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Simultaneous determination of prostaglandin E_1, prostaglandin E_0 and 15-keto-prostaglandin E_0 in human plasma by gas chromatography/negative-ion chemical-ionization tandem mass spectrometry

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Abstract

A sensitive and selective routine method for the simultaneous determination of prostaglandin E_1 (PGE_1), prostaglandin E_0 (PGE_0) and 15-keto-prostaglandin E_0 (15-keto-PGE_0) in human plasma is described using deuterated internal standards. The analytes were isolated from acidified human plasma by solid-phase extraction by means of Bond Elut C_{18} cartridges and derivatized to the pentafluorobenzyl (PFB) ester methoxime. The analytes were purified on Bond Elut Si cartridges and converted to the trimethylsilyl (TMS) ether. Quantitation was achieved by gas chromatography–negative-ion chemical-ionization tandem mass spectrometry. The precursor ion $[M-PFB]^- = [P]^-$ carried more than 80% of the total ion current. Collision activated decomposition (CAD) of $[P]^-$ resulted in characteristic product ions of which the $[P-2(CH_3)_3SiOH]^-$ ion (PGE_1) and the $[P-(CH_3)_3SiOH]^-$ ion (PGE_0 and 15-keto-PGE_0) were used for quantitation. The lower limit of quantitation (LLQ) was 2 pg/ml (PGE_1 and PGE_0) and 10 pg/ml (15-keto-PGE_0) extracted from 2 ml of human plasma. Linear calibration curves were obtained over the concentration range 2–100 pg/ml (PGE_1 and PGE_0) and 10–500 pg/ml (15-keto-PGE_0). In all cases, the precision and accuracy were $<17\%$. The present method has been applied successfully to pharmacokinetic and clinical studies in humans.

Keywords: Plasma; Prostaglandins; Human plasma; Tandem mass spectrometry; Negative-ion chemical ionization

1. Introduction

Prostaglandin E_1 (PGE_1) has been used therapeutically for the intravenous (i.v.) treatment of peripheral arterial occlusive disease [1] and of erectile disfunction as an intracavernous (i.c.) injection [2]. PGE_1 is rapidly converted to 15-keto-prostaglandin E_0 (15-keto-PGE_0), the major circulating metabolite, and prostaglandin E_0 (PGE_0), a metabolite with activity comparable with PGE_1[3].

Analyses of PGE_1 alone or together with its main metabolites PGE_0 and 15-keto-PGE_0 have been conducted by means of high-performance liquid chromatography (HPLC) with UV [4–6], fluorometric [7–11], combined with radioimmunoassay [12–16], laser-induced fluorometric [17] and fluorometric detection after double antibody extraction [18]. Furthermore, gas chromatography (GC) methods with electron-capture [19–21] and mass spectrometric detection have been described [22–25].

Recently, mass spectrometric detection in combination with HPLC has become an effective and convenient analytical technique for the quantitation of drugs in biological fluids [26]. Different detection

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methods including thermospray, electrospray and atmospheric pressure chemical ionization mass spectrometry have been published for the quantitation of PGE_1 [27–30].

However, the lower limits of quantitation (LLQs) of the above-mentioned methods did not allow the simultaneous determination of PGE_1, PGE_0 and 15-keto-PGE_0 in human plasma in the low pg range.

Selectivity and the sensitivity were significantly increased by gas chromatography negative-ion chemical-ionization tandem mass spectrometry (GC–NICI–MS–MS). The analytes were isolated from human plasma by solid-phase extraction followed by derivatization to the pentafluorobenzyl (PFB) ester methoxime. The samples were purified by thin-layer chromatography and converted to the trimethylsilyl (TMS) ether. The LLQ was 1 pg/ml for PGE_1 and PGE_0 and 2 pg/ml for 15-keto-PGE_0 determined from 5 ml of human plasma [31]. By this method, PGE_1, PGE_0 and 15-keto-PGE_0 were quantified in several pharmacokinetic and clinical studies in human subjects and volunteers [32–34].

The major challenge is the reliable quantitation of PGE_1, PGE_0 and 15-keto-PGE_0 after i.v. infusion or i.c. injection of PGE_1 (Prostavasin and Viridal, Schwarz Pharma, PGE_1-α-cyclodextrin) resulting in plasma levels of PGE_1 and PGE_0, slightly higher than the endogenous plasma levels.

Our studies were aimed at improving the sensitivity and selectivity of the assay by modifying the derivatization procedure of the carboxylic acid and the ketone moieties. Compared to the PFB ester methoxime of PGE_1, PGE_0 and 15-keto-PGE_0, both the esterification with 3,5-bis-(trifluoromethyl)-benzylbromide [35] or 4-(trifluoromethyl)-2,3,5,6-tetrafluoro-benzylbromide [36], the oximation with *O*-2,3,4,5,6-pentafluorobenzyl-hydroxylamine [37] and the combination of both steps did not increase the sensitivity or the selectivity [38].

A further refinement became necessary when the analytes had to be quantified in large numbers of plasma samples collected in clinical studies. Compared to the clean-up procedure described in Ref. [31], the purification of the PFB ester methoxime derivatives was carried out by means of solid-phase cartridges. This modification facilitates a high sample throughput by enabling the extraction and purification of 24 samples simultaneously per day.

In this paper, a highly sensitive and selective routine method for the simultaneous determination of PGE_1, PGE_0 and 15-keto-PGE_0 extracted from 2 ml of human plasma is presented. The quantitation by GC–NICI–MS–MS provided reliable validated data.

2. Experimental

2.1. Chemicals and reagents

PGE_1 was purchased from Acros Organics (St. Augustin, Germany), PGE_0 and 15-keto-PGE_0 (Fig. 1) from Cascade Biochem (Reading, UK). The internal standards d_6-PGE_1, d_4-PGE_0 and d_6-15-keto-PGE_0 were synthesized by the Chemistry Department (Schwarz Pharma, Monheim/Rhein, Germany). Ethyl acetate, acetonitrile, dichloromethane,

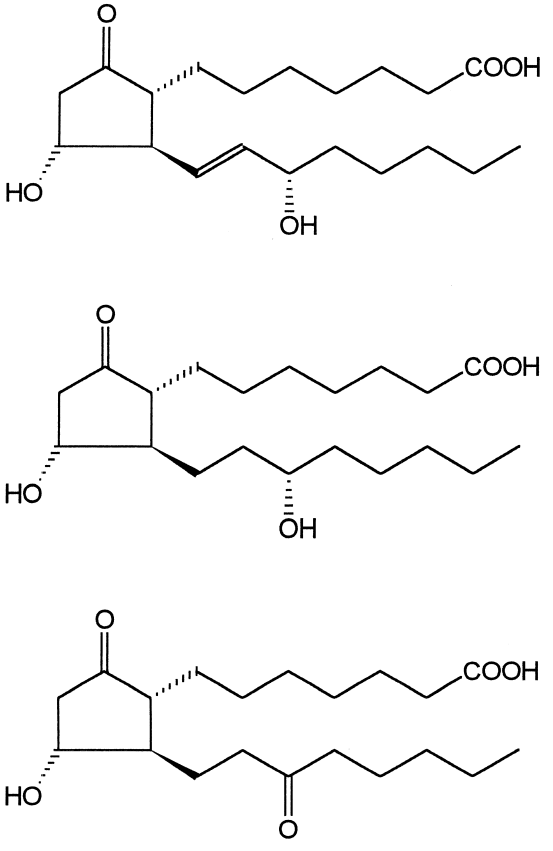

Fig. 1. Chemical structure of PGE_1 (top), PGE_0 (middle) and 15-keto-PGE_0 (bottom).

hexane and methanol were obtained from Promochem (Wesel, Germany), ethanol and formic acid from Merck (Darmstadt, Germany) and pentafluorobenzyl bromide (PFBBr), *N*,*N*-diisopropylethylamine (DIPEA), MOX reagent (2% methoxyamine hydrochloride in pyridine) and *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) from Pierce (Oud Beijerland, Netherlands). Indomethacin was from Sigma (Deisenhofen, Germany) and Biostabil from Biotrans (Dreieich, Germany). All chemicals were of the highest grade available and used without further purification. Bond Elut C_{18}/Si cartridges and the VacElut were obtained from ICT (Frankfurt/Main, Germany). Water was purified with the NANOpure system delivered by Werner (B.-Gladbach, Germany).

