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Short Communication

Determination of seven prostanoids in 1 ml of urine by gas chromatography–negative ion chemical ionization triple stage quadrupole mass spectrometry

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Abstract

In an isotope dilution assay, prostaglandin (PG) E_2, 6-keto-$PGF_{1\alpha}$, thromboxane (Tx) B_2 and their metabolites PGE-M (11α-hydroxy-9,15-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid), 2,3-dinor-6-keto-$PGF_{1\alpha}$, 2,3-dinor-TxB_2 and 11-dehydro-TxB_2 were determined in urine by gas chromatography–triple stage quadrupole mass spectrometry (GC–MS–MS). After addition of deuterated internal standards, the prostaglandins were derivatized to their methoximes and extracted with ethyl acetate–hexane. The sample was further derivatized to the pentafluorobenzylesters and purified by thin-layer chromatography (TLC). Three zones were scraped from the TLC plate. The prostanoid derivatives were converted to their trimethylsilyl ethers and the products were quantified by GC–MS–MS. In each run, two or three prostanoids were determined.

1. Introduction

After the discovery of primary prostanoids, *e.g.* PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2, in human urine, their urinary excretion rates have been used for the *in vivo* determination of renal prostanoid activity. This approach appears to be justified because systematically administered or generated prostanoids are excreted primarily as metabolites, *e.g.* PGE-M, 2,3-dinor-6-keto-$PGF_{1\alpha}$, 2,3-dinor-TxB_2 and 11-dehydro-TxB_2. Thus, simultaneous determination of urinary levels of primary prostanoids and their metabolites has been employed in many studies to assess renal as well as systemic prostanoid synthesis.

Initially prostanoid assays using electron-impact ionization GC–MS of the methyl ester–methoxime (MO)–trimethylsilyl ether (TMS) needed up to two liquid–liquid or solid-phase extractions and one high-performance liquid chromatography (HPLC) step for purification of the sample [1–6]. A technician could work up only two or three samples a day, each sample containing up to three prostaglandins. The subsequent introduction of GC–negative ion chemical ionization (NICI) MS of the pentafluorobenzylester (PFB)–MO–TMS derivatives of prostanoids [7] allowed reduction of the sample volume from 20–50 ml of urine [8–11] and also,

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because of the higher efficiency of GC–NICI-MS, the determination of prostanoids in plasma [10,12,13]. The clean-up procedure consisted of solid-phase and liquid–liquid extraction and one or two TLC steps. Switching from HPLC to TLC allowed to increase the number of analyses by a factor of three. In one sample, only one or two prostanoids were determined.

Next improvements were the introduction of immunoaffinity chromatography [14–17] and of GC–MS–MS [18–23]. Both methods allow to reduce the clean-up procedure. The first has the advantage of using a cheaper instrument, butnot all antibodies, *e.g.* the one against PGE-M and 9α,11α-dihydroxy-15-oxo-2,3,4,5,20-pentanor-19-carboxyprostanic acid, the main metabolite of $PGF_{2\alpha}$ in urine, used to prepare an immunoaffinity column are available. From our experience, the clean-up method with only one liquid–liquid or solid-phase extraction and one TLC separation is more rapid than immunoaffinity chromatography.

Using the assays reported in the literature, it is not possible to determine both the the primary prostanoids and their metabolites in several hundreds of samples within a reasonable time. Therefore, a new method had to be developed.

2. Experimental

2.1. Prostanoids and reagents

[3,3,4,4-$^2H_4$]-PGE_2, [3,3,4,4-$^2H_4$]-6-keto-$PGF_{1\alpha}$, [3,3,4,4-$^2H_4$]-TxB_2, [18,18,19,19-$^2H_4$]-2,3-dinor-6-keto-$PGF_{1\alpha}$ [18,18,19,19-$^2H_4$]-2,3-dinor-TxB_2, their undeuterated analogues and PGE-M were a kind gift from Dr. Udo Axen (Upjohn, Kalamazoo, MI, USA). [18,18,19,19-$^2H_4$]-11-Dehydro-TxB_2, [13,17,17,18,18,19,19-$^2H_7$]-PGE-M and 11-dehydro-TxB_2 were obtained from Dr. Claus O. Meese (Dr. Margarete-Fischer-Bosch-Institut für Klinische Pharmakologie, Stuttgart, Germany).

