Journal of Chromatography, 497 (1989) 1–16
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4986

ANALYSIS OF THE MAJOR URINARY THROMBOXANE METABOLITES, 2,3-DINORTHROMBOXANE B_2 AND 11-DEHYDROTHROMBOXANE B_2, BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY AND GAS CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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(First received March 23rd, 1989; revised manuscript received July 17th, 1989)

SUMMARY

2,3-Dinorthromboxane B_2 and 11-dehydrothromboxane B_2, the two major metabolites of thromboxane B_2, are considered to be indices of thromboxane A_2 activity in humans. The determination of these metabolites in urine was comparatively performed by gas chromatography–mass spectrometry and gas chromatography–mass spectrometry–mass spectrometry using the corresponding chemically synthesized tetradeuterated analogues as internal standards. The urine samples of five females and two males, all healthy, were prepurified by solid-phase extraction. The corresponding pentafluorobenzyl ester derivatives were repurified by high-performance liquid chromatography. The concentrations of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 ranged from 21 to 266 pg/ml and 47 to 942 pg/ml, respectively. The ratio of urinary 2,3-dinorthromboxane B_2 to 11-dehydrothromboxane B_2 varied from 1:3 to 1:5, except for one sample with nearly equal concentrations of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2.

0378-4347/89/$03.50

INTRODUCTION

Thromboxane A_2, the predominant product of arachidonic acid metabolism in the platelet, is a potent vasoconstrictor and platelet agonist. The determination of thromboxane A_2 itself is difficult owing to its extremely short half-life of ca. 30 s. Thromboxane B_2, which is formed spontaneously from thromboxane A_2, also undergoes rapid degradation. The more stable, biologically inactive major urinary metabolites of thromboxane B_2, 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2, are considered to be representative indices of thromboxane A_2 formation in vivo [1,2].

The published analytical methods for thromboxane B_2, 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 quantification in urine or plasma include one or more purification steps by thin-layer chromatography (TLC) [3–6] or column chromatography [7–10]. Generally, the described techniques provide a fast and comparatively simple extraction. However, significant losses of material may occur owing to exposure to oxygen and/or incomplete extraction from the solid phase.

Recently a purification technique for 2,3-dinorthromboxane B_2 in urine has been published, which is based on the use of an immobilized antibody column [1,11,12]. The selectivity of this method is rather high, but the required materials are not easily available, and their preparation is difficult and time-consuming. Experiments in our laboratory have shown that 2,3-dinorthromboxane B_2, when chromatographed as the free acid, deteriorates during the high-performance liquid chromatographic (HPLC) procedure. HPLC of the underivatized thromboxane metabolite [8–10] is therefore unsatisfactory. HPLC separation of prostaglandin methyl esters [13–15] is limited to the mass spectrometric (MS) detection using the less sensitive positive electron ionization (EI) or chemical ionization (CI) mode.

The determination of 2,3-dinorthromboxane B_2 in human body fluids has so far been performed only by means of single-stage quadrupole MS. For the determination of 11-dehydrothromboxane B_2, however, a method including cartridges for extraction and tandem (triple-stage) MS for quantification has been published [2].

This paper describes a sensitive and specific quantitation technique for the determination of both enzymic metabolites of thromboxane B_2 in urine. In this method, HPLC of the pentafluorobenzyl derivatives of the thromboxanes for purification, and gas chromatography (GC) in combination with negative ion CI tandem MS (GC–NICI–MS–MS) for quantitation are utilized. The results of the quantitative determination of both thromboxane metabolites, with and without HPLC purification, are compared, as are detection by single-stage GC–MS and by triple-stage quadrupole GC–MS–MS.

EXPERIMENTAL

Materials

All solvents were of analytical grade and were purchased from Merck (Darmstadt, F.R.G.). Synthetic 2,3-dinorthromboxane B_2, 11-dehydrothromboxane B_2 and the low-blank tetradeuterated analogues ($^2H_0$ with respect to $^2H_4$ less than 0.1%) were synthesized as described in detail elsewhere [2,16]; the isotopomers were labelled at C-18 and C-19. [^{3}H]-6-Ketoprostaglandin $F_{1\alpha}$ and [^{3}H]-11-dehydrothromboxane B_2 were obtained from NEN (Dreieich, F.R.G.). Methoxyamine hydrochloride and pentafluorobenzyl bromide were purchased from Sigma (St. Louis, MO, U.S.A.), and N,O-bis(trimethylsilyl)trifluoroacetamide was from Pierce (Rockford, IL, U.S.A.). Silica and C_{18} cartridges were obtained from Waters Assoc. (Milford, MA, U.S.A.).

Instrumentation

For HPLC an Ultrasphere ODS column (5 μm, 25 mm$\times$4.6 mm I.D.) from Beckman Instruments (Munich, F.R.G.) was used; the solvent-delivery pumps (Model 600 A) were from Waters Assoc. The mass spectra were run on a triple-stage quadrupole mass spectrometer (Model TSQ 70) from Finnigan MAT (San Jose, CA, U.S.A.) attached to a gas chromatograph (Model 3400) from Varian (Sunnyvale, CA, U.S.A.).