2.2. Chromatographic system and detection

The MS–MS analyses were performed on a Finnigan TSQ 700 triple stage mass spectrometer (Finnigan MAT, Bremen, Germany) equipped with an HP 5890 Series II gas chromatograph, a HP 7673A autosampler (Hewlett-Packard, Palo Alto, CA, USA) and a temperature-programmable split/splitless injector (Gerstel, Mülheim/Ruhr, Germany). A fused-silica capillary column (Ultra 2, 12 m×0.2 mm I.D., 0.33 μm film thickness; Hewlett-Packard) was inserted directly into the ion source. The injector temperature was programmed from 150°C to 300°C at 10°C/s (held for 5 min) and the oven temperature from 150°C (held for 0.1 min) to 300°C at 40°C/min (held for 7 min). The transfer line, the manifold and the ion source were kept at 300°C, 70°C and 150°C, respectively. The mass spectrometer was operated in the negative ion mode with an emission current of 300 μA and an electron energy of 85 eV. Helium was used as carrier gas at an inlet pressure of 30 kPa, methane as reagent gas (ion source pressure: 120 Pa) and argon as collision gas (collision cell pressure: 0.2 Pa).

2.3. Calibration and quality control

Calibration curves were generated daily by adding known amounts of PGE_1, PGE_0, 15-keto-PGE_0 and the deuterated internal standards to 2 ml of human plasma in the concentration range 4–200 pg (PGE_1, PGE_0) and 20–1000 pg (15-keto-PGE_0). Quality control (QC) samples contained PGE_1 and PGE_0 in concentrations of 10 and 50 pg/2 ml and 15-keto-PGE_0 in concentrations of 50 and 250 pg/2 ml. Two QC samples were freshly prepared and analysed together with the calibration curve and the unknown samples. Quantitation was achieved by calculating the peak area ratio of the analytes to the deuterated internal standards versus the drug concentration followed by linear regression analysis with $1/x$ weighting.

2.4. Standard and working solutions

For the preparation of the separate stock solutions 1 mg PGE_1 was dissolved in 100 ml of ethanol and 1 mg PGE_0 or 15-keto-PGE_0 both delivered in 100 μl of methyl acetate were diluted to 100 ml of ethanol in order to obtain a concentration of 10 ng/μl. From these stock solutions ethanolic working solutions with a concentration of 100 pg/μl were prepared. These working solutions were further diluted with ethanol in order to obtain concentrations of 10 pg/μl and 1 pg/μl (PGE_1, PGE_0) and of 50 pg/μl and 5 pg/μl (15-keto-PGE_0) for the preparation of the calibration curves.

The separate stock solutions of the deuterated internal standards contained 5 μg d_6-PGE_1, 10 μg d_4-PGE_0 and 5 μg d_6-15-keto-PGE_0 in 1 ml of ethanol, respectively. These stock solutions were further diluted with ethanol in order to obtain concentrations of 20 pg/μl (PGE_1, PGE_0) and of 50 pg/μl (15-keto-PGE_0). Aliquots (10 ml) of each of these working solutions were mixed and 30 μl were added to each plasma sample.

2.5. Blood sampling

The endogenous PGE_1 concentration depends significantly on the blood sampling procedure. In order to avoid further production of PGE_1, the following procedure has to be carried out within 20 min. An indwelling venous catheter is fitted approximately 30 min before the start of the sampling procedure. The polypropylene tubes containing 1 ml of a mixture of Biostabil as anticoagulant and 50 μg

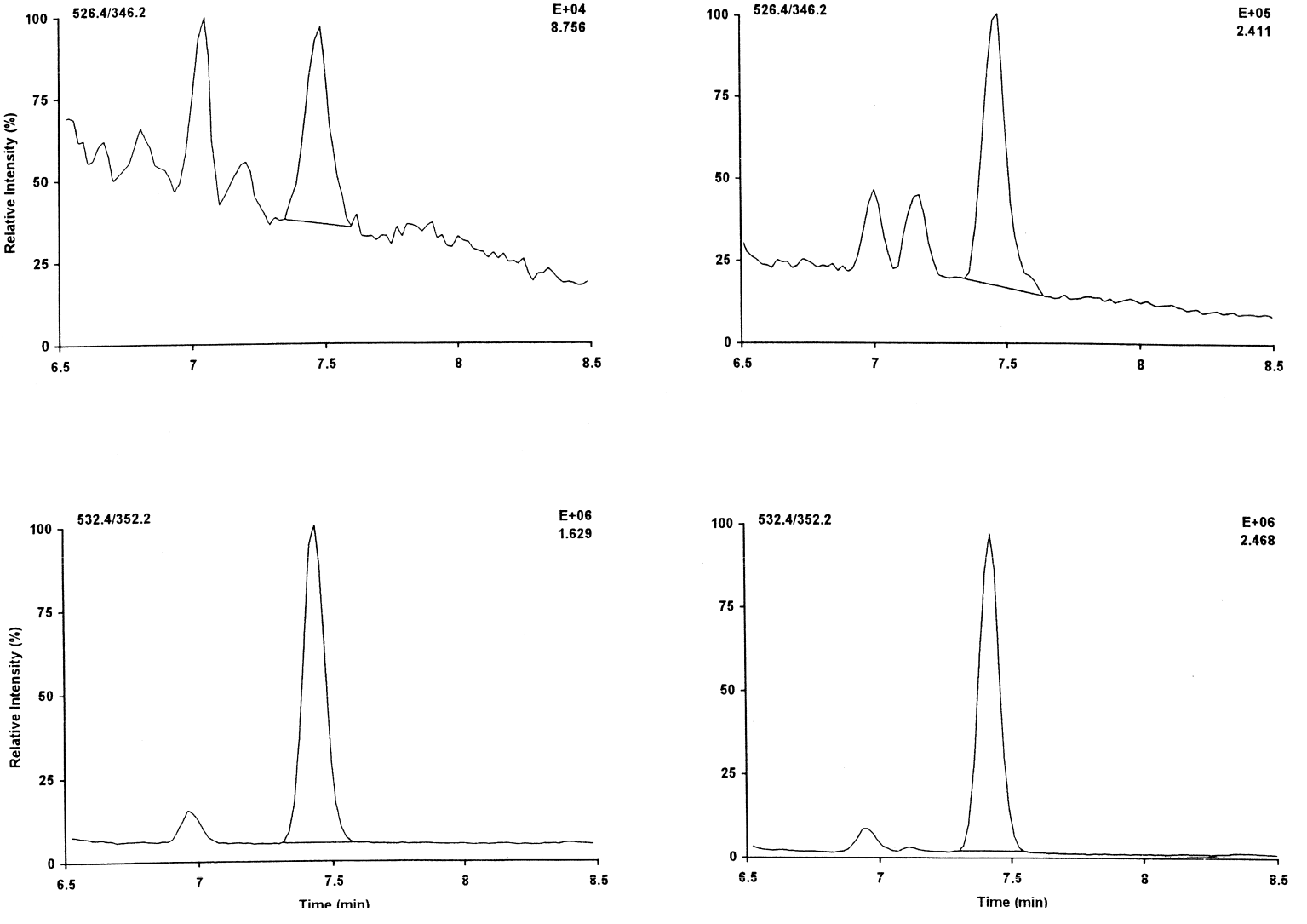

Fig. 2. MS–MS chromatograms of blank human plasma (2 ml) spiked with 4 pg PGE_1 and 200 pg d_6-PGE_1 (left) and with 15 pg PGE_1 and 200 pg d_6-PGE_1 (right).

