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Solid- and liquid-phase extraction for the gas chromatographic–tandem mass spectrometric quantification of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ in human urine

Dimitrios Tsikas*, Frank-Mathias Gutzki, Marianne Böhme, Ilka Fuchs, Jürgen C. Frölich

Institute of Clinical Pharmacology, Hannover Medical School, Carl-Neuberg-Str. 1, D-30623 Hannover, Germany

Abstract

Whole body synthesis of thromboxane A_2 is best assessed by quantifying non-invasively its major urinary metabolite, i.e., 2,3-dinor-thromboxane B_2 (2,3-dn-TxB_2), by gas chromatography–mass spectrometry (GC–MS) or GC–tandem MS. Methods based on these techniques usually require a series of extraction and purification procedures including solid-phase extraction (SPE) and thin-layer chromatography (TLC) or liquid chromatographic separation of authentic or derivatized 2,3-dn-TxB_2. Taking advantage of the inherent accuracy of GC–tandem MS and the high selectivity of the extraction of methoximated 2,3-dn-TxB_2 on phenylboronic acid SPE cartridges we developed a method that involves only SPE steps prior to quantification by GC–tandem MS. The method was validated by performing in parallel an additional TLC step. Method mean accuracy and precision were of the order of 103% and 95%, respectively. The method allows furthermore co-processing of the same urine sample to quantify accurately and rapidly the major urinary metabolite of prostacyclin, i.e., 2,3-dn-6-oxo-prostaglandin (PG) $F_{1\alpha}$, by GC–tandem MS. The limit of detection of the method was below each 5 pg of 2,3-dn-TxB_2 and 2,3-dn-6-oxo-$PGF_{1\alpha}$ per 5 ml of urine. Our study suggests that dinor metabolites of isothromboxanes and isoprostacyclins are not abundantly present in human urine.

Keywords: Thromboxanes; Prostaglandins

1. Introduction

Thromboxane A_2 (TxA_2) is a vasoconstrictor and stimulator of platelet aggregation mainly produced in the platelets [1]. At physiological pH, TxA_2 is nonenzymatically converted to TxB_2. Systemically administered TxB_2 in man has been shown to undergo extensive metabolism [2]. The most abundant urinary metabolites of TxB_2 have been identified as 2,3-dinor-thromboxane B_2 (2,3-dn-TxB_2) (Fig. 1) and 11-dehydro-TxB_2 [3]. Urinary 2,3-dn-TxB_2 and 11-dehydro-TxB_2 have been shown to closely reflect whole body biosynthesis of TxA_2 in vivo. Therefore, these thromboxane metabolites are frequently measured to assess whole body TxA_2 synthesis and also to monitor effects of drugs on cyclooxygenase activity in vivo [4]. Gas chromatography–mass spectrometry (GC–MS) and GC–tandem MS especially in the negative-ion chemical ionization (NICI) mode are the most reliable analytical techniques to quantify urinary levels of 2,3-dn-TxB_2 and 11-dehydro-TxB_2 (reviewed in [4]).

The greatest improvement in the GC–MS measurement of TxB_2 and its metabolites in human urine was achieved by introducing a highly selective solid-phase extraction (SPE) of methoximated TxB_2 and

*Corresponding author. Tel.: +49-511-532-3959; fax: +49-511-532-2750.

E-mail address: tsikas.dimitros@mh-hannover.de (D. Tsikas)