Ethyl acetate was obtained from Promochem (Wesel, Germany), O-methylhydroxylamine hydrochloride and pentafluorobenzyl bromide from Serva (Heidelberg, Germany), water, formic acid, pyridine and acetonitrile from Merck (Darmstadt, Germany) and hexane and sodium acetate from Riedel-de Haen (Seelze, Germany). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Macherey and Nagel (Düren, Germany) and N,N-diisopropylethylamine from Pierce (Oud Beijerland, Netherlands) and silica-gel TLC plates (LK6D, 5 × 20 cm) were from Whatman (Maidstone, UK). Helium (99.996%), methane (99.995%), and argon (99.998%) were from Messer Griesheim (Herborn, Germany).

2.2. Sample preparation

Urine (1 ml) and 4–12 ng of deuterated prostanoids were acidified with formic acid (10%) to pH 3.2 and O-methylhydroxylamine hydrochloride (130 mg) in 1.5 ml of acetate buffer (1.5 *M*, pH 5) were added to form the methoxime. After acidification with formic acid to pH 2.5, the prostanoid derivatives were extracted twice with 3 ml of ethyl acetate–hexane (70:30, v/v). After evaporation of the solvent, acetonitrile (80 μl), pentafluorobenzyl bromide (7 μl) and N,N-diisopropylethylamine (25 μl) were added, The mixture was allowed to react at 40°C for 25 min. The dry sample was purified by TLC (developing solvent: ethyl acetate–hexane 90:10, v/v). Three broad zones (zone 1: R_F 0.03–0.16, zone 2: R_F 0.17–0.39, zone 3: R_F 0.40–0.80) were eluted with the TLC developing solvent (800 μl) and water (50 μl) was added. After centrifugation at 10 000 *g* for 5 min, the ethyl acetate phase was withdrawn, the solvent was evaporated and the prostanoids derivatized with BSTFA (25 μl; 40°C, 1 h). A 2-μl aliquot of this solution was injected.

2.3. GC–MS–MS

A Finnigan MAT TSQ700 tandem mass spectrometer equipped with a Varian 3400 gas chromatograph and a CTC A200S autosampler (Finnigan MAT, Bremen, Germany) was employed. Gas chromatography of prostanoid derivatives was carried out on a J&W DB-1 (20 m × 0.25 mm I.D., 0.25 μm film thickness)

Table 1
Parent and daughter ions of endogenous prostanoids and their [^2H_n]-analogues ($n = 4–7$) used for quantification

Prostanoid	m/z (endogenous compound)		m/z (^2H_n-prostanoid)	
	Parent ion	Daughter ion	Parent ion	Daughter ion
2,3-Dinor-6-keto-$PGF_{1\alpha}$	586	240	590	244
6-Keto-$PGF_{1\alpha}$	614	312	618	316
2,3-Dinor-TxB_2	586	240	590	244
PGE_2	524	344	528	348
TxB_2	614	268	618	272
11-Dehydro-TxB_2	511	243	515	247
PGE-M	637	349	644	356

capillary column (Carlo Erba, Hofheim, Germany) in the splitless mode at an inlet pressure of 100 kPa. The oven temperature program for all prostanoids analyzed was: initial temperature of 100°C was held for 2 min, then increased at 30°/min to 280°C and at 5°/min to 310°C. This temperature was held for 2 min. Mass spectrometer conditions were: interface temperature 300°C, source temperature 150°C, methane CI gas pressure 50 Pa, electron energy 70 eV, emission current 0.4 mA, conversion dynode 15 kV, and electron multiplier 1600 V. The collision cell pressure was 0.2 Pa and the collision energy was 12–16 eV. Daughter ions used for quantification were $[P - 2(CH_3)_3SiOH]^-$ (PGE_2), $[P - 3(CH_3)_3SiOH - CH_3OH]^-$ (6-keto-$PGF_{1\alpha}$), $[P - 3(CH_3)_3SiOH - CH_3OH - CO_2]^-$ (TxB_2, 2,3-dinor-6-keto-$PGF_{1\alpha}$, 2,3-dinor-TxB_2), $[P - 2(CH_3)_3SiOH - 2CO_2]^-$ (11-dehydro-TxB_2), and $[P - (CH_3)_3SiOH - C_6F_5CH_2OH]^-$ (PGE-M), respectively (Table 1).

