipped with a Teknivent–IBM PC-XT data system and directly interfaced with a DANI (Monza, Italy) 6500 gas chromatograph, was used GC operating conditions were CP-Sil 5 CB (Chrompack, The Netherlands) fused-silica capillary column (25 m $\times$ 0.32 mm I D ; 0 12 μm film thickness), oven temperature, isothermal at 160 °C for 1 min, then programmed from 160 °C to 290 °C at 6 or 8 °C/min, septumless injection mode using a programmed temperature vaporizer (DANI) at 60 °C for 6 s (solvent split) then at 290 °C (splitless), helium as carrier gas Negative ion chemical ionization (NICI) operating conditions were: selected-ion monitoring of carboxylate anions (M − 181), *m/z* 586 for 2,3-dinor-TXB_2 and 2,3-dinor-6-keto-$PGF_{1\alpha}$, *m/z* 590 for 2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4, *m/z* 614 for TXB_2 and 6-keto-$PGF_{1\alpha}$, *m/z* 618 for 6-keto-$PGF_{1\alpha}$-d_4 and *m/z* 622 for TXB_2-d_8, measuring time, 80 ms, interchannel time, 8 ms, ion source pressure, 0 12 Torr, ammonia as reagent gas, ionizer temperature, 200 °C, electron energy, 100 eV

Standard curves

Standard curves were obtained with increasing amounts (0–5 ng) of 6-keto-$PGF_{1\alpha}$ (r = 0 9998), 2,3-dinor-6-keto-$PGF_{1\alpha}$ (r = 0 9999), TXB_2 (r = 0 9998), 2,3-dinor-TXB_2 (r = 0.9997) in the presence of constant amounts of the respective deuterated analogues, by plotting the unlabelled-to-labelled peak-area ratio for each metabolite against the amount of unlabelled compound TXB_2-d_8 was used as internal standard for 2,3-dinor-TXB_2

Human urine

Urine was collected between midnight and 8 a.m. from eight healthy non-smoker male volunteers (age 19–28 years) who had not taken any drugs in the preceding two weeks. Samples were frozen and stored at −20 °C until analysed.

Rat urine

Urine was collected for 24 h from five male rats (200 g body weight, Sprague–Dawley CD COBS, Charles River, Calco, Italy) kept in metabolic cages with free access to food and water Samples were frozen and stored at −20 °C until analysed.

RESULTS

Extraction of 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$

The efficiency and reproducibility of the immunoaffinity extraction of 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$ was first assessed separately, as previously done for TXB_2 and 2,3-dinor-TXB_2 [13] For these experiments, columns containing only the anti-6-keto-$PGF_{1\alpha}$ antibodies were used

Recovery of unlabelled 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$ was first evaluated at the nanogram level by spiking phosphate buffer aliquots (50 m*M*, pH 7.4, 20 ml) with 10 or 30 ng of 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$, and extracting the samples as described in Experimental Constant amounts of deuterated analogues (40 ng) were added after the extraction, before derivatization and by high-resolution GC–MS The loss of unlabelled (d_0) 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$ during the extraction was then calculated by comparing the d_0/d_4 peak-area ratios of extracted versus corresponding unextracted standard mixtures. Recovery was 98% and 94%, respectively, for 10 and 30 ng.

Labelled and unlabelled 6-keto-$PGF_{1\alpha}$ or 2,3-dinor-6-keto-$PGF_{1\alpha}$ were identically recovered, as demonstrated in separate experiments where d_0–d_4 mixtures (100–800 pg d_0 plus 500 pg d_4) were extracted from buffer The d_0/d_4 peak-area ratios were found to be identical with those of the corresponding unextracted mixtures (data not shown). Typical recovery from urinary samples was 70–90% for 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$

The accuracy of the assay was evaluated after extraction and high-resolution GC–MS analysis of 2-ml aliquots of a human urine pool (five healthy males) spiked with increasing amounts (0, 100, 400, 800 pg/ml) of unlabelled 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$ and a constant amount (500 pg) of deuterated analogues. The calculated amounts (y) of each metabolite were plotted against the amounts added (x) Each calculated amount (pg/ml) corresponded to the added plus endogenous level ($y=0\,98x+93.5$, $r=0\,999$ for 6-keto-$PGF_{1\alpha}$, $y=0.93x+144\,8$, $r=0\,998$ for 2,3-dinor-6-keto-$PGF_{1\alpha}$) The endogenous concentrations of 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$ (y-axis

intercepts) in this urine pool were 93 5 pg/ml for 6-keto-$PGF_{1\alpha}$ and 144.8 pg/ml for 2,3-dinor-6-keto-$PGF_{1\alpha}$.