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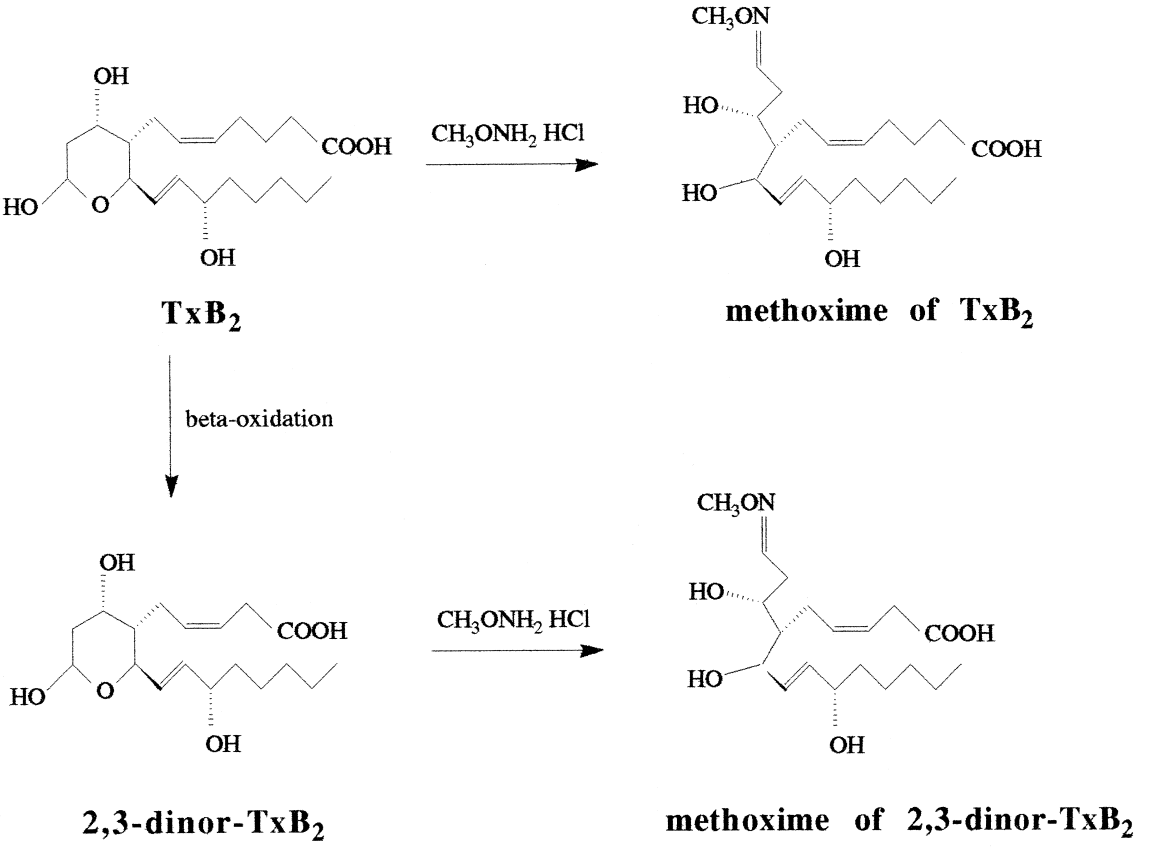

Fig. 1. Chemical structures of underivatized and methoximated TxB_2 and 2,3-dn-TxB_2.

2,3-dn-TxB_2 (Fig. 2) and of the open ring form of 11-dehydro-TxB_2 with phenylboronic acid (PBA) cartridges [5]. However, despite selective extraction of methoximated 2,3-dn-TxB_2 and TxB_2 from human urine on PBA cartridges, their quantification by GC–MS in the NICI mode required an additional TLC purification step [5]. Similar results have been reported by Weber et al. [6]. Lorenz et al. [7] have reported that quantification of 11-dehydro-TxB_2 in human urine can simply be performed by a single SPE of 11-dehydro-TxB_2 on PBA cartridges followed by GC–tandem MS of the pentafluorobenzyl-trimethylsilyl (PFB-TMS) derivative without the need of any further purification step. This has not been shown so far for urinary 2,3-dn-TxB_2. The inherent accuracy of GC–tandem MS and the high selectivity of the extraction of methoximated 2,3-dn-TxB_2 on PBA cartridges could also enable accurate quantification of this metabolite by GC–tandem MS without the need of any other purification steps analogous to 11-dehydro-TxB_2 [7]. By modifications of previously described SPE procedures for 2,3-dn-TxB_2 [5,6] we developed a simple and rapid method that allows accurate quantification of urinary 2,3-dn-TxB_2 by GC–tandem MS without the need of any additional purification step. This method was validated by performing an additional TLC step. Modifications were performed in such a way that the index metabolite of prostacyclin, i.e., 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (2,3-dn-6-oxo-$PGF_{1\alpha}$) [8], can also be determined in parallel using only liquid-phase extraction (LPE) procedures. Preliminary results of the present study have been reported recently elsewhere [4].

