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DETERMINATION OF AN ANTISECRETORY TRIMETHYL PROSTAGLANDIN E_2 ANALOG IN HUMAN PLASMA BY COMBINED CAPILLARY COLUMN GAS CHROMATOGRAPHY—NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY

B.H. MIN*

Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)

J. PAO

Department of Pharmacokinetics and Biopharmaceutics, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)

W.A. GARLAND

Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)

J.A.F. de SILVA

Department of Pharmacokinetics and Biopharmaceutics, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)

and

M. PARSONNET

Department of Medical Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)

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SUMMARY

A method is described for measuring a trimethyl prostaglandin E_2 analog, TM-PGE_2, in human plasma. Trideuterated and monofluorinated analogs of TM-PGE_2 are added to plasma as internal standard and carrier, respectively. The plasma is adjusted to pH 3.0 and is extracted with a mixture of benzene—dichloromethane (9:1). The residue, following removal of the extracting solvent, is reacted consecutively with pentafluorobenzyl bromide and bistrimethylsilyltrifluoroacetamide. The excess derivatizing reagents are removed by evaporation, and an aliquot of the reconstituted residue is analyzed by capillary column gas chroma-

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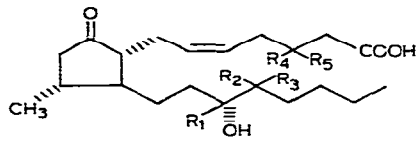
tography using methane as the carrier gas. A quadrupole mass spectrometer is set to monitor in the gas chromatographic effluent the $(M - C_7H_2F_5)^-$ fragment ion of TM-PGE_2 (*m/e* 449) and trideuterated TM-PGE_2 (*m/e* 452) generated by methane negative chemical ionization. Quantitation of unknowns is based on a comparison of the *m/e* 449 to *m/e* 452 ion ratio in each unknown to that obtained from the analysis of control plasma spiked with known amounts of TM-PGE_2 and fixed amounts of internal standard and carrier. The sensitivity limit of the assay is approximately 100 pg ml^{-1}, which is equivalent to 1 pg injected. The assay was used to measure the concentration of TM-PGE_2 in the plasma of two subjects following a single 10 $\mu g\ kg^{-1}$ oral dose of the drug.

INTRODUCTION

The trimethyl prostaglandin E_2 analog, TM-PGE_2 (I), is an orally active inhibitor of gastric acid secretion in dog and man [1,2]. An assay for the compound is required to establish its pharmacokinetic profile and to verify its systemic absorption in toxicology studies. The assay must be extraordinarily sensitive because the drug is effective at a dose of only 10 $\mu g\ kg^{-1}$ and highly specific because TM-PGE_2 is structurally similar to many naturally occurring prostaglandins. The procedure should be relatively simple for it to be useful in analyzing the large number of samples generated by a typical pharmacokinetic or toxicology study.



Compound	R_1	R_2	R_3	R_4	R_5
I	H	CH_3	CH_3	H	H
II	^2H	CH_3	CH_3	^2H	^2H
III	H	F	H	H	H

This paper reports a sensitive, specific and relatively simple gas chromatographic—mass spectrometric (GC—MS) procedure for TM-PGE_2. The method is based on the conversion of TM-PGE_2 to an electron-capturing (EC) pentafluorobenzyl ester and the GC analysis of the latter as its trimethylsilyl ether. A similar derivative has been used in an EC—GC assay for prostaglandin $F_{2\alpha}$ [3]. Sensitivity and specificity are provided by mass spectral analysis using both negative chemical ionization (NCI) and selected ion monitoring. Specificity is aided by capillary GC using a SCOT column. The assay features the use of a trideuterated analog of TM-PGE_2, compound II, as internal standard. Compound III is used as a carrier substance to ensure high recoveries at low drug concentrations. Because of the assay's relatively simple workup procedure, it can be used to analyze a great number of plasma samples in a relatively short period of time. Assay sensitivity is approximately 100 pg ml^{-1}.

EXPERIMENTAL

Equipment and operating conditions

Gas chromatograph. A Finnigan Model 9500 gas chromatograph was equipped with a 19 m × 0.5 mm I.D. glass SCOT column (SGE, Austin, TX, U.S.A.) and an SGE splitless SCI-AK injector. Prior to use, the column was conditioned overnight at 250°C with methane (69 kPa) as carrier gas. The injection port, column oven, interface oven and transfer line were operated at 280°C, 250°C,

230°C, and 225°C, respectively. A methane column pressure of 14 kPa was used for the assay. Under these conditions, the retention time of TM-PGE_2 was approximately 3 min.