The precision was calculated by extracting four 2-ml aliquots of the same urine pool without addition of exogenous metabolites The mean ± S D concentrations were 100 1 ± 2 7 pg/ml [relative standard deviation (R S D) = 2 7%] for 6-keto-$PGF_{1\alpha}$ and 135 5 ± 3 4 pg/ml (R S D. = 2 5%) for 2,3-dinor-6-keto-$PGF_{1\alpha}$

Simultaneous extraction of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2 and 2 3-dinor-TXB_2

The precision and accuracy of the immunoaffinity–GC–MS method were evaluated for the simultaneous extraction of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2 and 2,3-dinor-TXB_2, by using mixed-bed columns prepared with anti-6-keto-$PGF_{1\alpha}$ and anti-TXB_2 immobilized antibodies

To check the validity of the assay over a concentration range close to the physiological levels of the four metabolites, we added 0, 50, 200 and 500 pg/ml 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$, 0, 25, 100 and 250 pg/ml TXB_2, and 0, 250, 1000 and 2500 pg/ml 2,3-dinor-TXB_2 to triplicate aliquots (2 ml) of a urine pool (corresponding to an average diuresis of ca 4 min), together with constant amounts of the deuterated analogues (500 pg of 6-keto-$PGF_{1\alpha}$-d_4, 2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4 and TXB_2-d_8) Linear regression plots [calculated amounts (y) of each metabolite against added amounts (x)] and R S D at the endogenous concentrations were $y = 0.92x + 108\ 5$, $r = 0\ 999$, R S.D = 3 4% for 6-keto-$PGF_{1\alpha}$, $y = 0\ 86x + 133\ 5$, $r = 0\ 997$, R S D = 2.0% for 2,3-dinor-6-keto-$PGF_{1\alpha}$, $y = 0\ 92x + 44\ 5$, $r = 0\ 997$, R S D = 9 1% for TXB_2; $y = 0\ 93x + 414.5$, $r = 0\ 999$, R S D = 3 4% for 2,3-dinor-TXB_2 Intercepts on the y-axis corresponded to the endogenous levels (pg/ml)

The validity of the assay was also assessed using different human urine volumes with constant amounts of deuterated analogues Different size samples (2,4 and 8 ml) of a urine pool were carried through the analysis after addition of 1 ng of 6-keto-$PGF_{1\alpha}$-d_4, 2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4 and TXB_2-d_8 (Table

TABLE I

ASSAY REPRODUCIBILITY WITH DIFFERENT SAMPLE SIZES AND CONSTANT INTERNAL STANDARD CONCENTRATION

Urine volume (ml)	Metabolite concentration (pg/ml)			
	6-Keto-$PGF_{1\alpha}$	2,3-Dinor-6-keto-$PGF_{1\alpha}$	TXB_2	2,3-Dinor-TXB_2
2 0	98 5	138 5	56 5	391 5
4 0	94 7	142 2	52 2	379 5
8 0	87 7	145 5	51 1	378 5

I) Results were similar and independent of sample size Urine volumes of up to 8 ml (corresponding to an average of 16 min diuresis) can be extracted by this method

High-resolution GC–NICIMS

Figs 1 and 2 show representative selected-ion recording results obtained using a mixed-bed immunoaffinity column for the simultaneous extraction of endogenous 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2 and 2,3-dinor-TXB_2 from human and rat urine, in the presence of added 6-keto-$PGF_{1\alpha}$-d_4, 2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4 and TXB_2-d_8 The 2,3-dinor-6-keto-$PGF_{1\alpha}$ derivative gave two chromatographic peaks, corresponding to the *syn* and *anti* isomers of the methoxime derivative The most abundant isomer was considered for quantitation.