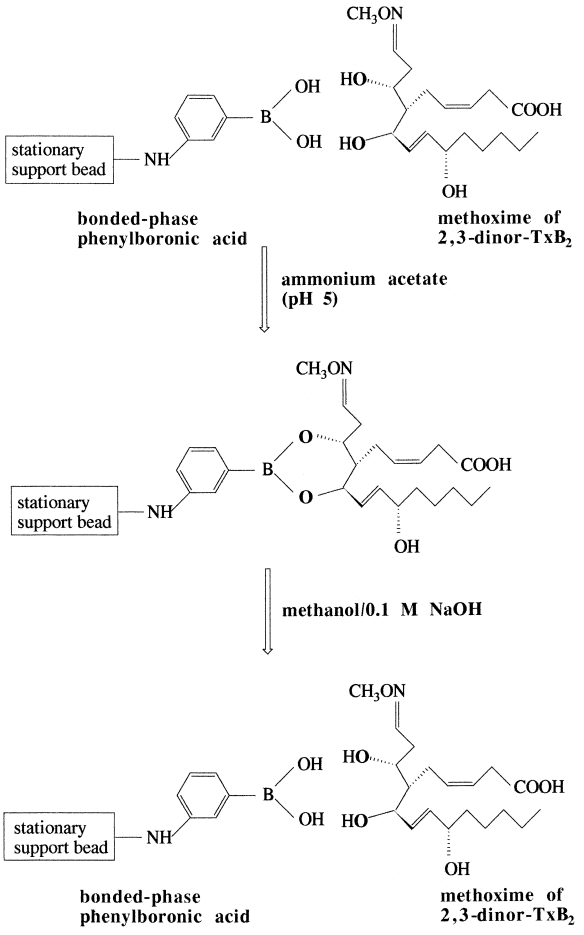

Fig. 2. The methoxime derivative of 2,3-dn-TxB_2 is a 1,3-diol that condensates with bonded-phase phenylboronic acid to form a stable complex. Cleavage of the complex with methanolic aqueous NaOH recovers the methoxime derivative of 2,3-dn-TxB_2 and the bonded-phase phenylboronic acid. A part of this scheme has been published elsewhere [4].

2. Experimental

2.1. Materials

Pentafluorobenzyl (PFB) bromide, methoxyamine hydrochloride and *N,N*-diisopropylethylamine were obtained from Aldrich (Steinheim, Germany). *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, IL, USA). Acetonitrile of gradient grade and all other chemicals were from Merck (Darmstadt, Germany). Tritiated TxB_2 was purchased from Amersham Buchler (Braunschweig, Germany). Unlabeled compounds and their tetradeuterated analogs, i.e., 2,3-dn-[3,3′,4,4′-$^2H_4$]TxB_2 and 2,3-dn-6-oxo-[3,3′,4,4′-$^2H_4$]$PGF_{1\alpha}$ were a kind gift from Dr. U. Axen, Upjohn (Kalamazoo, MI, USA). Octadecyl silica (ODS) cartridges (500 mg and 300 mg) were purchased from Macherey–Nagel (Düren, Germany). Phenylboronic acid (PBA) cartridges (100 mg) were obtained from Varian (Harbor City, CA, USA).

2.2. Extractions and derivatization procedures

Fig. 3 shows schematically the SPE and LPE extraction procedures and the derivatization steps used in the present method. In some experiments tritiated TxB_2 was added to urine samples to determine the recovery rate of the various procedures.

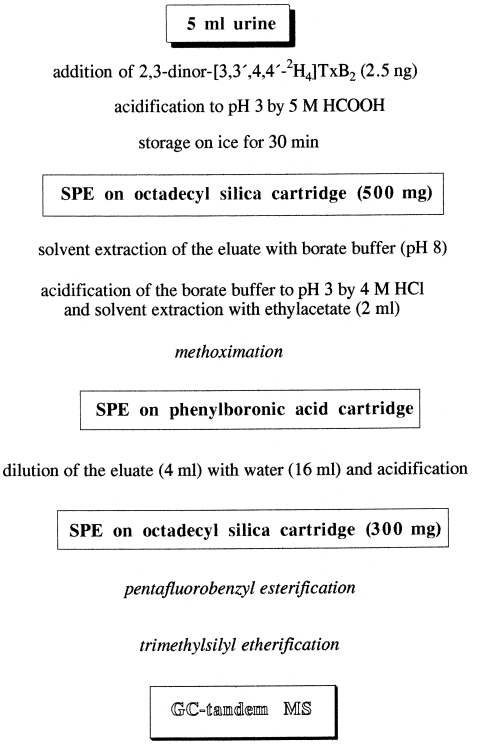

Fig. 3. Summary of the extraction and derivatization steps of the method for 2,3-dn-TxB_2. This scheme is a part of the scheme reported elsewhere [4].