3. Results and discussion

Besides for clean-up, TLC was used in this assay to remove the derivatization agents. Under the chosen conditions (developing solvent: ethyl acetate–hexane, 90:10, v/v), the prostanoids were separated by TLC only partially (Table 2). Three TLC zones containing 6-keto-$PGF_{1\alpha}$ and its major urinary metabolite 2,3-dinor-6-keto-$PGF_{1\alpha}$ (zone 1, R_F 0.03–0.16), PGE_2, TxB_2 and its metabolite 2,3-dinor-TxB_2 (zone 2, R_F 0.17–0.39) and 11-dehydro-TxB_2 and PGE-M, the major metabolites of TxB_2 and PGE_2 (zone 3, R_F 0.40–0.80) were scrapped off and derivatized for GC–MS–MS analysis.

The prostanoids contained in one TLC zone can be separated by GC. To obtain a higher sensitivity, the prostanoids were measured consecutively within one run. The scan time for the endogenous compound was 0.4 s, whereas the scan time for the deuterated internal standard was only 0.05 s because of its higher concentration. The chromatograms of a human urine sample (Fig. 1: 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$ Fig. 2: 2,3-dinor-TxB_2, PGE_2, and TxB_2, and Fig. 3: 11-dehydro-TxB_2 and PGE-M) show that there is nearly no background noise from biological compounds.

The limit of detection of PFB–MO–TMS

Table 2
R_F values of MO-PFB derivatives

Prostanoid	R_F	
	First isomer	Second isomer
2,3-Dinor-6-keto-$PGF_{1\alpha}$	0.08	0.11
6-Keto-$PGF_{1\alpha}$	0.09	0.12
PGE_2	0.20	0.31
2,3-Dinor-TxB_2	0.23	0.31
TxB_2	0.25	0.31
11-Dehydro-TxB_2	0.45[a]	
PGE-M		0.63[b]

Developing solvent: ethyl acetate–hexane, 90:10, v/v.
[a] Only one isomer.
[b] Maximum as determined by GC–MS.