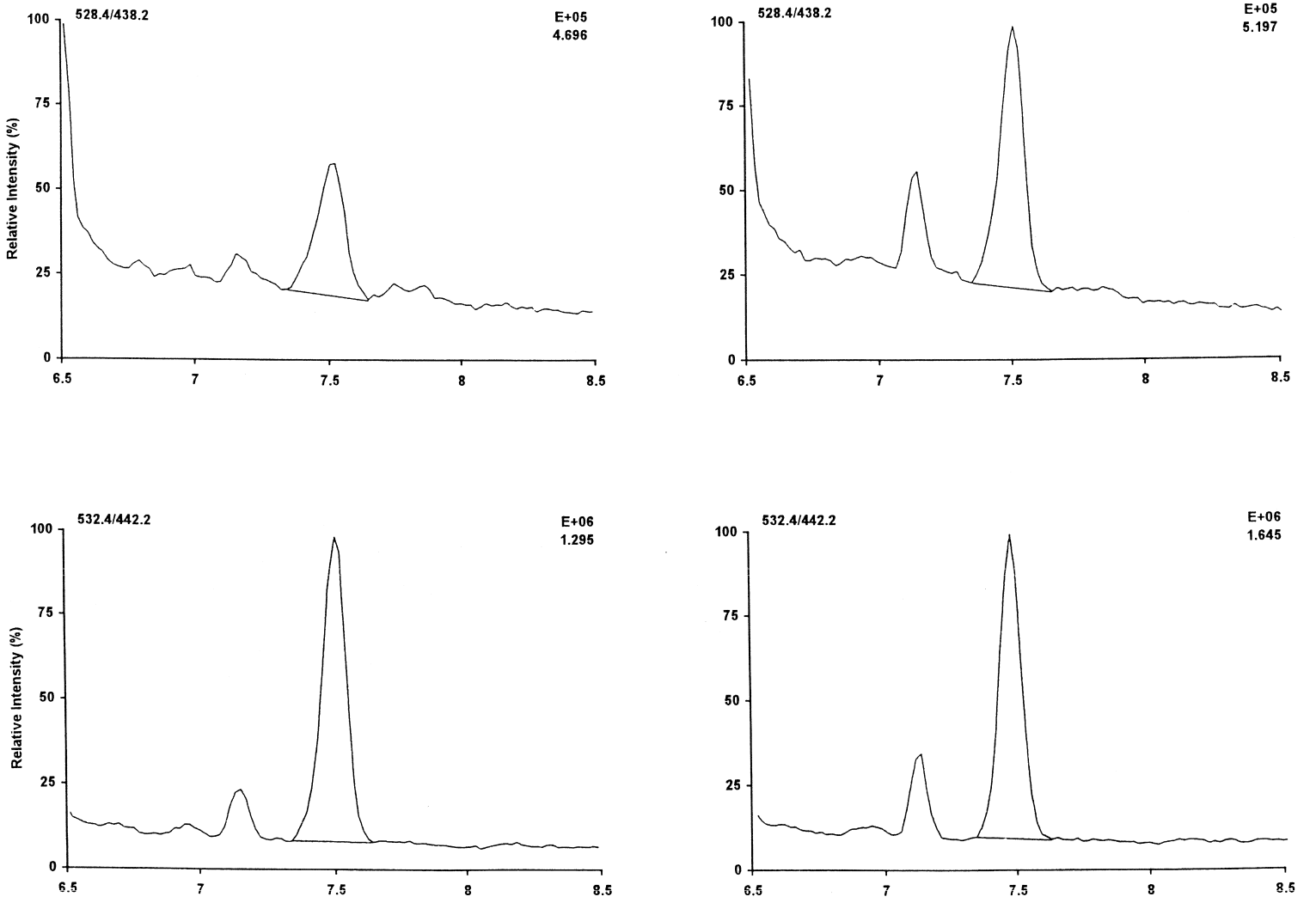

Fig. 3. MS–MS chromatograms of blank human plasma (2 ml) spiked with 4 pg PGE_0 and 200 pg d_4-PGE_0 (left) and with 15 pg PGE_0 and 200 pg d_4-PGE_0 (right).

of Indomethacin were pre-cooled in crushed ice. Prior to blood withdrawal, approximately 2 ml of blood were collected in a centrifuge tube and discarded. In order to determine the prostaglandins, 10 ml of human blood was collected – without a major stasis – in the prepared polypropylene tubes by slow dripping. The contents of the sampling tubes were carefully mixed and immediately cooled for 2 min in crushed ice. The samples were centrifuged for 10 min at 4°C and 2000 *g*. The supernatant plasma was transferred to polypropylene tubes and stored at $<-$ 80°C until extraction. After each blood withdrawal the indwelling catheter was rinsed with 2 ml of 0.9% sodium chloride solution.

2.6. Sample preparation

The Bond Elut C_{18} cartridges were preconditioned by sequential treatment with 6 ml of methanol and 6 ml of water. Human plasma samples unknowns or standards (2 ml) containing the deuterated internal standards were acidified with 200 μl of 3% formic acid to pH 3–3.5, allowed to stand at room temperature for 10 min and transferred to the preconditioned Bond Elut C_{18} cartridges. After successive washing with 3 ml of water and 6 ml of hexane, the cartridges were dried for 10 min at 80 kPa. The analytes were eluted with 3 ml of ethyl acetate (recovery over the concentration range validated: 95–100% for PGE_1, 86–89% for PGE_0 and 91–93% for 15-keto-PGE_0). The eluates were evaporated to dryness at room temperature.

The residues were incubated with PFBBr (10 μl) and DIPEA (20 μl) in acetonitrile (70 μl) for 10 min at 40°C. The samples were evaporated to dryness and converted to the methoxime with 200 μl of MOX reagent for 30 min at 70°C. After evaporation to dryness, the samples were transferred three times by 1 ml of dichloromethane to Bond Elut Si cartridges pre-washed with 6 ml of dichloromethane. The cartridges were washed with ethyl acetate–dichloromethane (5:95, v/v) and the analytes were eluted with 3 ml of ethyl acetate. The extracts were incubated with 100 μl of BSTFA for 60 min at 50°C. The samples were evaporated to dryness at room temperature, redissolved in 40 μl of BSTFA and 1 μl of this solution was injected.

3. Results

3.1. Chromatography and selectivity

Under the experimental conditions described, the retention times of PGE_1/d_6-PGE_1 and PGE_0/d_4-PGE_0 were about 7.5 min and of 15-keto-PGE_0/d_6-15-keto-PGE_0 approximately 7.7 min. Typical MS–MS chromatograms of blank human plasma (2 ml; taken from different volunteers) spiked with 4 pg or 15 pg PGE_1/PGE_0, with 50 pg or 250 pg 15-keto-PGE_0 and the deuterated internal standards (200 pg d_6-PGE_1/d_4-PGE_0 and 500 pg d_6-15-keto-PGE_0) are shown in Figs. 2–4, respectively.