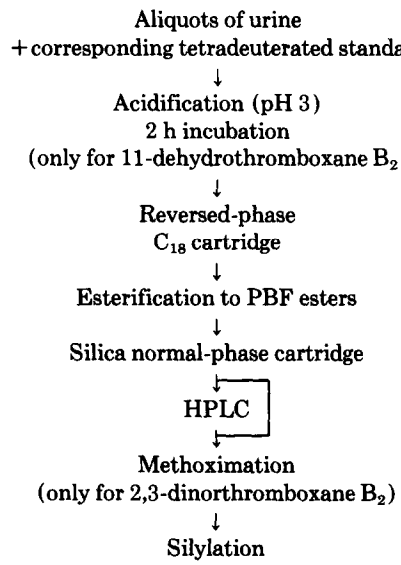

Fig. 1. Scheme of the sample purification and derivatization procedure with and without HPLC.

Sample collection

Healthy adults who had not taken any medication for two weeks were instructed to collect urine for 24 h. Samples were kept in a refrigerator until the end of the collection. Aliquots of 50 ml were stored at −20°C.

Urine purification and derivatization

A scheme of the purification and derivatization procedure with and without the HPLC option is shown in Fig. 1.

Extraction

For the determination of 11-dehydrothromboxane B_2, 10 ng of [$^2H_4$]-11-dehydrothromboxane B_2 were added to a 10-ml aliquot of urine. For 2,3-dinorthromboxane B_2 determination, 3 ng of tetradeuterated internal standard were added to a 5-ml aliquot of urine. The urine samples were then acidified to ca. pH 3 with formic acid (10%, v/v, in water). The 11-dehydrothromboxane B_2 sample was equilibrated at room temperature for 2 h to produce the lactone form.

Both samples were applied to a C_{18} cartridge preconditioned with 20 ml of ethanol and 20 ml of water. The sample was washed with 20 ml of water, 20 ml of ethanol–water (1:10, v/v) and 20 ml of light petroleum (boiling point range 40–60°C), then eluted with 20 ml of acetonitrile–methanol (9:1, v/v). The solvent was then evaporated.

Esterification

The sample was esterified with 90 μl of pentafluorobenzyl bromide–N,N-diisopropylethylamine–acetonitrile (2:2:5) at 45°C for 25 min. To remove excess pentafluorobenzyl bromide, the sample was dried under nitrogen, redissolved in 2 ml of dichloromethane and applied to a silica cartridge preconditioned with 20 ml of dichloromethane. The sample was washed with 20 ml of dichloromethane, then eluted with 20 ml of ethyl acetate. The solvent was then evaporated.

High-performance liquid chromatography

Chromatography was performed on an Ultrasphere ODS column. The solvent system was acetonitrile–water–methanol (4:3:3, v/v) acidified to pH 4.0 with acetic acid. The flow-rate was 1.0 ml/min. The sample was dissolved in 200 μl of the mobile phase.

The retention time of the pentafluorobenzyl (PFB) derivative of 11-dehydrothromboxane B_2 was determined with analogously treated [^{3}H]-11-dehydrothromboxane B_2, for which forty 1-ml fractions were collected and the corresponding radioactivity counted.

A radioactive standard for 2,3-dinorthromboxane B_2 was not available. The retention time, therefore, was determined using a non-deuterated standard of

the esterified compound, for which fractions 5–30 were measured with GC–MS detection.

Further derivatization

For the methoximation 100 μl of methoxyamine hydrochloride (10% in pyridine) were added to the 2,3-dinorthromboxane B_2 sample. After incubation at 50 °C for 30 min the sample was dried under nitrogen.

Both thromboxane samples were dissolved in 50 μl of dry pyridine and 50 μl of bis(trimethylsilyl)trifluoroacetamide (BSTFA), equilibrated for 1 h at room temperature, and dried under nitrogen. In order to remove excess derivatizing agent, the samples were dissolved in 2 ml of dichloromethane and purified by means of a silica cartridge as described above.

Finally, the samples were treated with 100 μl of BSTFA for 1 h at room temperature and dissolved in ethyl acetate before detection by GC–MS and/or GC–MS–MS.

Preliminary studies have shown that the best results for sample purity and recovery were obtained from urine samples treated for silylation first with BSTFA–pyridine followed by pure BSTFA. The different reactivity of the hydroxyl groups present in thromboxanes may cause this effect.