The eluate (2 ml ethyl acetate) of the first SPE was treated with borate buffer (2 ml; 50 mmol/l, pH 8.0) and mixed by vortexing for 2 min. The organic phase contains the lactone form of 2,3-dn-6-oxo-$PGF_{1\alpha}$ while in the aqueous phase 2,3-dn-TxB_2, TxB_2 and other prostanoids are present as their carboxylate anions. The aqueous phase of the first SPE was acidified and prostanoids were extracted by vortex-mixing for 1 min with ethyl acetate. The solvent was removed under nitrogen, and methoximation was performed using a saturated solution of methoxyamine hydrochloride in pyridine (100 μl) by heating at 60°C for 60 min. Pyridine was completely removed under nitrogen, the residue was reconstituted in ammonium acetate buffer (1.4 ml; 6 mol/l, pH 5.0) and applied to PBA cartridges. SPE was performed as described by Lawson et al. [5]. SPE of prostanoids from the eluate (4 ml of methanol–0.1 mol/l NaOH, 1:1, v/v) was performed as described [6]. After solvent evaporation under nitrogen PFB esterification with PFB bromide and subsequent TMS etherification with BSTFA (20 or 50 μl) were performed by standard derivatization procedures [4].

2.3. Experiments on precision, accuracy and validation of the method

The inter-day precision of the method was assessed by analyzing in duplicate four identical unspiked 5-ml urine samples for 2,3-dn-TxB_2 on five consecutive days. Intra-day precision was further determined by analyzing in duplicate 5-ml aliquots from 24-h urines collected unspiked from ten volunteers. Accuracy of the assay was assessed by analyzing in duplicate urine samples (5-ml aliquots) of four volunteers to which increasing amounts of 2,3-dn-TxB_2 were spiked to obtain added concentrations of 50, 100, 200 and 500 pg/ml.

The method was validated as follows. Unspiked and spiked urine samples were subjected to SPE as described above and PFB esterification was performed. One third of the methoxime PFB ester fraction was derivatized by BSTFA and analyzed directly by GC–tandem MS. Two thirds of the methoxime PFB ester fraction was subjected to TLC on 20×20 cm silica gel 60 plates from Merck performed with a TLC-Applicator AS 30 and a DC-MAT, both of which were from Desaga (Wiesloch, Germany). The residue containing the methoxime PFB esters was reconstituted in ethanol (15 μl) and aliquots (10 μl) thereof were subjected to TLC using ethyl acetate–methanol (98:2, v/v) for elution. A 0.6-cm band, centered around the reference compound (R_F=0.8±0.2, mean±SD, n=5), was scraped off the TLC plate, compounds were extracted with ethanol (500 μl) and suspensions were centrifuged (4000 *g*, 10 min). Supernatants were decanted, ethanol was removed under nitrogen, and the methoxime PFB esters were converted to their TMS ether derivatives.

2.4. GC–tandem MS

GC–tandem MS analyses were performed on a Thermoquest TSQ 7000 triple-stage quadruple mass spectrometers interfaced with a Thermoquest gas chromatograph model Trace 2000 (Egelsbach, Germany). A fused-silica capillary column Optima 17 (30 m×0.25 mm I.D., 0.25 μm film thickness) from Macherey–Nagel was used. Helium was used as a carrier gas at a constant pressure of 55 kPa. For NICI methane was used as a reagent gas at a pressure of 65 Pa. Argon was used for collisionally-activated dissociation (CAD) at a pressure of 0.15 Pa. The collision energy was set to 25 eV. The electron energy was 200 eV and the emission current 600 μA. Injector, interface and ion source were kept at 280°C, 290°C and 180°C, respectively. The column was held at 70°C for 2 min, then programmed to 280°C at 25°/min followed to 320°C at 4°/min. The daughter ions $[M–PFB–3\times TMSOH–CH_3OH–CO_2]^-$ at m/z 240 for endogenous 2,3-dn-TxB_2 and 2,3-dn-6-oxo-$PGF_{1\alpha}$, and m/z 244 for their tetradeuterated analogs were monitored in the selected reaction monitoring mode generated by CAD of the parent ions $[M–PFB]^-$ at m/z 586 and 590, respectively. Aliquots (1 μl) were injected into the GC–tandem MS instrument in the splitless mode.

3. Results

The recovery rate of tritiated TxB_2 from urine after the first SPE and the LPE steps was determined as 85±9% (mean±SD, n=10). After the second SPE step on PBA cartridges, 22±5% (mean±SD, n=10)

Table 1
Intra-day reproducibility of the GC–tandem MS method for 2,3-dn-TxB_2 conducted with 24-h unspiked urine samples from ten healthy volunteers