The selectivity of the method was examined by assaying blank human plasma spiked with the deuterated internal standards. Only blank human plasma with an endogenous plasma level in the range of the LLQ was used for the calibration curves and the quality control samples. Typical MS–MS chromatograms of blank human plasma (2 ml) processed with the described method are shown in Figs. 8–10.

3.2. GC–MS and GC–MS–MS

In the NICI mode, the $[M-PFB]^-=[P]^-$ ions of the PFB ester methoxime TMS ether derivatives are the predominant precursor ions with more than 80% of the total ion current (Fig. 5). Collision activated decomposition (CAD) of $[P]^-$ resulted in characteristic product ions (Fig. 6). For quantitation in the multiple ion detection (MID) mode, the precursor→product ions monitored in the negative ion mode were m/z 526.4→m/z 346.2=$[P-2(CH_3)_3SiOH]^-$ ion for PGE_1, m/z 483.3→m/z 393.2=$[P-(CH_3)_3SiOH]^-$ ion for 15-keto-PGE_0 in both cases at a collision energy of 13 eV and m/z 528.4→m/z 438.2=$[P-(CH_3)_3SiOH]^-$ ion for PGE_0 at a collision energy of 11 eV. The scan times were 0.2 s (PGE_1 and PGE_0), 0.1 s (15-keto-PGE_0) and 0.06 s (each deuterated internal standard).

3.3. Linearity and sensitivity

Linear calibration curves were obtained over the concentration range 2–100 pg/ml (PGE_1 and PGE_0) and 10–500 pg/ml (15-keto-PGE_0) of human plasma. A typical calibration curve was described by the

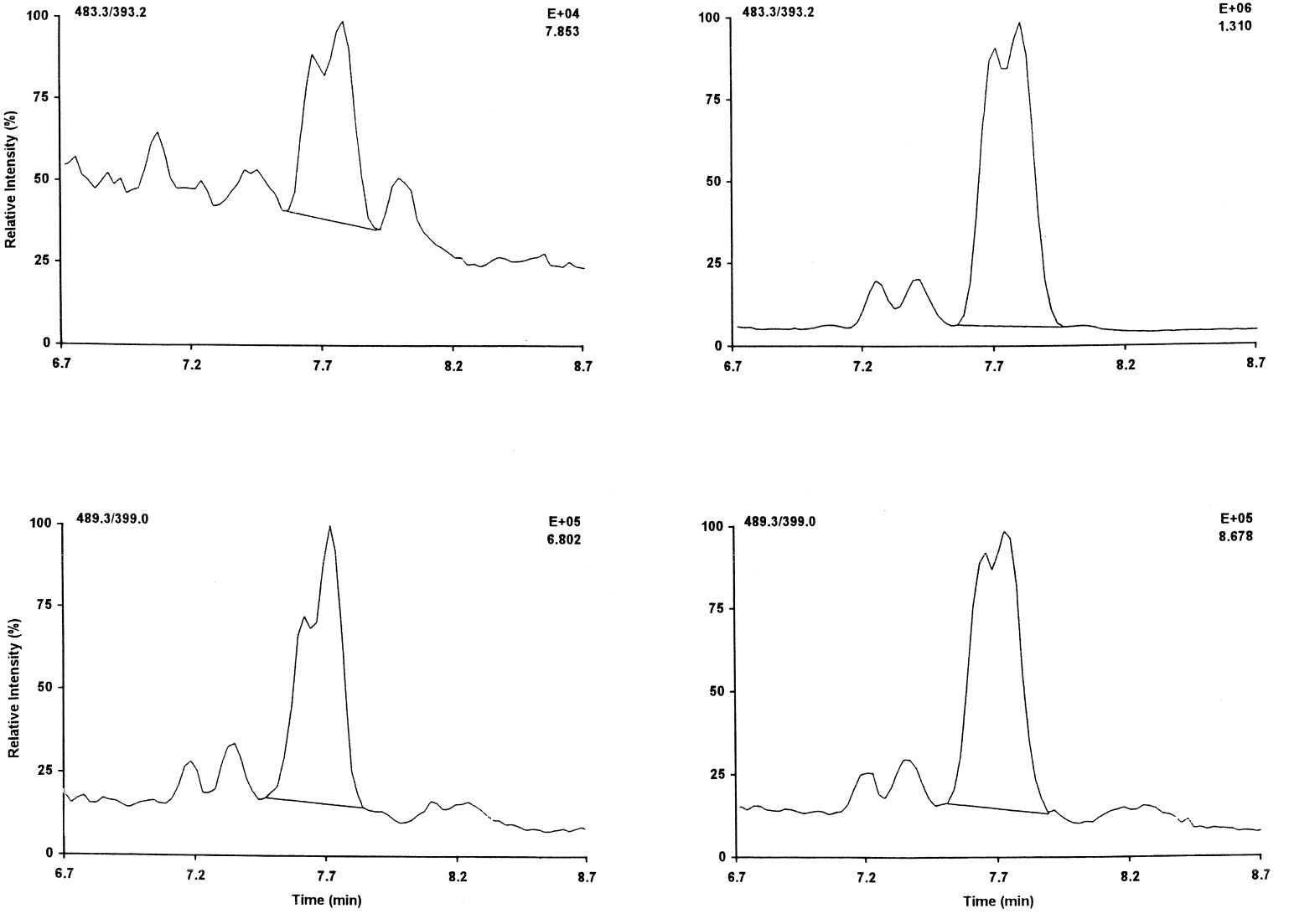

Fig. 4. MS–MS chromatograms of blank human plasma (2 ml) spiked with 20 pg 15-keto-PGE_0 and 500 pg d_6-15-keto-PGE_0 (left) and with 250 pg 15-keto-PGE_0 and 500 pg d_6-15-keto-PGE_0 (right).