Gas chromatography and mass spectrometry

GC was performed on a Varian 3400 gas chromatograph in the splitless mode using a J&W fused-silica capillary column (DB-1, 30 m × 0.25 mm I.D., coating

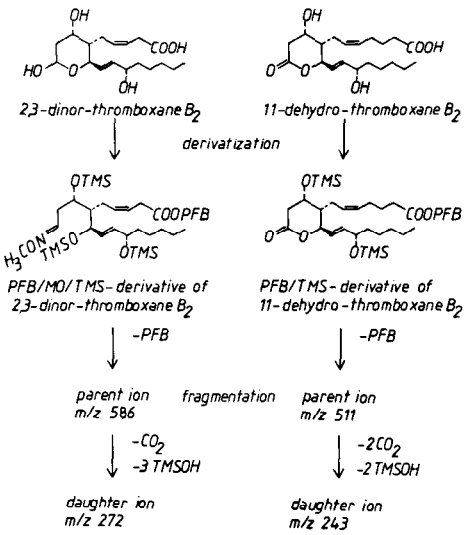

Fig. 2. Derivatization and MS detection of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2. PFB = pentafluorobenzyl ($CH_2(C_6F_5)$); TMS = trimethylsilyl ($Si(CH_3)_3$); MO = methoxime ($=NOCH_3$).

thickness 0.25 μm; ICT, Frankfurt, F.R.G.). The conditions were: injector temperature, 280 °C; interface temperature, 300 °C; oven temperature programme, 2 min at 100 °C, 100 to 250 °C at 30 °C/min, 250 °C to 300 °C at 5 °C/min, 10 min at 300 °C; carrier gas, helium; column head pressure, 750 Torr.

For MS a Finnigan TSQ 70 was used in the NICI mode; argon was used as reagent gas in the MS–MS experiments. Operating conditions were: source temperature, 130 °C; electron energy, 70 eV; emission current 0.2 mA; methane CI gas pressure, 10 Torr; electron multiplier, 1300 V. In the MS–MS experiments, the argon collision cell pressure was 2 mTorr and the collision energy

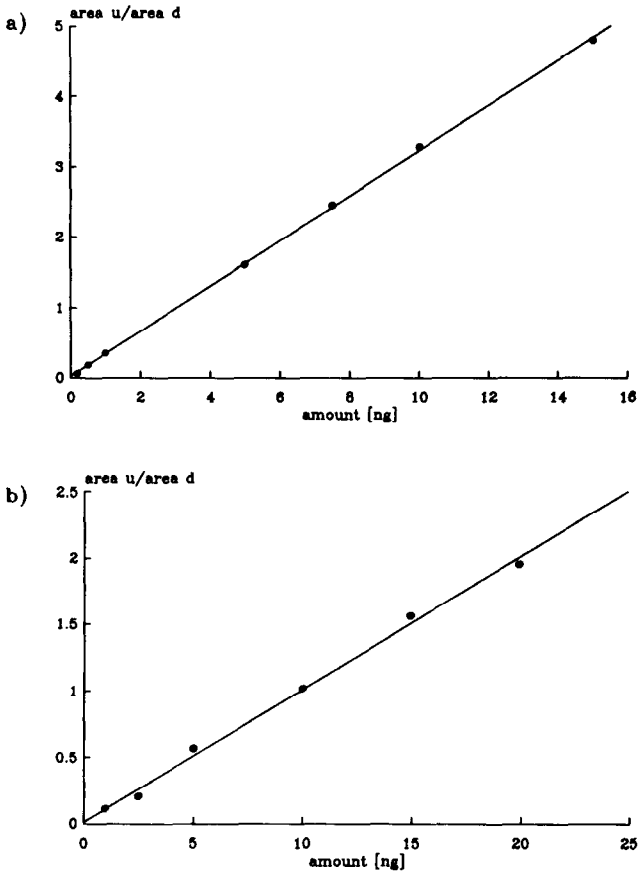

Fig. 3. Calibration curves for (a) daughter ions m/z 272/276 of 2,3-dinorthromboxane B_2 (3 ng of deuterated and 0.2–15 ng of non-deuterated standard) and (b) daughter ions m/z 243/247 of 11-dehydrothromboxane B_2 (10 ng of deuterated and 1–20 ng of non-deuterated standard). The ratio of the area of the non-deuterated (u) to the deuterated (d) compound (y-axis) is plotted against the amount of the non-deuterated standard (x-axis). The linear equations and r values obtained from the plots are $y=0.321x+0.022$, $r=1.0$ and $y=0.099x+0.013$, $r=0.996$ for (a) and (b), respectively.

was 14 eV for 2,3-dinorthromboxane B_2 and 16 eV for 11-dehydrothromboxane B_2. The electron multiplier was set to 2000 V.

MS was performed in the multiple ion detection (MID) mode. In the single MS mode, selected masses were *m/z* 586/590 for undeuterated and deuterated 2,3-dinorthromboxane B_2 and *m/z* 511/515 for undeuterated and deuterated 11-dehydrothromboxane B_2. For tandem MS, fragments of *m/z* 272/276 and *m/z* 243/247 were chosen as characteristic daughter ions of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2, respectively (Fig. 2).