Urine sample	2,3-dn-TxB_2 (pg/ml)	RSD (%)
1	54.0	9.2
1	47.4	
2	38.5	2.9
2	40.1	
3	96.7	6.7
3	87.9	
4	10.5	13.2
4	8.7	
5	85.9	0.3
5	86.3	
6	44.6	4.6
6	41.8	
7	300.0	3.9
7	283.8	
8	50.7	4.7
8	54.2	
9	233.3	3.3
9	244.6	
10	6.5	10.1
10	7.5	
Mean RSD (%)		5.9

of total radioactivity was counted in the eluate of the SPE on the PBA cartridges. We were not able to improve the recovery rate of tritiated TxB_2 by means of various modifications (not shown) of the methoximation and the SPE procedure on the PBA cartridges originally described by Lawson et al. [5]. This group has reported a recovery rate of the order of 80% prior to and approximately 50% after the TLC step. Under the assumption that TxB_2 and 2,3-dn-TxB_2 behave similarly in all procedures of our method the final recovery of 2,3-dn-TxB_2 should be of the order of 20%.

Table 1 summarizes the data from the experiment on the intra-day reproducibility of the method. 2,3-dn-TxB_2 was measured in urine in the concentration range 5–300 pg/ml at a mean RSD of 5.9%. The lowest 2,3-dn-TxB_2 levels were measured in urine samples from five volunteers who had taken aspirin orally (numbers 1,2,4,6,10).

Table 2 summarizes the data from the experiments on the accuracy of the method for human urine samples with different basal 2,3-dn-TxB_2 levels. Mean accuracy and precision (RSD) of 103% and 5.2%, respectively, demonstrate the high accuracy and precision of the method for the quantification of 2,3-dn-TxB_2 in human urine in a relevant concentration range.

To further validate the method we measured 2,3-dn-TxB_2 in unspiked and spiked urine samples without and after additional TLC of the PFB ester methoxime derivatives. The data of Table 3 showing the concentration of 2,3-dn-TxB_2 in human urine samples measured by these methods and the 2,3-dn-TxB_2 concentration ratio measured without and with TLC — that is close to unity — demonstrate the validity of the method. Representative partial chromatograms from GC–tandem MS analyses of 2,3-dn-

Table 2
Accuracy and precision of the method for 2,3-dn-TxB_2

2,3-dn-TxB_2 spiked (pg/ml)	2,3-dn-TxB_2 measured minus the basal level[a] (pg/ml)				RSD/accuracy (%)				Mean±SD of RSD/accuracy (%)
	Subject				Subject				
	1	2	3	4	1	2	3	4	
0	0	0	0	0	10.5/n.a.	1.7/n.a.	4.6/n.a.	6.3/n.a.	5.8±3.7/n.a.
50	63.2	42.2	56.0	45.0	5.3/126	4.5/84	6.2/112	5.5/90	5.4±0.7/103±20
100	109.2	106.9	94.0	100.5	3.4/109	9.8/107	0.5/94	7.8/101	5.4±4.2/103±7
200	220.6	209.4	194.5	223.0	2.8/110	5.3/105	5.3/97	5.1/111	4.6±1.2/106±6
500	495.1	481.9	485.0	519.0	9.0/99	5.1/96	3.3/97	2.8/104	5.1±2.8/99±4
Mean±SD of RSD					6.2±3.4	5.3±2.9	3.9±2.2	5.5±1.8	5.2±0.9
Mean±SD of accuracy					111±11	98±10	100±8	102±9	103±6

[a] Mean basal levels were 124, 103, 45 and 236 pg/ml, respectively. n.a., not applicable.

Table 3
Urinary 2,3-dn-TxB_2 levels measured by GC–tandem MS prior to and after an additional TLC step

Urine sample	2,3-dn-TxB_2 (pg/ml, mean±SD)[a]		Concentration ratio without TLC to with TLC
	without TLC	with TLC	
1	290.3±6.3	325.2±44.3	0.893
2	93.2±14.6	100.7±15.1	0.926
3	78.8±0.14	70.3±2.5	1.121
4	317.2±15	304±3.74	1.043
5	250.4±11.1	336.6±10.2	0.744
6	437.6±29.1	487.2±74	0.898
7	575.5±55.5	544.8±35.9	1.056
8	1201±45	1245±58	0.965
Mean±SD (RSD, %) of the ratio without/with TLC			0.956±0.118 (12.4%)

[a] Note: $n=4$ for sample 1; $n=2$ for samples 2 to 8; samples 1 to 6: unspiked; samples 7 and 8: spiked.

TxB_2 in a urine sample without additional TLC purification is shown in Fig. 4 (top). The chromatogram of this Figure shows that no other compounds interfere with the measurement of 2,3-dn-TxB_2.