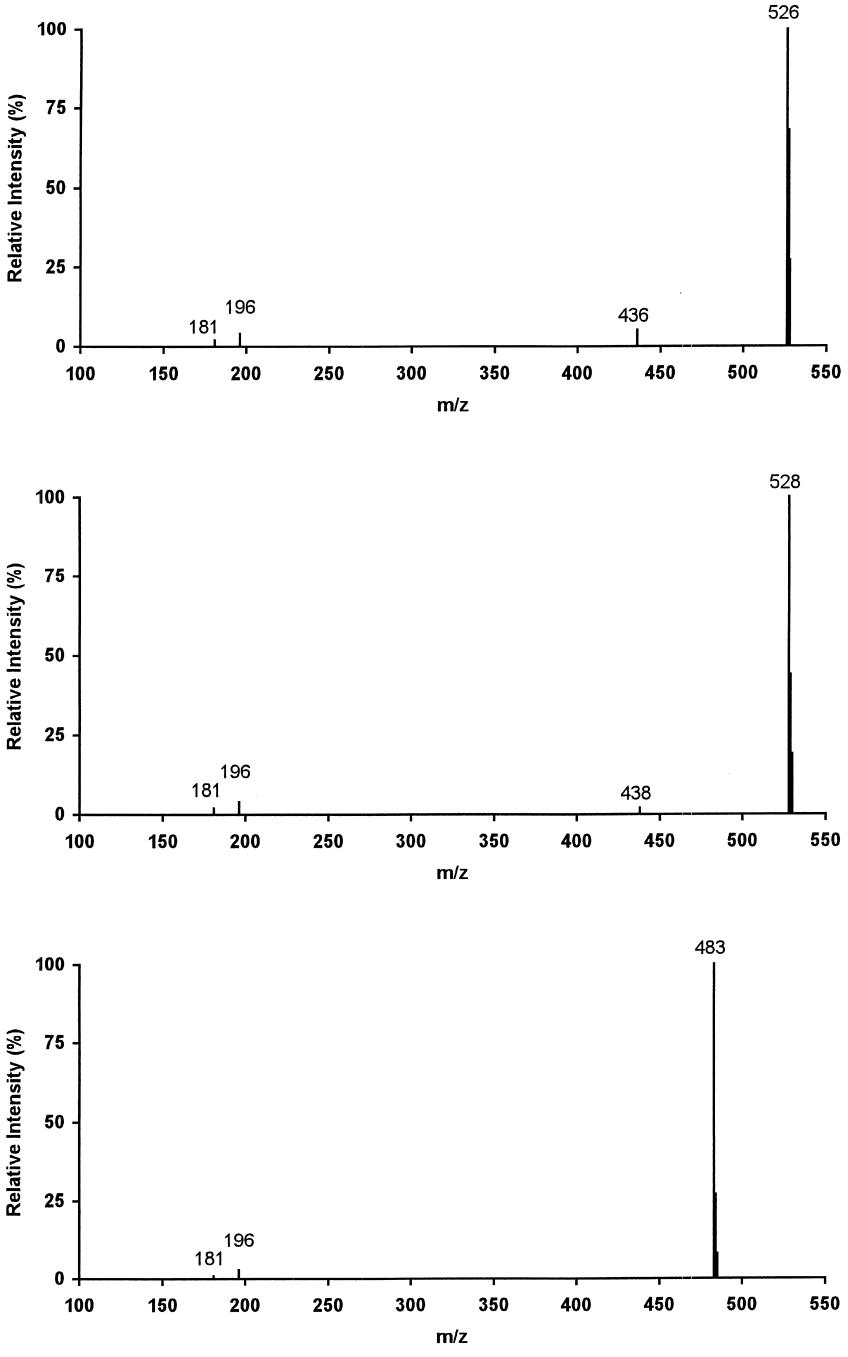

Fig. 5. Full scan NICI mass spectra of the PFB ester methoxime TMS ether derivatives of PGE_1 (top), PGE_0 (middle) and 15-keto-PGE_0 (bottom).

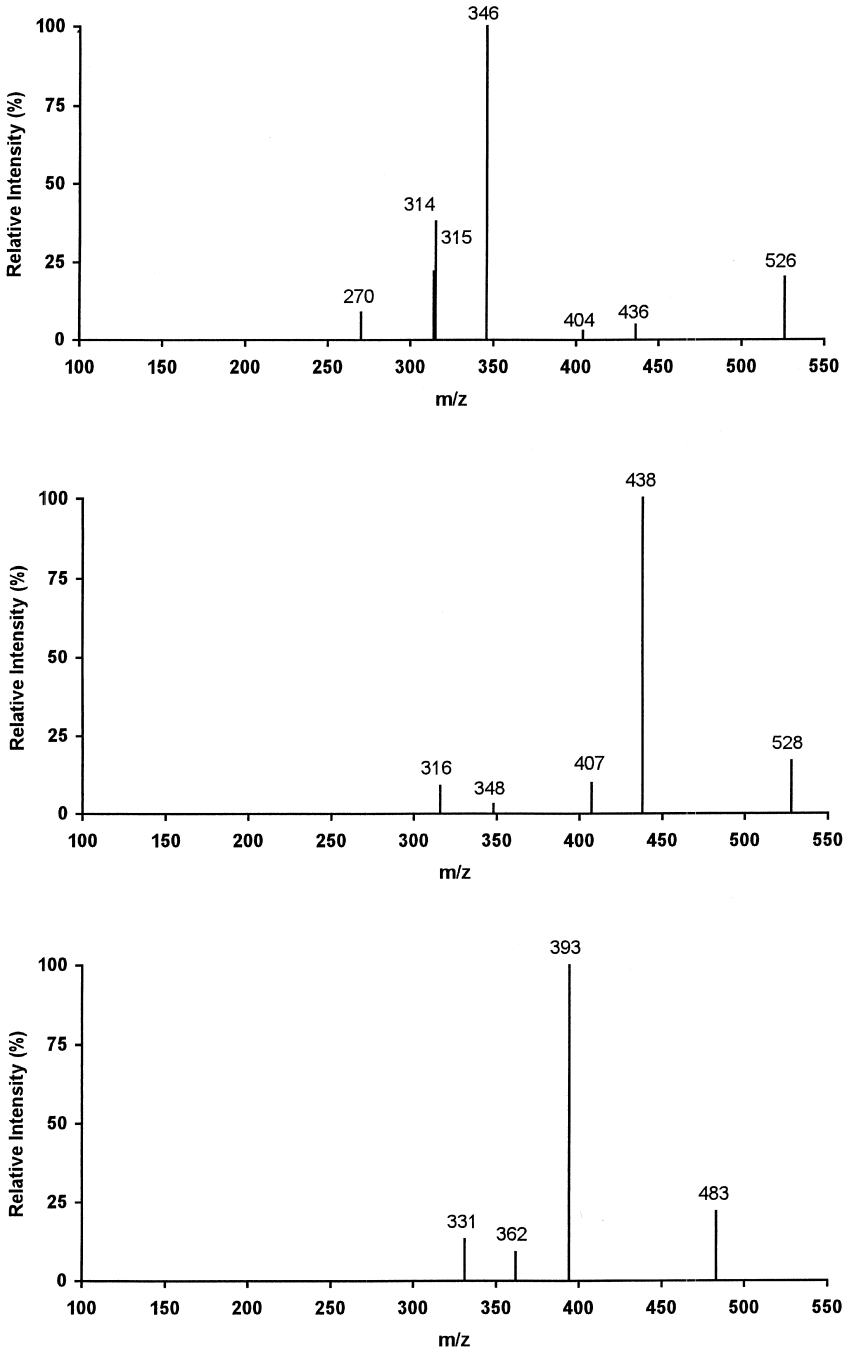

Fig. 6. MS–MS spectra of the $[M-PFB]^-$ ions of PGE_1 (top), PGE_0 (middle) and 15-keto-PGE_0 (bottom).

equation $y=0.0033x+0.0035$, $r=0.9992$ (PGE_1); $y=0.0357x+0.0049$, $r=0.9992$ (PGE_0) and $y=0.1178x+0.0123$, $r=0.9991$ (15-keto-PGE_0). The LLQ of the assay, i.e., the concentration with an accuracy and a precision ≤20%, was 2 pg/ml (PGE_1 and PGE_0) and 10 pg/ml (15-keto-PGE_0).

3.4. Reproducibility

The precision and the accuracy of the method were determined by analysis of blank human plasma (2 ml) spiked with PGE_1/PGE_0 in the concentration range 4–200 pg and with 15-keto-PGE_0 in the concentration range 20–1000 pg. All intra- and inter-assay data are summarized in Tables 1–6. In any case, both the precision and the accuracy were <17% and indicated good reproducibility.

Table 1
Inter-assay data of PGE_1 ($n=6$)

Added (pg/ml)	Found[a] (pg/ml)	Accuracy (%)	Precision (%)
2	2.20±0.48	10.0	21.8
2[b]	2.08±0.28	4.0	13.5
5	5.22±0.52	4.4	10.0
10	10.27±0.50	2.7	4.9
50	49.4±2.0	−1.2	4.0
100	90.7±2.6	−9.3	2.9

[a] Mean value±standard deviation.
[b] $n=5$.

Table 2
Inter-assay data of PGE_0 ($n=6$)

Added (pg/ml)	Found[a] (pg/ml)	Accuracy (%)	Precision (%)
2	2.17±0.13	8.5	6.0
5	4.54±0.49	−9.2	10.8
10	9.79±0.87	−2.1	8.9
50	50.2±3.8	0.4	7.6
100	95.4±5.0	−4.6	5.2

[a] Mean value±standard deviation.