Although no interfering peaks are eluted at the retention times corresponding to the metabolites to be measured and their deuterated analogues, in human urine we noted an unknown compound eluting close to 6-keto-$PGF_{1\alpha}$ on

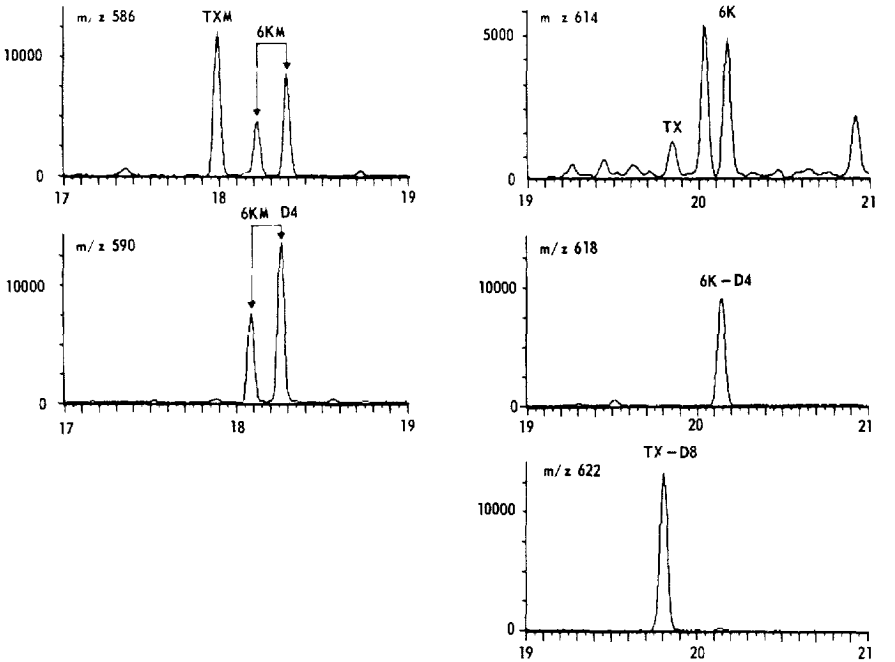

Fig 1 Typical selected-ion recording tracings from human urine spiked with 6-keto-$PGF_{1\alpha}$-d_4, 2,3-dinor-6-keto-$PGF_{1\alpha}$-d_4 and TXB_2-d_8 and extracted by mixed-bed immunoaffinity column The extract was analysed after derivatization of metabolites to pentafluorobenzyl (PFB) ester, methyloxime, trimethylsilyl ether derivatives Carboxylate anions (M – PFB) of the derivatives were recorded Peaks TX = TXB_2, TXM = 2,3-dinor-TXB_2, 6K = 6-keto-$PGF_{1\alpha}$, 6KM = 2,3-dinor-6-keto-$PGF_{1\alpha}$ (*syn/anti* isomers)