EC—GC measurements were made using a Micro-Tek MT-220 (Tracor, Austin, TX, U.S.A.) gas chromatograph equipped with a ^{63}Ni detector. The detector was operated in the d.c. pulsed mode. The EC—GC parameters were adjusted to give maximum sensitivity.

Mass spectrometer. A Finnigan Model 3200 mass spectrometer was modified to permit negative ion chemical ionization as previously described [4]. The ion source voltages were set to give the maximum signal response consistent with satisfactory ion peak shape and unit mass resolution. Methane was used as GC carrier gas and was also added directly after the GC column, before the interface, as makeup gas and NCI gas. The ion source pressure was approximately 130 Pa. The continuous dynode electron multiplier was operated at −2100 V and the conversion dynode was operated at +2500 V.

Peak monitor. Selected ion recordings were obtained using a Finnigan PROMIM peak monitor. The responses were recorded on a multichannel paper-chart recorder (Rikadenki KA-41). The mass spectrometer was set to monitor *m/e* 449 and *m/e* 452. Both channels were operated at a gain of 10^8 V A^{-1}, 100 msec dwell time and a filter setting of 0.5 Hz. The recorder was operated at a chart speed of 2 cm min^{-1}.

Glassware. Culture tubes (16 ml, Pyrex No. 9825) equipped with Teflon®-lined screw-caps were used for plasma extraction. Conical centrifuge tubes (5 ml, Pyrex No. 8061) were used for evaporation of the solvent extracts. Both types of tubes were purchased from SGA, Bloomfield, NJ, U.S.A. All glassware, including the GC column, was cleaned with detergent, rinsed thoroughly with distilled water, treated with Prosil-28 (PCR Research Chemical, Gainesville, FL, U.S.A.) and, finally, was rinsed in an ultrasonic bath with methylene chloride and then methanol prior to drying in an oven at 105°C. Blood samples were collected in Vacutainer® No. 6527 from Becton-Dickinson.

Solvent evaporation. Solvents were removed at 50°C using a nitrogen evaporator (N-Evap, Organomation Assoc.).

Shaker. Extractions were performed by shaking (80—100 strokes min^{-1}) on a variable-speed reciprocating shaker (Eberbach Inc.).

Centrifuge. Centrifugation was done using a Damon/IEC Model CRU-5000 refrigerated centrifuge operated at 10°C and 1000 *g*.

Chemicals

TM-PGE_2 and compounds II and III were obtained from Dr. G. Holland, Chemical Research Department, Hoffmann-La Roche, Nutley, NJ, U.S.A. Nanograde methylene chloride, acetonitrile and benzene and reagent grade monobasic potassium phosphate and 85% phosphoric acid were purchased from Mallinckrodt (St. Louis, MO, U.S.A.). Pentafluorobenzyl bromide (PFBB) and diisopropylethylamine were obtained from Aldrich (Milwaukee, WI, U.S.A.). Bis-trimethylsilyltrifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, IL, U.S.A.).

Solutions

1 M phosphate buffer (pH 3.0). Monobasic potassium phosphate (136 g) was dissolved in 1 l of distilled water and the pH of the solution was adjusted to 3.0 with 85% phosphoric acid.

TM-PGE_2 [I]. TM-PGE_2 (1.0 mg) was dissolved in 1 ml of methanol. A 0.010-ml aliquot of this solution was diluted to 100 ml with distilled water (solution A, 100 ng ml^{-1}). Ten milliliters of this solution were diluted to 100 ml with distilled water to give solution B (10 ng ml^{-1}).

Compound II. Compound II (1.0 mg) was dissolved in 1.0 ml of methanol. A 0.010-ml aliquot of this solution was diluted to 100 ml with distilled water (solution C, 100 ng ml^{-1}).

Compound III. Compound III (2.0 mg) was dissolved in 100 ml of methanol (solution D, 20 μg ml^{-1}).

35% PFBB in acetonitrile. PFBB (3.5 ml) was diluted to 10 ml with acetonitrile.

Procedure

Extraction. Depending on the expected concentration, either 0.5 or 1.0 ml of plasma and either 1.0 ng or 2.5 ng of compound II (10 or 25 μl of solution C) were added to a 16-ml culture tube containing 200 ng of compound III (10 μl of solution D). To this solution were added 3 ml of 1 *M* phosphate buffer (pH 3.0) and 6 ml of benzene—methylene chloride (9:1). The mixture was vortexed vigorously for 30 sec on a Vortex Genie mixer and was shaken for 30 min on a mechanical shaker. The sample was centrifuged and the organic phase was transferred to a 5-ml centrifuge tube and evaporated to dryness under a stream of nitrogen. If necessary, the residue can be stored overnight at this point in a vacuum desiccator.