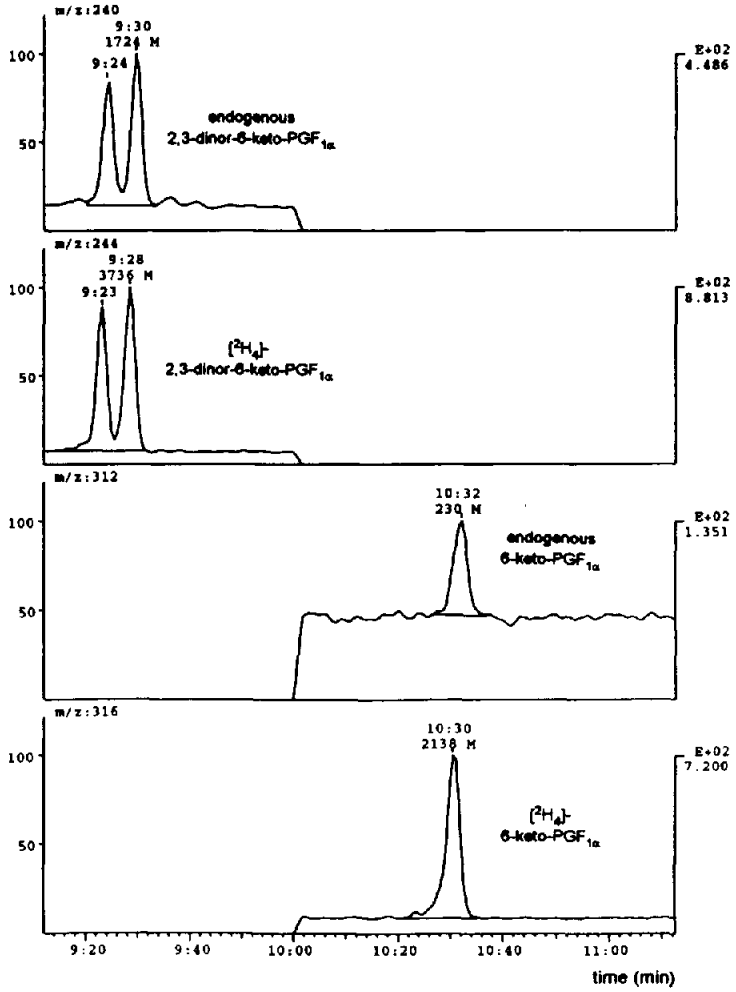

Fig. 1. Chromatogram of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$. 2,3-Dinor-6-keto-$PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$ form two methoxime isomers, but only the dinor-metabolite shows two peaks in the chromatogram. The concentrations of the prostanoids are 2.30 ng/ml (2,3-dinor-6-keto-$PGF_{1\alpha}$) and 0.41 ng/ml (6-keto-$PGF_{1\alpha}$), respectively.

derivatives of prostanoids in the selective-ion monitoring mode is in the high femtogram to the low picogram range [7]. In the collision-induced dissociation (CID) mode, the limit of detection increases by a factor of *ca.* 10, but there are no problems with the higher limit of detection because of the relatively high concentrations of prostanoids in urine. At a signal-to-noise ratio of 5:1, the detection limit is 1–5 pg on column or 10–50 pg/ml of urine, respectively. The highest sensitivity with 1–2 pg on column was obtained for PGE_2, PGE-M, TxB_2, 2,3-dinor-TxB_2 and 11-dehydro-TxB_2, whereas the limit of detection for 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$ is *ca.* 5 pg on column. The calibration curves are linear over a wide range from 10 pg to 10 ng added to 1 ml of urine. The coefficients of variation (one sample worked up five times) are less than 10% at the limit of detection and less than 5% when concentrations of *ca.* 1 ng/ml of urine were observed.

The great advantage of GC–MS–MS is the requirement of less purification. The new method allows the purification and derivatization of