The applicability of the method for biological samples was demonstrated by performing a small study. Five healthy humans collected their urine for 24 h one day before and one day after oral administration of six 500-mg of aspirin tablets as a single dose. Urinary 2,3-dn-TxB_2 was determined as described in this work. Urinary 2,3-dn-6-oxo-$PGF_{1\alpha}$ was determined by GC–tandem MS in the same urine sample, i.e., by extracting the borate phase from the first SPE extraction on ODS of this method as described previously [4] using modifications of previously described methods [9,10]. A typical chromatogram from the GC–tandem MS analysis of urinary 2,3-dn-6-oxo-$PGF_{1\alpha}$ is shown in Fig. 4 (bottom). The intra-day precision (RSD) of the method was 2.8±1.9% (mean±SD, $n=8$) for urinary 2,3-dn-6-oxo-$PGF_{1\alpha}$ concentrations in the range 41–2500 pg/ml. The accuracy of the method for 2,3-dn-6-oxo-$PGF_{1\alpha}$ spiked to human urine at added concentrations of 50, 100, 200 and 500 pg/ml was 98±9% (mean±SD). The ratio of the urinary 2,3-dn-6-oxo-$PGF_{1\alpha}$ concentration without and after additional TLC separation of its PFB ester methoxime derivative (as described above for 2,3-dn-TxB_2 except for the solvent which was a mixture of ethylacetate/isooctane, 75:25, v/v; $R_F=0.09$) was determined as 0.962±0.044 (mean±SD, $n=8$). Oral administration of aspirin (3 g) was found to decrease urinary excretion of 2,3-dn-TxB_2 and 2,3-dn-6-oxo-$PGF_{1\alpha}$ by (mean±SD) 85.9±7.7% and 51.1±20.9%, respectively. The lowest concentrations of urinary metabolites measured in this study after aspirin administration were 4.2 pg for 2,3-dn-TxB_2/mg creatinine and 24 pg for 2,3-dn-6-oxo-$PGF_{1\alpha}$/mg creatinine. The highest concentrations were measured before aspirin administration as 171.7 pg for 2,3-dn-TxB_2/mg creatinine and 129.7 pg for 2,3-dn-6-oxo-$PGF_{1\alpha}$/mg creatinine. Similar basal urinary concentrations of 2,3-dn-TxB_2 and 2,3-dn-6-oxo-$PGF_{1\alpha}$ have been reported by other groups [5,10–12].

4. Discussion

Lawson et al. have utilized the unique ability of methoximated TxB_2 and 2,3-dn-TxB_2 (Fig. 2), and of the open ring form of 11-dehydro-TxB_2 to react with the bonded phenylboronic acid to selectively extract these compounds from urine and to quantify them by GC–MS following an additional purification procedure by TLC [5]. The introduction of SPE on PBA cartridges and the NICI in mass spectrometry greatly improved and considerably shortened methods based on GC–MS for quantification of TxB_2 and its metabolites in human urine [4]. The development of tandem mass spectrometry further increased method specificity and considerably decreased labor and working time for most prostanoids including 11-dehydro-TxB_2 [4,7]. In the present work we show that a single SPE on PBA cartridges of methoximated 2,3-dn-TxB_2 suffices for the accurate and