Calibration curve and recovery experiments

For calibration, mixtures of 10 ng of deuterated and 1–20 ng of non-deuterated 11-dehydrothromboxane B_2 or 3 ng of deuterated and 0.2–15 ng of non-

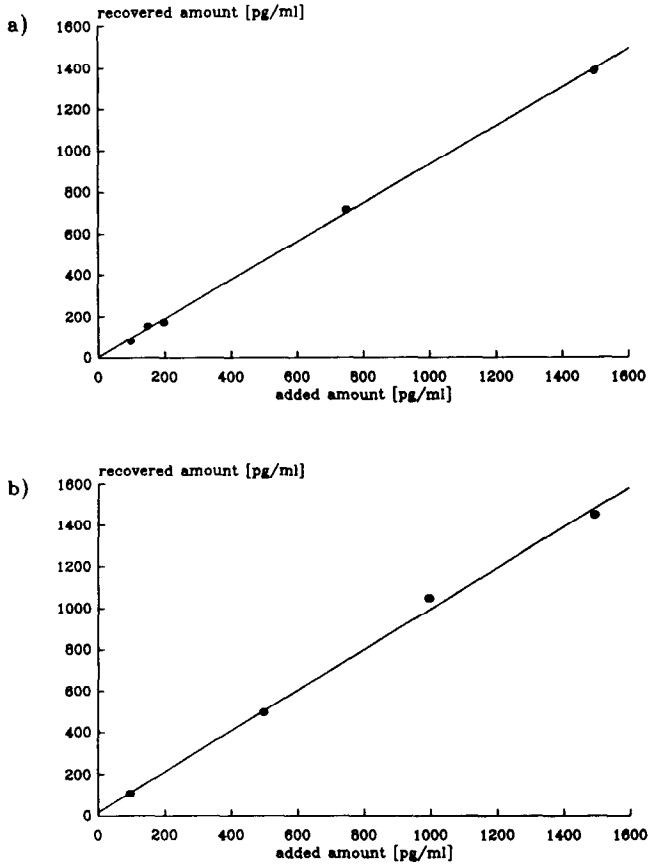

Fig. 4. Recovery experiment for (a) daughter ions *m/z* 272/276 of 2,3-dinorthromboxane B_2 (5 ml of urine, spiked with 3 ng of deuterated and 500–7500 pg non-deuterated standard) and (b) daughter ions *m/z* 243/247 of 11-dehydrothromboxane B_2 (10 ml of urine, spiked with 10 ng of deuterated and 1–15 ng of non-deuterated standard). Sample clean-up according to Fig. 1 with HPLC.

deuterated 2,3-dinorthromboxane B_2 were measured by GC–MS–MS.

Two approaches were used in the recovery experiment. In the first, a defined urine sample was spiked with increasing amounts of non-deuterated thromboxane standard, i.e. 100–1500 pg/ml for both thromboxane metabolites.

In the second approach, all urine samples extracted by HPLC were spiked with 100 and 500 pg/ml of the corresponding non-deuterated standard of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2, respectively. The reproducibility and accuracy of the method were controlled by running the original and the spiked urine sample one after the other and comparing the results of endogenous and added thromboxane.

RESULTS

Calibration curve

The calibration curves for both thromboxane metabolites (Fig. 3) were linear, i.e. the ratio between the signals of the non-deuterated and deuterated derivatives were directly proportional to the amount of the non-deuterated derivative.

Owing to the high deuterium content of the labelled isotopomers ($^2H_4 > 98\%$), the ratio of the corresponding signals ($^2H_0/^2H_4$) equals 1.0, when equal amounts of the deuterated and the non-deuterated standards are measured.

Recovery experiment

In the recovery experiments the sample clean-up was carried out, as de-

TABLE I

ENDOGENOUS CONCENTRATIONS OF 2,3-DINORTHROMBOXANE AND 11-DEHYDROTHROMBOXANE B_2 IN SEVEN URINE SAMPLES PREPURIFIED BY HPLC AND DETERMINED BY GC-MS AND GC-MS-MS

N.D. = not detectable.

Sample No.	Concentration (pg/ml)			
	2,3-Dinorthromboxane B_2		11-Dehydrothromboxane B_2	
	GC-MS	GC-MS-MS	GC-MS	GC-MS-MS
1	20	21	114	109
2	27	36	339	173
3	45	170	173	226
4	77	143	867	574
5	117	266	722	696
6	122	176	874	942
7	N.D.	N.D.	140	47

scribed in Fig. 1, with the HPLC step included. The results are shown in Fig. 4. The average ($\pm$S.D.) recoveries for 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 were 93 ± 7 and $95 \pm 4\%$, respectively. This demonstrated the reproducibility of the method for both metabolites. The concentration range examined in this experiment, 100–1500 pg/ml, corresponds to the concentrations of both metabolites found in urine of healthy volunteers. By