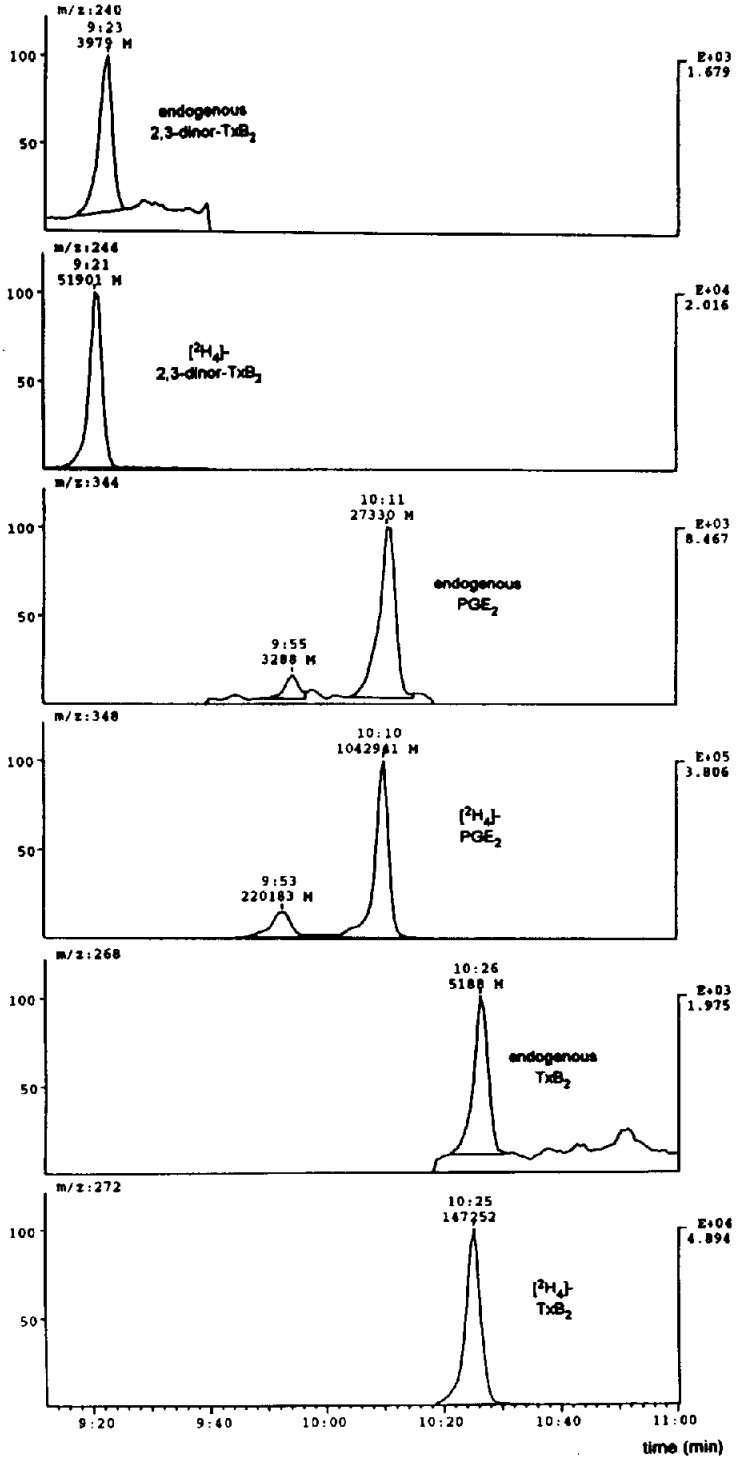

Fig. 2. Chromatogram of 2,3-dinor-TxB_2, PGE_2 and TxB_2. PGE_2 shows two peaks in the chromatogram because of two methoxime isomers. The concentrations of the prostanoids are 0.73 ng/ml (2,3-dinor-TxB_2), 0.26 ng/ml (PGE_2) and 0.33 ng/ml (TxB_2), respectively.