Table 3
Inter-assay data of 15-keto-PGE_0 ($n=6$)

Added (pg/ml)	Found[a] (pg/ml)	Accuracy (%)	Precision (%)
10	10.66±0.81	6.6	7.6
20	20.7±1.5	3.5	7.2
50	52.7±2.5	5.4	4.7
250	252.4±14.0	1.0	5.5
500	482.2±34.9	−3.6	7.2

[a] Mean value±standard deviation.

Table 4
Intra-assay data of PGE_1 ($n=6$)

Added (pg/ml)	Found[a] (pg/ml)	Accuracy (%)	Precision (%)
5	5.64±0.50	12.8	8.9
10	10.62±0.50	6.2	4.7
50	50.5±1.3	1.0	2.6

[a] Mean value±standard deviation.

Table 5
Intra-assay data of PGE_0 ($n=6$)

Added (pg/ml)	Found[a] (pg/ml)	Accuracy (%)	Precision (%)
5	5.83±0.38	16.6	6.5
10	10.70±0.79	7.0	7.4
50	52.4±2.3	4.8	4.4

[a] Mean value±standard deviation.

Table 6
Intra-assay data of 15-keto-PGE_0 ($n=6$)

Added (pg/ml)	Found[a] (pg/ml)	Accuracy (%)	Precision (%)
50	49.1±2.3	−1.8	4.7
250	255.3±11.8	2.1	4.6
500	492.3±23.0	−1.5	4.7

[a] Mean value±standard deviation.

3.5. Application of the method

The method has been applied successfully for the determination of PGE_1, PGE_0 and 15-keto-PGE_0 in human plasma after a 2-h i.v. infusion of 60 μg of PGE_1 in order to investigate the pharmacokinetics of PGE_1, PGE_0 and 15-keto-PGE_0 in healthy male volunteers. A plasma concentration–time profile of PGE_1, PGE_0 and 15-keto-PGE_0 of one healthy male volunteer is shown in Fig. 7. Peak plasma levels of 13.0 pg/2ml (PGE_1), 24.0 pg/2ml (PGE_0) and 374.6 pg/2ml (15-keto-PGE_0) were determined at the end of the i.v. infusion. The corresponding MS–MS chromatograms of the analytes in the plasma sample collected 115 min after the start of the i.v. infusion are shown in Figs. 8–10. The precision and the accuracy data of the calibration curve and of the quality control samples processed with this method

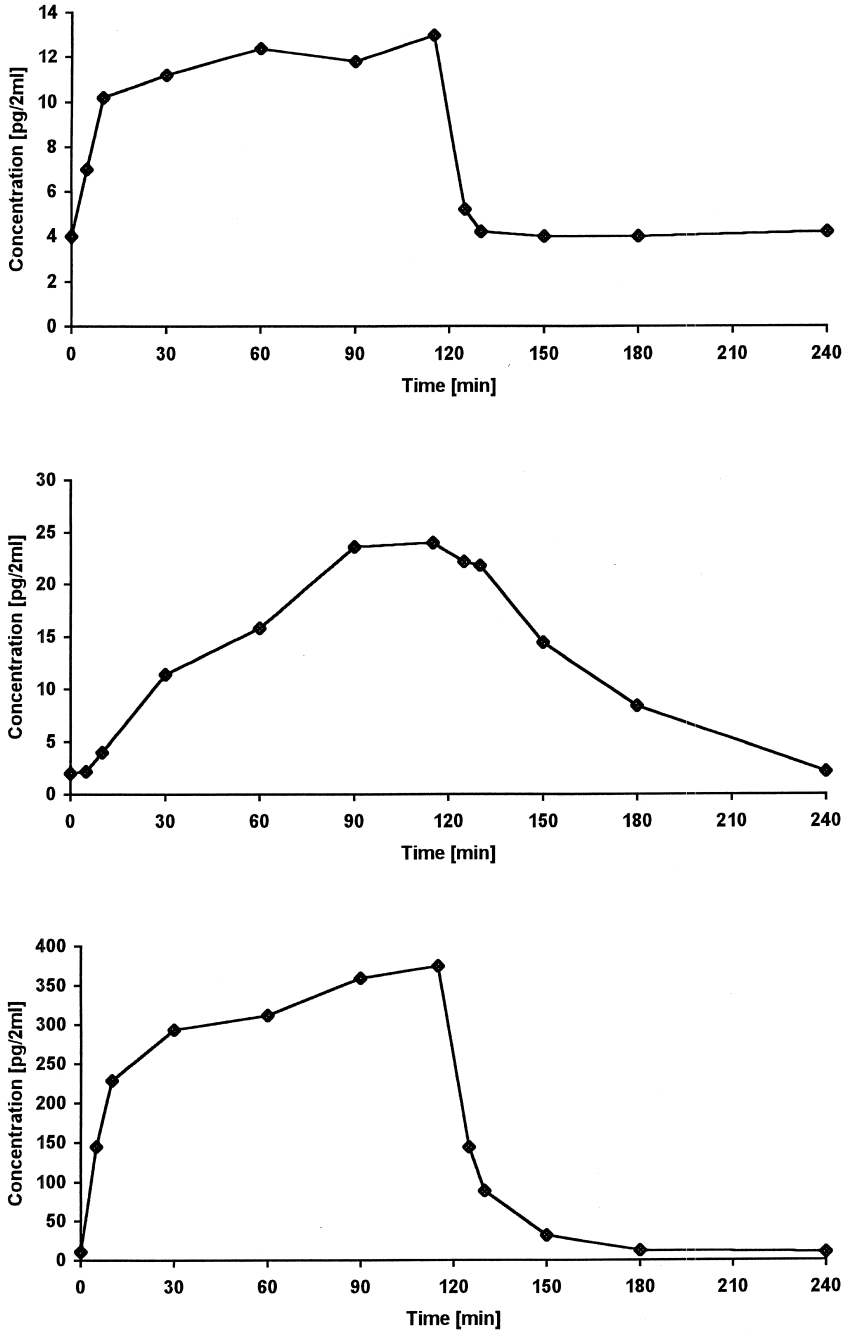

Fig. 7. Representative plasma concentration–time profile of PGE_1 (top), PGE_0 (middle) and 15-keto-PGE_0 (bottom) of one human volunteer after i.v. infusion of 60 μg PGE_1.