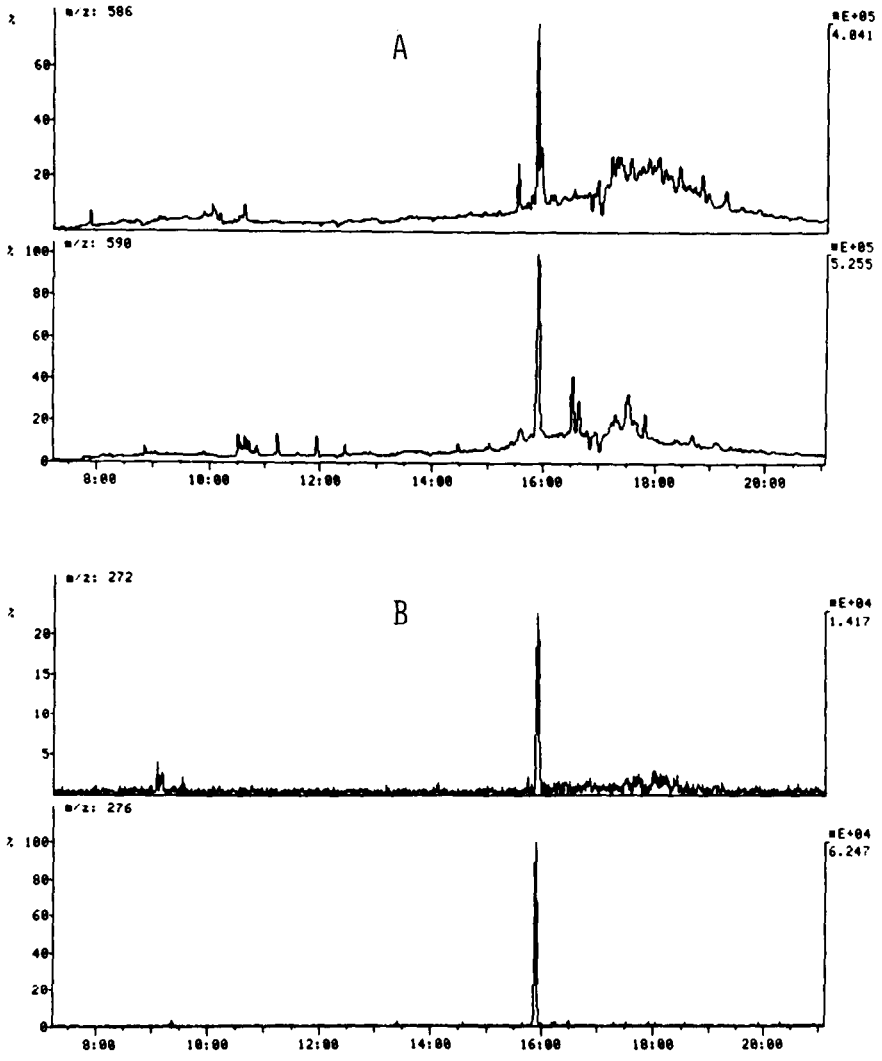

Fig. 5. Parent ion (A) and daughter ion (B) chromatograms of PFB-MO-TMS derivatives of urinary 2,3-dinorthromboxane B_2 (upper trace, retention time 15.56 min) and [$^2H_4$]-2,3-dinorthromboxane B_2 (lower trace, retention time 15.54 min). Sample purification according to Fig. 1 with HPLC.

this method, therefore, the levels of endogenous 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 in urine can be determined with sufficient accuracy.

Urine samples

Purification with HPLC. The endogenous levels of 11-dehydrothromboxane B_2 and 2,3-dinorthromboxane B_2 were determined in seven urine samples, us-