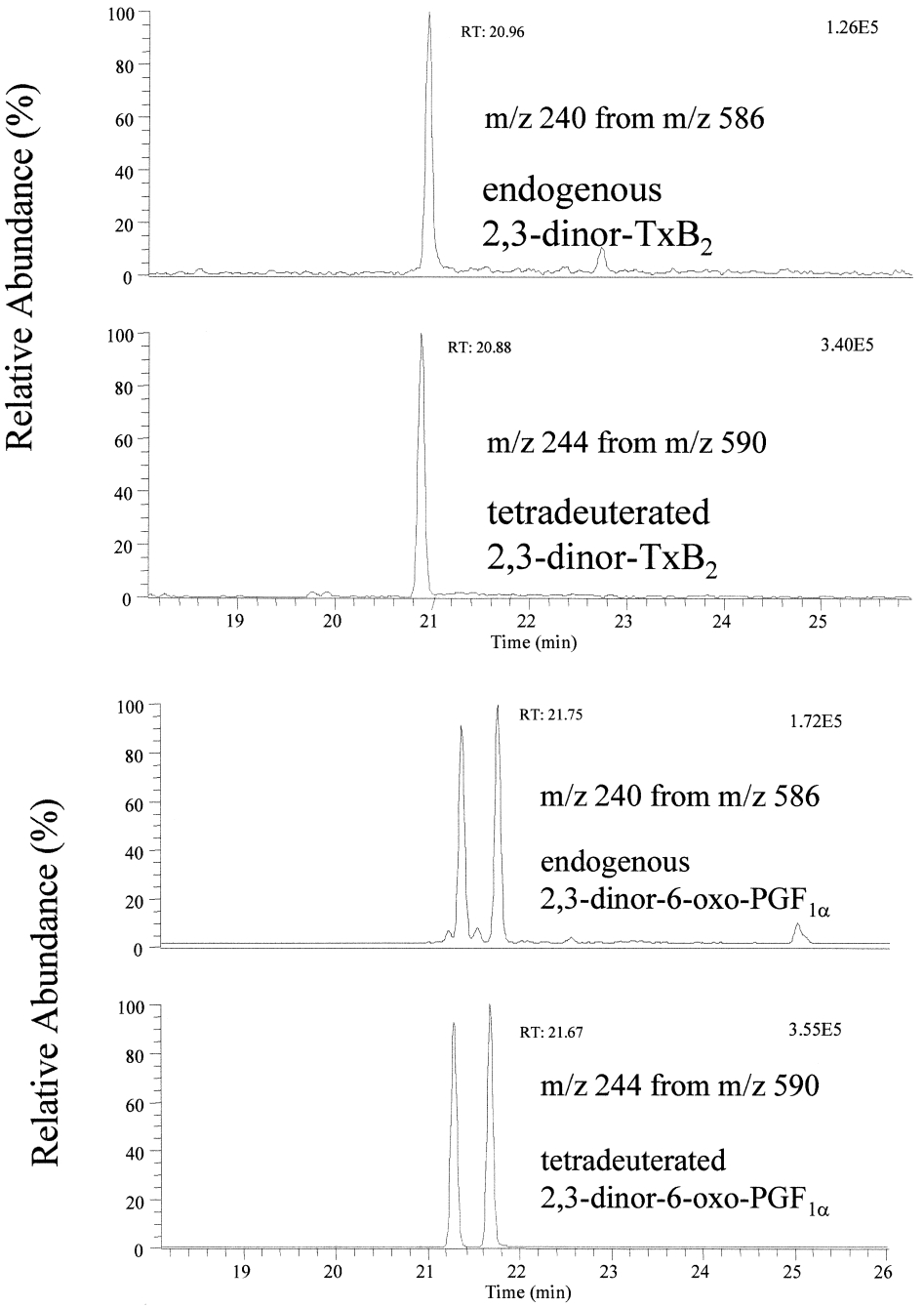

Fig. 4. Partial chromatograms from the GC–tandem MS analyses by the present method of 2,3-dn-TxB_2 (top) and of 2,3-dn-6-oxo-$PGF_{1\alpha}$ (bottom) in a urine sample from a healthy human who did not receive aspirin. A 3-ml aliquot of urine was spiked each with 5 ng of the tetradeuterated standards 2,3-dn-[3,3′,4,4′-$^2H_4$]TxB_2 and 2,3-dn-6-oxo-[3,3′,4,4′-$^2H_4$]$PGF_{1\alpha}$. The aqueous phase (borate buffer) of the first SPE procedure described for 2,3-dn-TxB_2 was processed by LPE procedures as described [4], and the methoxime PFB ester TMS ether derivative was analyzed by GC–tandem MS without any further purification. The concentrations of 2,3-dn-TxB_2 and of 2,3-dn-6-oxo-$PGF_{1\alpha}$ in this urine sample were determined as 462 pg/ml and 585 pg/ml (first methoxime isomer) and 604 pg/ml (second methoxime isomer), respectively. Similar chromatograms from the use of the present method have been reported elsewhere [4].

rapid quantification of 2,3-dn-TxB_2 in human urine by GC–tandem MS.

The results obtained from the use of SPE on PBA cartridges are comparable with those from the use of immunoaffinity extraction of prostanoids including TxB_2 and its metabolites from biological fluids [11,12]. Therefore, SPE of methoximated 2,3-dn-TxB_2 and TxB_2 and of the open ring form of 11-dehydro-TxB_2 on PBA cartridges could be characterized as a chemoaffinity chromatography. The results of the present study support this statement. Lawson et al. [5] have shown that SPE on PBA cartridges and subsequent TLC permitted GC–MS analysis both of 2,3-dn-TxB_2 and its parent molecule TxB_2 in the same urine sample. Our study confirms this observation but demonstrates that use of GC–tandem MS instead of simple GC–MS makes TLC superfluous (Fig. 4, top).