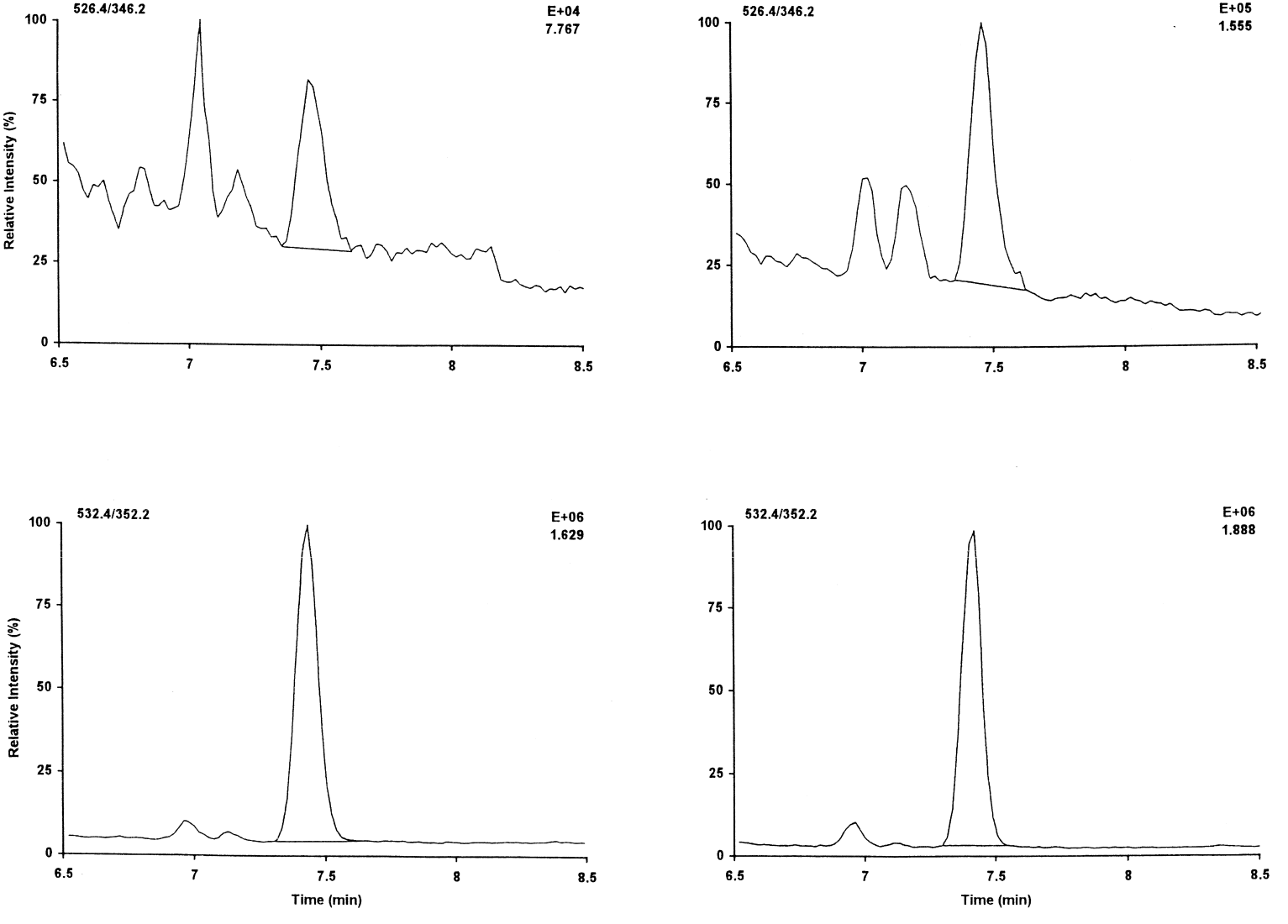

Fig. 8. MS–MS chromatograms of human plasma samples both spiked with 200 pg d_6-PGE_1; blank human plasma sample containing 3.6 pg/2 ml PGE_1; sample collected before the start of the i.v. infusion of 60 μg PGE_1 (left); human plasma sample containing 13.0 pg/2 ml PGE_1; sample collected 115 min after the start of the i.v. infusion of 60 μg PGE_1 (right).

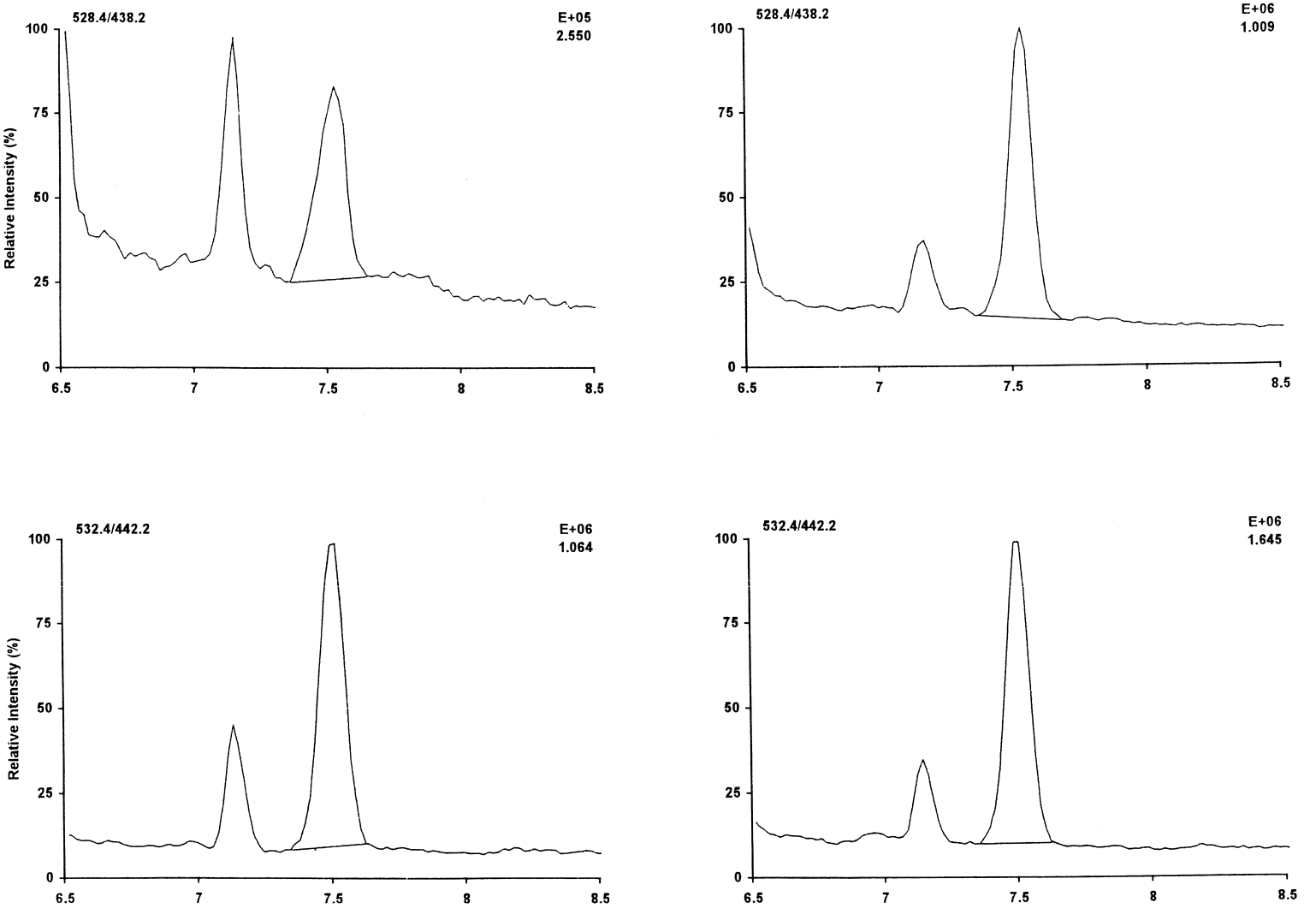

Fig. 9. MS–MS chromatograms of human plasma samples both spiked with 200 pg d_4-PGE_0; blank human plasma sample containing 2.8 pg/2 ml PGE_0; sample collected before the start of the i.v. infusion of 60 μg PGE_1 (left); human plasma sample containing 24.0 pg/2 ml PGE_0; sample collected 115 min after the start of the i.v. infusion of 60 μg PGE_1 (right).