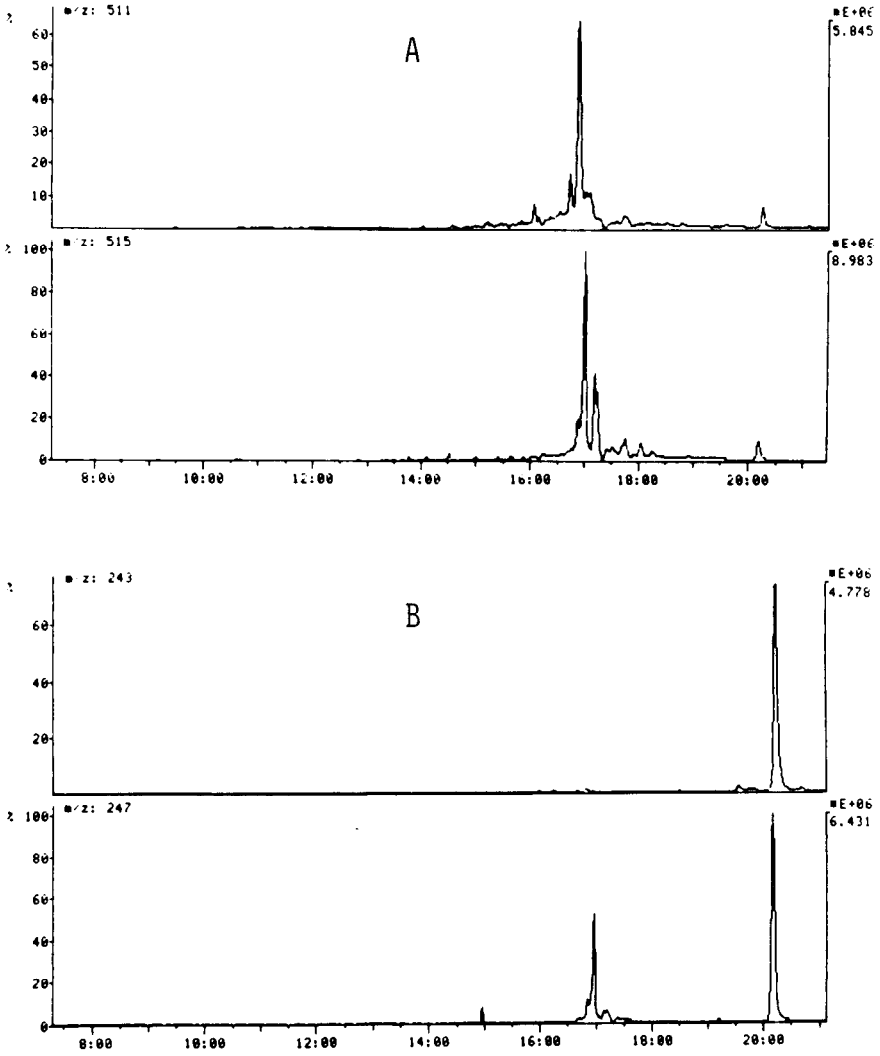

Fig. 6. Parent ion (A) and daughter ion (B) chromatograms of PFB-TMS derivatives of urinary 11-dehydrothromboxane B_2 (upper trace, retention time 20.14 min) and [$^2H_4$]-11-dehydrothromboxane B_2 (lower trace, retention time 20.12 min). Sample clean-up according to Fig. 1 with HPLC.

ing the triple-stage mass spectrometer in the MS as well as in the MS–MS mode (Table I).

The corresponding chromatograms of urine samples 4 and 5 are shown in Figs. 5 and 6, respectively.

Although the urine samples were prepurified by two solid-phase extractions and HPLC, the chromatograms contain signals other than those of the pentafluorobenzyl–methoxime–trimethylsilyl (PFB–MO–TMS) or penta-fluorobenzyl–trimethylsilyl (PFB–TMS) derivatives of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 (Figs. 5A and 6A), when run in the MS mode.

TABLE II

RECOVERY EXPERIMENT FOR 2,3-DINORTHROMBOXANE B_2 AND 11-DEHYDROTHROMBOXANE B_2 WITH SEVEN URINE SAMPLES, PREPURIFIED BY HPLC

N.D. = not detectable.

Sample No.	2,3-Dinorthromboxane B_2[a] (pg/ml)	11-Dehydrothromboxane B_2[b] (pg/ml)
1	102	498
2	93	450
3	110	476
4	116	494
5	105	432
6	104	476
7	N.D.	486

[a]Amount added 100 pg/ml.
[b]Amount added 500 pg/ml.

TABLE III

ENDOGENOUS CONCENTRATIONS OF 11-DEHYDROTHROMBOXANE B_2 IN SEVEN URINE SAMPLES PREPURIFIED WITHOUT HPLC AND DETERMINED BY GC-MS AND GC-MS-MS

Sample No.	Concentration (pg/ml)	
	GC-MS	GC-MS-MS
1	216	91
2	-[a]	221
3	-[a]	278
4	-[a]	539
5	2705	692
6	-[a]	904
7	-[a]	N.D.[b]

[a]Quantification impossible because of impurities.
[b]N.D. = not detectable.

If an impurity is coeluted with the compound to be determined, a precise quantification is not possible. When the interfering compound forms a fragment of the same *m/z* value as the derivative of the deuterated standard, the calculated urinary concentration will be underestimated. Samples 3, 4 and 5 of the 2,3-dinorthromboxane B_2 determination show this effect (Table I). If, in contrast,