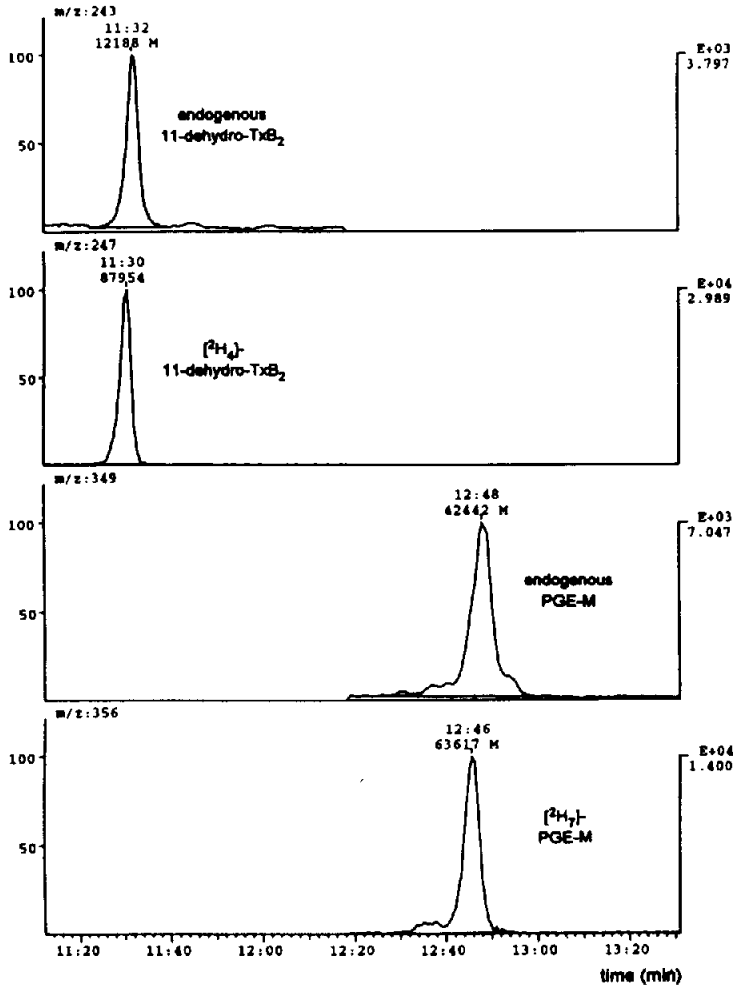

Fig. 3. Chromatogram of 11-dehydro-TxB_2 and PGE-M. PGE-M with its two keto groups forms four methoxime isomers resulting in two broad peaks. The concentrations of the prostanoids are 0.58 ng/ml (11-dehydro-TxB_2) and 7.9 ng/ml (PGE-M), respectively.

12 urine samples/day by one person. GC–MS–MS quantification of the seven prostanoids per sample occurs in three runs with two or three prostanoids per run. Using an autosampler, 24 urine samples/day can be analyzed by GC–MS–MS.

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5. References

[1] H.W. Seyberth, B.J. Sweetman, J.C. Frölich and J.A. Oates, *Prostaglandins*, 11 (1976) 381.
[2] A. Ferretti and V.P. Flanagan, *Biomed. Mass. Spectrom.*, 6 (1979) 431.
[3] T. Erlenmaier, H. Müller and H.W. Seyberth, *J. Chromatogr.*, 163 (1979) 289.

[4] H. Müller, R. Mrongovius and H.W. Seyberth, *J. Chromatogr.*, 226 (1981) 450.
[5] P. Falardeau, J.A. Oates and A.R. Brash, *Anal. Biochem.*, 115 (1981) 359.
[6] H.W. Seyberth, H. Müller, K. Soeding, L. Wille and E. Hackenthal, *Adv. Prostaglandin Thromboxane Res.*, 11 (1983) 533.
[7] K.A. Waddell, I.A. Blair and J. Wellby, *Biomed. Mass Spectrom.*, 10 (1983) 8.
[8] S.E. Barrow, K.A. Waddell, M. Ennis, C.T. Dollery and I.A. Blair, *J. Chromatogr.*, 239 (1982) 71.
[9] J.A. Lawson, A.R. Brash, J. Doran and G.A. FitzGerald, *Anal. Biochem.*, 150 (1985) 463.
[10] F. Catella, D.H. Healy, J.A. Lawson and G.A. FitzGerald, *Proc. Natl. Acad. Sci. USA*, 83 (1986) 5861.
[11] F. Catella and G.A. FitzGerald, *Thrombosis Res.*, 47 (1987) 647.
[12] A. Martineau and P. Falardeau, *J. Chromatogr.*, 417 (1987) 1.
[13] H. Schweer, J. Kammer and H.W. Seyberth, *J. Chromatogr.*, 333 (1985) 273.
[14] 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.
[15] C. Chiabrando, A. Benigni, A. Piccinelli, C. Carminati, E. Cozzi, G. Remuzzi and R. Fanelli, *Anal. Biochem.*, 163 (1987) 255.
[16] C. Chiabrando, V. Pinciroli, A. Campoleoni, A. Benigni, A. Piccinelli and R. Fanelli, *J. Chromatogr.*, 495 (1989) 1.
[17] G. Mackert, M. Reinke, H. Schweer and H.W. Seyberth, *J. Chromatogr.*, 494 (1989) 13.
[18] H. Schweer, H.W. Seyberth and R. Schubert, *Biomed. Environ. Mass Spectrom.*, 13 (1986) 611.
[19] H. Schweer, C.O. Meese, O. Fürst, P.G. Kühl and H.W. Seyberth, *Anal. Biochem.*, 164 (1987) 156.
[20] H. Schweer, H.W. Seyberth, C.O. Meese and O. Fürst, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 143.
[21] R. Lorenz, P. Helmer, W. Uedelhoven, B. Zimmer and P.C. Weber, *Prostaglandins*, 38 (1989) 157.
[22] W.M. Uedelhoven, C.O. Meese and P.C. Weber, *J. Chromatogr.*, 497 (1989) 1.
[23] H. Schweer, C.O. Meese and H.W. Seyberth, *Anal. Biochem.*, 189 (1990) 54.