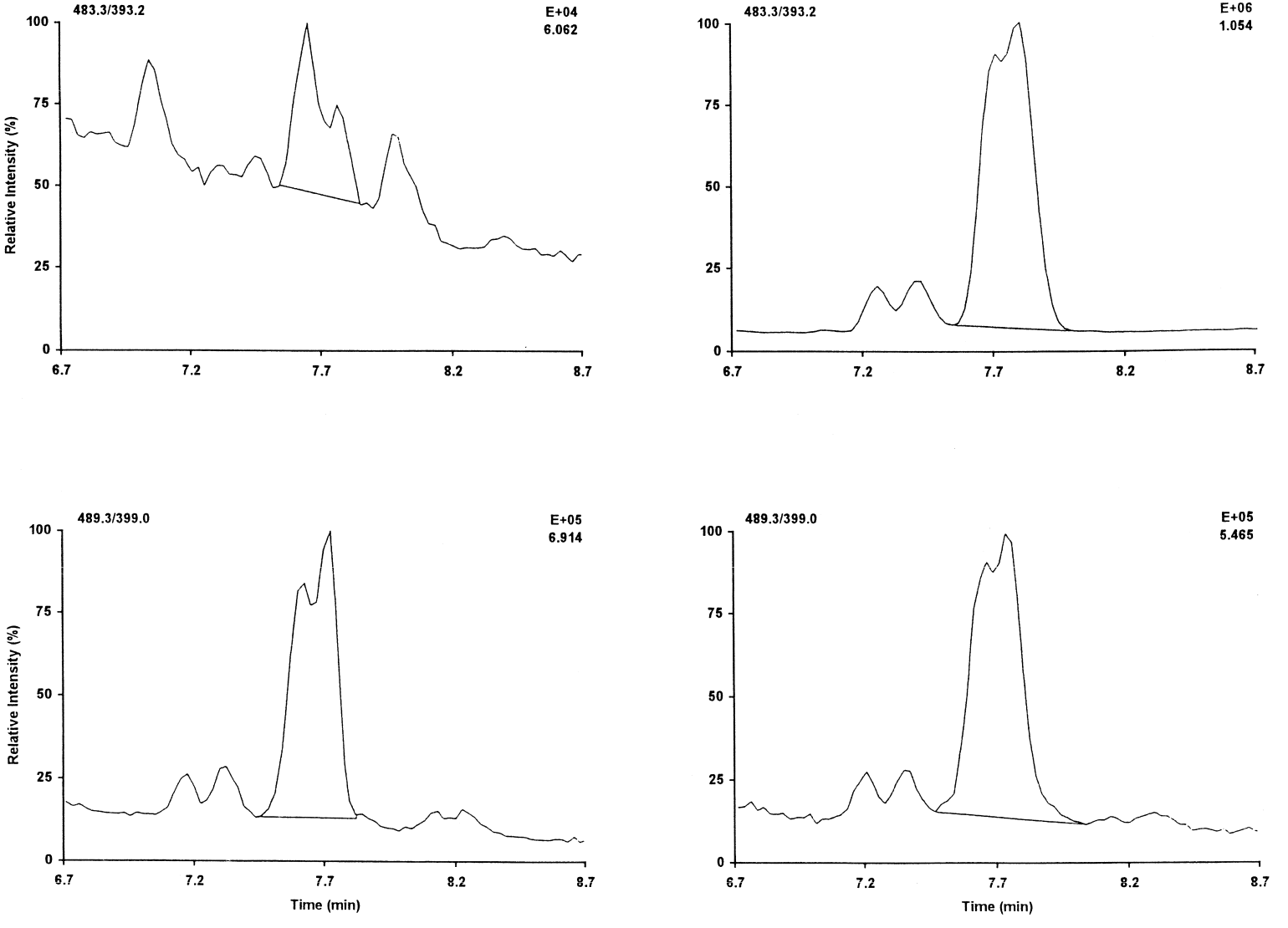

Fig. 10. MS–MS chromatograms of human plasma samples both spiked with 500 pg d_6-15-keto-PGE_0; blank human plasma sample containing 15.6 pg/2 ml 15-keto-PGE_0; sample collected before the start of the i.v. infusion of 60 μg PGE_1 (left); human plasma sample containing 374.6 pg/2 ml 15-keto-PGE_0; sample collected 115 min after the start of the i.v. infusion of 60 μg PGE_1 (right).

Table 7
Precision and accuracy of the QC samples of PGE_1

Added (pg/2 ml)	Found[a] (pg/2 ml)	Accuracy (%)	Precision (%)
10 (n=12)	10.05±0.92	0.5	9.2
50 (n=12)	51.8±2.4	3.6	4.6

[a] Mean value±standard deviation.

Table 8
Precision and accuracy of the QC samples of PGE_0

Added (pg/2 ml)	Found[a] (pg/2 ml)	Accuracy (%)	Precision (%)
10 (n=12)	10.00±1.01	0.0	10.1
50 (n=12)	49.4±2.7	−1.2	5.5

[a] Mean value±standard deviation.

Table 9
Precision and accuracy of the QC samples of 15-keto-PGE_0

Added (pg/2 ml)	Found[a] (pg/2 ml)	Accuracy (%)	Precision (%)
50 (n=12)	45.4±5.8	−9.2	12.8
250 (n=12)	245.1±4.7	−2.0	1.9

[a] Mean value±standard deviation.

Table 10
Precision and accuracy data of the calibration curves samples of PGE_1 (n=12)

Added (pg/2 ml)	Found[a] (pg/2 ml)	Accuracy (%)	Precision (%)
4	4.05±0.31	1.3	7.7
6	5.79±0.35	−3.5	6.0
8	7.76±0.28	−3.0	3.6
10	9.95±0.56	−0.5	5.6
15	15.2±1.0	1.3	6.6
20	20.5±0.7	2.5	3.4
50	52.1±1.9	4.2	3.6
100	98.6±1.9	−1.4	1.9
200	198.9±1.8	−0.6	0.9

[a] Mean value±standard deviation.

and calculated by the corresponding calibration curve are given in Tables 7–12.

4. Conclusions

A highly selective and sensitive routine method for the simultaneous determination of PGE_1, PGE_0 and 15-keto-PGE_0 in human plasma was presented. Compared to the recently developed GC–NICI–MS–MS assay [31], the described method with a modified purification step of the PFB ester methoxime derivatives by means of Bond Elut Si cartridges allows the processing of at least 24 samples per day applying the available solid-phase extraction unit. The assay is suitable for the quantitative determination of PGE_1, PGE_0 and 15-keto-PGE_0 in plasma samples collected in several pharmacokinetic and clinical studies.

Table 11
Precision and accuracy data of the calibration curves samples of PGE_0 (n=12)

Added (pg/2 ml)	Found[a] (pg/2 ml)	Accuracy (%)	Precision (%)
4	3.84±0.21	−4.0	5.5
6	6.12±0.38	2.0	6.2
8	7.87±0.35	−1.6	4.4
10	10.03±0.33	0.3	3.3
15	15.2±0.7	1.3	4.6
20	20.2±0.7	1.0	3.5
50	50.2±1.5	0.4	3.0
100	101.2±2.2	1.2	2.2
200	198.0±2.3	−1.0	1.2

[a] Mean value±standard deviation.

Table 12
Precision and accuracy data of the calibration curves samples of 15-keto-PGE_0 (n=12)

Added (pg/2 ml)	Found[a] (pg/2 ml)	Accuracy (%)	Precision (%)
20	21.7±2.6	8.5	12.0
30	29.0±3.7	−3.3	12.8
40	40.1±2.5	0.3	6.2
50	49.7±1.8	−0.6	3.6
75	72.6±5.3	−3.2	7.3
100	96.6±2.1	−3.4	2.2
250	252.7±9.6	1.1	3.8
500	504.8±9.3	1.0	1.8
1000	997.5±16.7	−0.3	1.7

[a] Mean value±standard deviation.

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