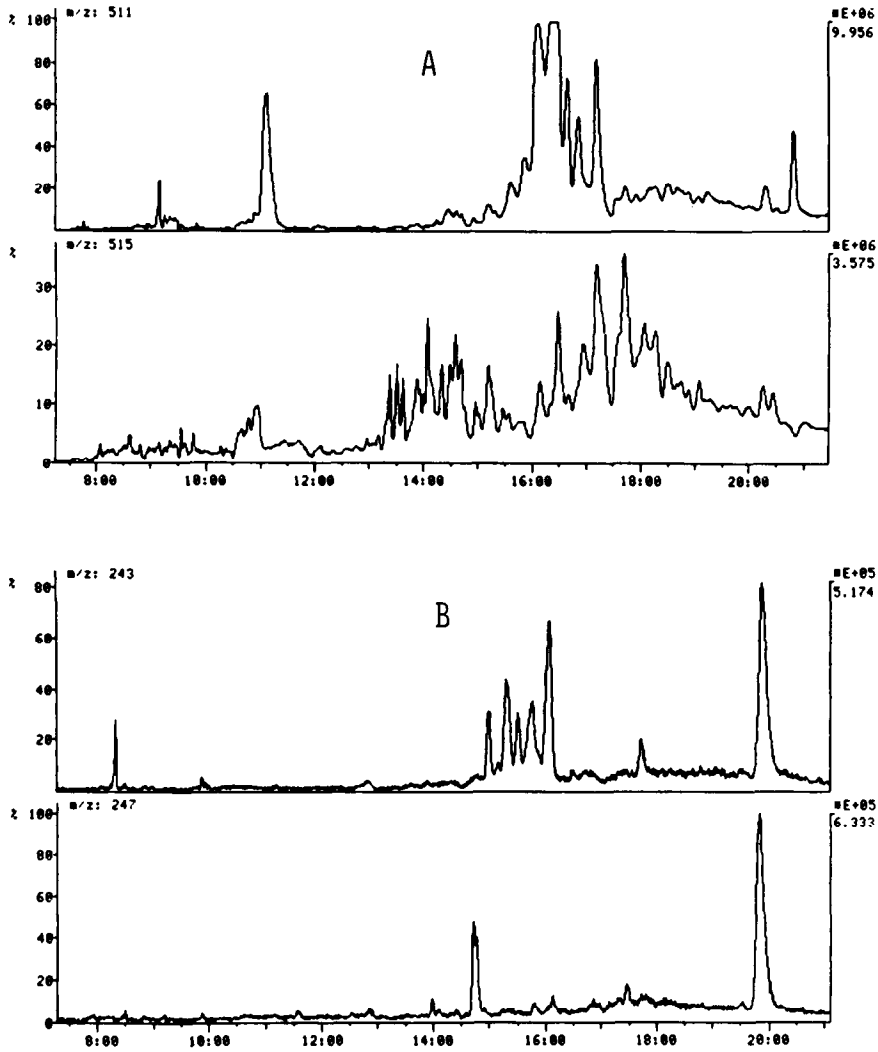

Fig. 7. Parent ion (A) and daughter ion (B) chromatograms of PFB–TMS derivatives of urinary 11-dehydrothromboxane B_2 (upper trace, retention time 20.18 and 19.52 min, respectively) and [$^2H_4$]-11-dehydrothromboxane B_2 (lower trace, retention time 20.16 and 19.50 min, respectively). Sample clean-up according to Fig. 1 without HPLC. The sample is identical with that of Fig. 6.

the impurity forms a fragment of the same *m/z* value as the derivative of the non-deuterated thromboxane metabolite, the calculated amount of the compound to be determined will be too high. This situation arose during the measurement of 11-dehydrothromboxane B_2 in urine samples 2, 4 and 7 (Table I).

For 2,3-dinorthromboxane B_2, the concentrations found in urine ranged from 21 to 266 pg/ml. Those of 11-dehydrothromboxane B_2 ranged from 47 to 942 pg/ml (Table I). The ratio of 2,3-dinorthromboxane B_2 to 11-dehydrothromboxane B_2 varied from 1:3 to 1:5 in five of the six samples examined. In sample 3, however, the ratio was close to unity.

The reproducibility and accuracy of the obtained results were examined by additional spiking of all urine samples with 100 pg/ml non-deuterated 2,3-dinorthromboxane B_2 standard or 500 pg/ml non-deuterated 11-dehydrothromboxane B_2 standard (Table II). The average recovery ($\pm$S.D.) was $105 \pm 7.1\%$ for 2,3-dinorthromboxane B_2 and $94.6 \pm 4.4\%$ for 11-dehydrothromboxane B_2. Therefore, even small differences and low endogenous levels of both thromboxane metabolites can be determined with sufficient accuracy by the GC–MS–MS method.

Purification without HPLC. For 2,3-dinorthromboxane B_2 a reliable identification and quantification without HPLC purification was not possible, even in the MS–MS mode (data not shown).

In contrast, because of its different structure (one lactone ring) the PFB derivatives of 11-dehydrothromboxane B_2 have longer retention times than the PFB derivatives of other thromboxanes, prostaglandins and most of the impurities. For this reason, its quantification was possible even without HPLC purification (Table III). The corresponding MS and MS–MS chromatograms for urine sample 5 are shown in Fig. 7. (Corresponding chromatograms for this urine sample with HPLC purification are shown in Fig. 6.)

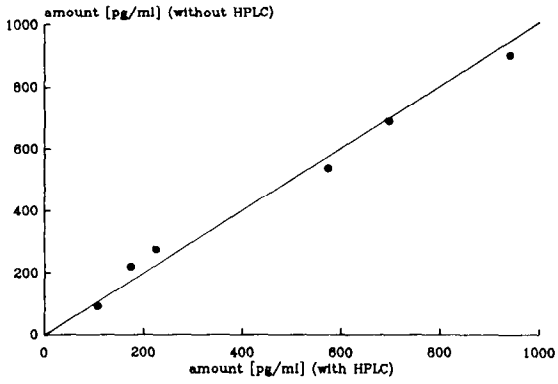

Fig. 8. Correlation of the concentrations of endogenous 11-dehydrothromboxane B_2 in urine, prepurified according to Fig. 1 with (x-axis) and without (y-axis) HPLC. Determination by tandem MS.

The urinary concentrations of 11-dehydrothromboxane B_2 obtained with (Table I) and without HPLC (Table III) purification were similar, when measured in the MS–MS mode. A good correspondence between the two methods was obtained within the concentration range measured ($103.8 \pm 16.0\%$) (Fig. 8).

In contrast, urine samples prepurified without HPLC contained considerable amounts of contaminants, which made reliable peak identification and quantification impossible in the MS mode (Fig. 7A). In the MS–MS mode, signals originating from impurities were also present (Fig. 7B); however, they did not interfere with peak quantification.