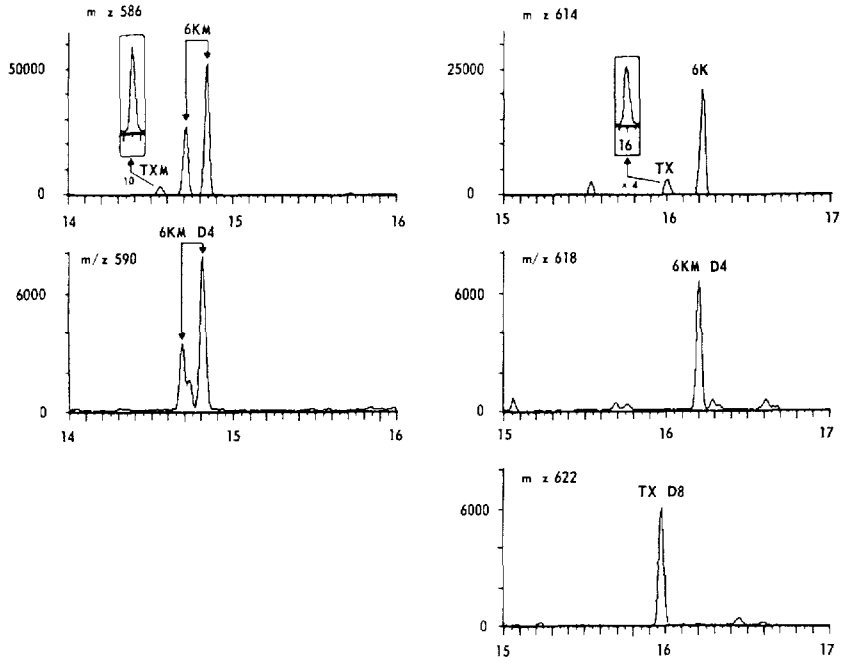

Fig 2 Typical selected-ion recording tracings from rat urine Experimental and peaks as in Fig 1

TABLE II

URINARY EXCRETION OF 6-KETO-PGF$_{1\alpha}$, 2,3-DINOR-6-KETO-PGF$_{1\alpha}$, TXB_2 AND 2,3-DINOR-TXB_2 IN HEALTHY NON-SMOKER MALES UNDER BASAL CONDITIONS

Compound	Urinary excretion (mean ± S D , $n=8$)		
	pg/mg of creatinine	pg/ml of urine	ng per day
6-Keto-PGF$_{1\alpha}$	53 1 ± 15 6	129 0 ± 41 5	97 6 ± 41 2
2,3-Dinor-6-keto-PGF$_{1\alpha}$	73 8 ± 25 0	177 9 ± 58 9	130 6 ± 33 4
TXB_2	23 9 ± 5 8	58 6 ± 17 6	43 5 ± 13 4
2 3-Dinor-TXB_2	190 6 ± 59 8	454 0 ± 128 3	336 7 ± 81 6

the m/z 614 trace Different amounts of this compound have been found in all human urines analysed so far, but never in rat urine It was retained by the anti-6-keto-PGF$_{1\alpha}$ but not by the anti-TXB_2 antibodies The NICI spectrum showed only an intense isotopic cluster at m/z 613 (100%), 614 (49 6%), 615 (23 8%), 616 (7 0%) The amount of the compound was not enough to obtain an electron impact spectrum

TABLE III

URINARY EXCRETION OF 6-KETO-$PGF_{1\alpha}$, 2,3-DINOR-6-KETO-$PGF_{1\alpha}$, TXB_2 AND 2,3-DINOR-TXB_2 IN THE RAT UNDER BASAL CONDITIONS

Compound	Urinary excretion (mean ± S D , $n=5$)		
	pg/g of creatinine	pg/ml of urine	ng per day
6-Keto-$PGF_{1\alpha}$	296 6 ± 38 9	221 2 ± 64 4	5 24 ± 1 63
2,3-Dinor-6-keto-$PGF_{1\alpha}$	852 4 ± 147 6	615 8 ± 56 9	14 73 ± 2 91
TXB_2	82 6 ± 22 4	61 8 ± 23 9	1 49 ± 0 65
2,3-Dinor-TXB_2	91 2 ± 15 6	68 2 ± 21 9	1 61 ± 0 51

The presence of three hydroxyl groups on this molecule could be demonstrated by the shift of 27 a m u ($3\times{}^2H_9$) using bis([$^2H_9$]trimethylsilyl)acetamide instead of BSTFA as silylating agent. The molecular structure of this compound is currently under investigation Other small peaks noted on the *m/z* 614 trace showed the same isotopic cluster and shift of 27 a m u after silylation with bis([$^2H_9$]trimethylsilylacetamide These contaminating compounds can be easily separated by high-resolution GC For this reason, human urine samples were analysed at lower GC temperature-programming rates than rat samples