The major modification of the methods previously described by Lawson et al. [5] and Weber et al. [6] is that we did not methoximate directly in urine but first extracted from urine presumably all eicosanoids by SPE on ODS cartridges. The procedure avoids methoximation of 2,3-dn-6-oxo-$PGF_{1\alpha}$ and gives thus the opportunity to measure by GC–tandem MS 2,3-dn-6-oxo-$PGF_{1\alpha}$ in addition to 2,3-dn-TxB_2 in the same urine sample utilizing highly specific LPE methods for 2,3-dn-6-oxo-$PGF_{1\alpha}$ [9,10]. Thus, the solvent extraction of the eluate from the first SPE separates the lactone form of 2,3-dn-6-oxo-$PGF_{1\alpha}$ (organic phase) from 2,3-dn-TxB_2 and other eicosanoids that are unable to form a stable lactone (aqueous phase). Our work shows that further treatment of the organic phase allows accurate and rapid quantification of 2,3-dn-6-oxo-$PGF_{1\alpha}$ by GC–tandem MS without the necessity of performing further purification steps. Moreover, the method described here offers the possibility to assess whole body synthesis both of thromboxane and of prostacyclin by GC–tandem MS in a single urine specimen in a single run [4]. The applicability of the present method to assess whole body synthesis of thromboxane and prostacyclin by measuring their index metabolites in a single urine sample before and after oral administration of aspirin is demonstrated.

Isoprostanes are prostaglandin-like compounds that are thought to be produced by non-enzymatic free radical-catalyzed peroxidation of arachidonic acid [13]. Morrow et al. have recently identified in vivo formation of thromboxane-like compounds, i.e., the isothromboxanes [14]. To date, prostacyclin-like compounds, i.e., the isoprostacyclins, have not been identified in vivo. The metabolism of isoprostanes in vivo is currently little investigated. The major urinary metabolite of 8-iso-$PGF_{2\alpha}$, one of the most abundant isoprostanes in human urine, has been shown to be 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ [15]. It can therefore be assumed that 8-iso-TxB_2 and 8-iso-6-oxo-$PGF_{1\alpha}$ could also be metabolized and excreted in human urine as their 2,3-dinor metabolites analogous to TxB_2 and prostacyclin.

Isoprostanes have been shown to have distinctly different chromatographic properties from their enzymatically formed analogs [4]. However, it can be expected that 2,3-dn-6-oxo-8-iso-$PGF_{1\alpha}$ and methoximated 2,3-dn-8-iso-TxB_2 would behave analogous to 2,3-dn-TxB_2 and 2,3-dn-6-oxo-$PGF_{1\alpha}$ in all procedures used in the method described in the present work. The chromatogram of Fig. 4 (top) from the analysis of urinary 2,3-dn-TxB_2 does not show other peaks in the close neighborhood of 2,3-dn-TxB_2 suggesting that 2,3-dn-8-iso-TxB_2 either is not present in human urine or its concentration is considerably smaller than that of 2,3-dn-TxB_2. The finding that additional TLC did not reduce urinary 2,3-dn-TxB_2 levels, strongly suggests that no other compounds co-eluted with 2,3-dn-TxB_2. In the chromatogram of Fig. 4 (bottom) from the analysis of the same urine sample for 2,3-dn-6-oxo-$PGF_{1\alpha}$ two peaks of comparable intensity emerged in the close neighborhood of the first 2,3-dn-6-oxo-$PGF_{1\alpha}$ peak. Whether these peaks, which have been found in all urine samples analyzed in the present study, are due to 2,3-dn-6-oxo-8-iso-$PGF_{1\alpha}$ or to dinor metabolites of other isoprostacyclins remains to be investigated. In the urine samples that had been additionally purified by TLC these compounds were not present in the TLC fractions of 2,3-dn-6-oxo-$PGF_{1\alpha}$ and did not appear in the GC–tandem MS chromatogram of this metabolite (not shown). Also, additional TLC did not reduce urinary levels of 2,3-dn-6-oxo-$PGF_{1\alpha}$, strongly suggesting that no other compounds co-eluted with this metabolite. Aspirin was found to reduce both 2,3-dn-6-oxo-$PGF_{1\alpha}$ and their close neighbor peaks. These and preliminary results of our group on 8-iso-PGE_2 excretion in human urine [15]

suggest that isothromboxanes, isoprostacyclins and E_2-isoprostaglandins are not abundantly present in human urine unlike F_2-isoprostanes [13,16].

In summary, the methods described in this work are useful to accurately and rapidly assess whole body synthesis of cyclooxygenase-dependent thromboxane and prostacyclin and should be useful in investigating formation and metabolism of cyclooxygenase-independent isothromboxanes and isoprostacyclins in humans.

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