DISCUSSION

The objectives of the present study were to compare the determination of urinary 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 using the MS–MS and the MS modes. In addition, two sample purification procedures, with and without HPLC, were compared.

Our results indicate that detection by GC–MS–MS is superior to that by GC–MS. Further, the use of HPLC in the purification of both metabolites is recommended.

MS–MS detection of daughter ions has several advantages over the MS detection of parent ions. For every signal appearing in an MS–MS chromatogram at a certain m/z value the following parameters have to be coordinated to one another: the m/z value of the parent ion; the m/z value of the daughter ion; the collision cell pressure; the kinetic energy of the daughter ion.

Compared with detection in the MS mode, where only the first of the above conditions has to be met (Figs. 5A and 6A), MS–MS detection produces chromatograms with only few signals in addition to the compound of interest (Figs. 5B and 6B). The increased selectivity of the MS–MS mode therefore leads to much more reliable results than the MS mode.

The identification and quantification of 2,3-dinorthromboxane B_2 by GC–MS and GC–MS–MS in urine can be achieved only with prepurification by solid-phase extraction and HPLC. In contrast, urinary 11-dehydrothromboxane B_2 can be detected by GC–MS–MS with sufficient accuracy from samples purified with two solid-phase extractions without HPLC. Under these conditions, however, the higher content of impurities remaining in the samples produces rapid contamination of the detector system, necessitating frequent and time-consuming cleaning procedures. An additional clean-up with HPLC is therefore recommended.

Prior to HPLC purification, derivatization of the thromboxane metabolites to the corresponding PFB esters is essential. In preliminary experiments we found that thromboxanes with unprotected carboxyl groups can undergo struc-

tural changes during HPLC, which invalidate the subsequent detection by GC–MS or GC–MS–MS. When standards of 2,3-dinorthromboxane B_2 or 11-dehydrothromboxane B_2 were applied to the HPLC column, the recovered amounts of both metabolites were ca. 100-fold higher if the compound had been esterified.

With HPLC prepurification, detection and qualitative estimation of both metabolites was also possible in the MS mode. The differences between the values obtained by GC–MS and by GC–MS–MS were, however, large (from 4 to 199% for 11-dehydrothromboxane B_2; from 137 to 291% for 2,3-dinorthromboxane B_2). Therefore, quantitative measurements using GC–MS are not possible.

In addition to the thromboxane metabolites, thromboxane B_2 and several other urinary prostaglandins can be determined by the described procedure. An overview of the retention times of the PFB derivatives of several eicosanoids, which have been separated in our laboratories using HPLC, is given in Fig. 9.

2,3-Dinorthromboxane B_2 and 11-dehydrothromboxane B_2 represent the two major metabolites of thromboxane in urine, reflecting the β-oxidation and the dehydrogenation, respectively, of the hemiacetal alcohol group at C-11 of the parent compound. The variation in the ratio of the two thromboxane metabolites obtained in the six urine samples (Table I) probably reflects the inter-individual variability in eicosanoid metabolism.

In five of the six samples the ratio of 2,3-dinorthromboxane B_2 to 11-dehydrothromboxane B_2 was in a narrow range (1:3 to 1:5) (see also Catella and FitzGerald [1]). Interestingly, urine sample 3, with a ratio close to unity (1:1.3), originated from a heavy smoker. Whether this finding reflects an alteration in thromboxane metabolism typical for smokers needs to be confirmed. Further measurements of 2,3-dinorthromboxane B_2 and 11-dehydro-

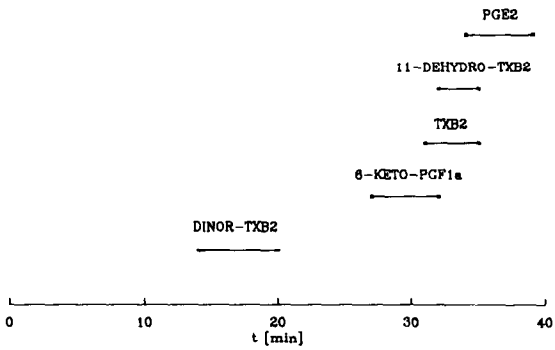

Fig. 9. HPLC of the PFB esters of 2,3-dinorthromboxane B_2 (DINOR-TXB2), 11-dehydrothromboxane B_2 (11-DEHYDRO-TXB2), thromboxane B_2 (TXB2), prostaglandin E_2 (PGE2) and 6-ketoprostaglandin $F_{1\alpha}$ (6-KETO-PGF1a). Chromatographic conditions as in Experimental.

thromboxane B_2 in urine samples from smokers and from patients who suffer from diseases associated with an increased thromboxane formation rate are therefore of interest. If a shift of the ratio of the two thromboxane metabolites could also be established in pathological state, the ratio of 2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2 might become relevant.

ACKNOWLEDGEMENTS

This work was supported by grants of the Deutsche Forschungsgemeinschaft (We 681/6-7 and Me 792/1-2) and by the Robert-Bosch-Foundation, Stuttgart. We thank B. Boehlig and U. Katzner for their excellent technical assistance.

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