Human urine

The method described above was applied to determine urinary levels of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2 and 2,3-dinor-TXB_2 in eight healthy male volunteers Mean excretion of prostacyclin and thromboxane metabolites is reported in Table II Mean ± S D ratios of 2,3-dinor-6-keto-$PGF_{1\alpha}$ to 6-keto-$PGF_{1\alpha}$ and of 2,3-dinor-TBX_2 to TXB_2 in each individual were 1 4 ± 0 3 and 8 2 ± 2 5%, respectively

Rat urine

Basal excretion values of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TXB_2 and 2,3-dinor-TXB_2 were determined in five control rats Table III shows the results with 24-h urinary collections Mean ± S D ratios of 2,3-dinor-6-keto-$PGF_{1\alpha}$ to 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-TXB_2 to TXB_2 in each rat were 2 9 ± 0 7 and 1 2 ± 0 3%, respectively

DISCUSSION

The measurement of urinary prostanoids by high-resolution GC–NICIMS is currently regarded as a reference method on account of its specificity and sensitivity Recently, we reported the use of immunoaffinity chromatography prior to GC–MS for one-step extraction and purification of urinary thrombox-

anes [13] This represents an impressive improvement over conventional chemico-physical methods in terms of rapidity, simplicity and recovery Others have reported on immunoaffinity purification of urinary 6-keto-$PGF_{1\alpha}$ [14] or TXB_2 [15] prior to GC–MS, but these methods included other extraction steps before immunoaffinity chromatography and after derivatization

We now present a novel assay based on the use of mixed-bed immunoaffinity columns employing different polyclonal antibodies (against 6-keto-$PGF_{1\alpha}$ and TXB_2) to extract simultaneously their corresponding haptens and cross-reacting related compounds (2,3-dinor metabolites) The antibodies are specific enough to give interference-free selected-ion recording chromatograms without pre-extracting or purifying the samples, but not too specific to prevent the extraction of closely related, cross-reacting metabolites We can therefore simultaneously extract, separate and measure a group of metabolites whose balance is of great importance in many physiopathological conditions

The rapidity and simplicity of the analytical procedure is such that, for example, sixteen samples can be run on two subsequent cycles on eight immunoaffinity columns, then be derivatized and ready for GC–MS analysis in ca 8 h The possibility of measuring four compounds in a single GC–MS run is also a most favourable feature

Using low-specificity antibodies and mixed-bed immunoaffinity columns, one could theoretically monitor any combination of related metabolites, provided there is a favourable combination of gas chromatographic and mass spectral properties to ensure high specificity in the GC–MS analysis

In our experimental conditions, we obtained accurate and precise measurements of the major urinary metabolites of prostacyclin and thromboxane in humans and rats An unknown compound [with an intense (100%) isotopic cluster at m/z 613, 614, 615 and 616] was constantly extracted by the anti-6-keto-$PGF_{1\alpha}$ but not by the anti-TXB_2 antibodies from human but not rat urine The presence of three hydroxyl groups on this molecule could be demonstrated by using bis($^2H_9$)trimethylsilylacetamide instead of BSTFA as silylating agent The molecular structure of this compound and other minor contaminants with similar characteristics is currently under investigation It should be noted that by high-resolution GC (with appropriate columns and temperature programming) one could easily separate these contaminating compounds However, this material might cause overestimates in radioimmunoassays for 6-keto-$PGF_{1\alpha}$ if low-specificity antibodies and/or purification techniques of insufficient specificity are used

The basal levels of urinary metabolites of prostacyclin and thromboxane in humans found by this method are consistent with those reported by other authors, who measured single metabolites by conventional extraction and purification procedures [7,9,10,18,19] or immunoaffinity purification preceded and/or followed by chemico-physical step(s) [14,15] The data on urinary excretion of thromboxane metabolites in rats are similar to those reported pre-

viously [13], while to our knowledge this is the first report on the in vivo basal excretion of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in this species The relatively high levels of urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ are in agreement with this metabolite being a major product of systematically infused PGI_2 or 6-keto-$PGF_{1\alpha}$ in rats [20,21]

Current studies suggest that the method presented here may be particularly useful for monitoring the effect of drugs or various physiopathological events on the in vivo differential modulation of prostacyclin and thromboxane biosynthesis

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