Derivatization. The residue was dissolved in 30 μl of acetonitrile. Ten microliters of the 35% PFBB solution and 10 μl of a 10% diisopropylethylamine in acetonitrile solution were added. The reaction mixture was allowed to stand for 15 min at 40°C in the N-Evap water-bath. The solution was evaporated at 50°C under nitrogen and the residue was dried in a vacuum desiccator for 15 min. The vacuum-dried residue was dissolved in 50 μl of acetonitrile and 10 μl of BSTFA. This mixture was heated at 60°C for 15 min and the solvent was evaporated under nitrogen. The residue was stored in a vacuum desiccator until analyzed.

GC—MS analysis. Depending on the expected concentration, the residue was dissolved in between 25 and 100 μl of hexane and a 1-μl aliquot of this solution was injected into the GC—MS system with the divert valve open and the electron filament off. Fifty-five seconds after injection, the GC divert valve was closed. The filament power supply was turned on 5 sec later.

Calculation

The peak heights of the *m/e* 449 and *m/e* 452 ions were measured with a ruler and the *m/e* 449 to *m/e* 452 ion ratio was converted to a concentration using a standard curve. The standard curve was generated from the analyses of control plasma (0.5 ml) to which was added either 0 μl (0 ng), 10 μl (0.1 ng), 25 μl (0.25 ng), 50 μl (0.5 ng), or 75 μl (0.75 ng) of solution B, or 10 μl (1 ng),

50 μl (5 ng), 75 μl (75 ng) or 100 μl (10 ng) of solution A, along with the 2.5 ng of compound II (internal standard) and 200 ng of compound III (carrier). The resulting data of ion ratio vs. amount added from the spiked plasma were subjected to linear regression analysis using an appropriate program on a digital computer. The concentration in an unknown was determined using the slope and intercept values from the regression analysis.

Clinical samples

Two healthy male volunteers were fasted for 7.5 h prior to receiving a 10 μg kg^{-1} oral dose of TM-PGE_2. Aliquots (10 ml) of whole blood were drawn at —5, 20, 40, 60, 75, 90, 120 and 150 min post dosing. The blood was centrifuged for 30 min and the plasma was isolated and stored at —10°C.

RESULTS AND DISCUSSION

The methane NCI mass spectra of derivatized TM-PGE_2 and compound II show no molecular ions (Fig. 1). Intense fragment ions corresponding to the loss of the pentafluorobenzyl radical are found at *m/e* 449 in the mass spectrum of TM-PGE_2 and at *m/e* 452 in the mass spectrum of compound II, and these ions are used for quantitation. Two smaller fragment ions corresponding to the loss of $(CH_3)_3$ SiOH and $CH_2 = Si(CH_3)_2$ from the base peak are found at *m/e* 359 and *m/e* 377 and at *m/e* 362 and *m/e* 380 in the mass spectra of TM-PGE_2 and compound II, respectively.

Typical ion chromatograms from the analysis of 1 ml of control plasma spiked with either 0 (A) or 0.5 ng (B) of TM-PGE_2, in addition to 2.5 ng of compound II and 200 ng of compound III, can be seen in Fig. 2. The small TM-PGE_2 response in chromatogram A from undeuterated TM-PGE_2 in compound II and "ghosting" from previous injections currently limits the assay sensitivity. This response typically represents 0.025 ng ml^{-1} of TM-PGE_2. For any given set of samples, the assay sensitivity limit is considered to be three times the TM-PGE_2 response at *m/e* 449 in the ion chromatogram from control plasma. The TM-PGE_2 response in ion chromatogram B represents, at most, 5 pg of derivatized TM-PGE_2 injected on-column. It is estimated from the analysis of pure derivatized standards that the NCIMS response of TM-PGE_2 at *m/e* 449 is approximately five times greater than the EC response of TM-PGE_2 and is approximately 25 times greater than the response of the $[MH - (CH_3)_3SiOH]^+$ base peak ion in the methane positive CI mass spectrum of the same derivative.

Assay precision and the recovery of TM-PGE_2 were determined by spiking six separate 1-ml plasma samples with 100 pg of the authentic compound and analyzing the samples using the procedure described. The mean (± S.D.) concentration found, 96 ± 5 pg ml^{-1}, indicates an inaccuracy of less than 10% and a precision (coefficient of variation) of 5% at this low concentration. This experiment was repeated with six 1-ml plasma samples each spiked with either 0.25 ng or 2.5 ng of TM-PGE_2. The mean concentrations (± S.D.) found were 0.26 ± 0.03 and 2.2 ± 0.03 ng ml^{-1} for the 0.25 and 2.5 ng ml^{-1} samples, respectively. The mean recovery (± S.D.) of the compound for these samples, based on a comparison of the response of derivatized TM-PGE_2 with the response

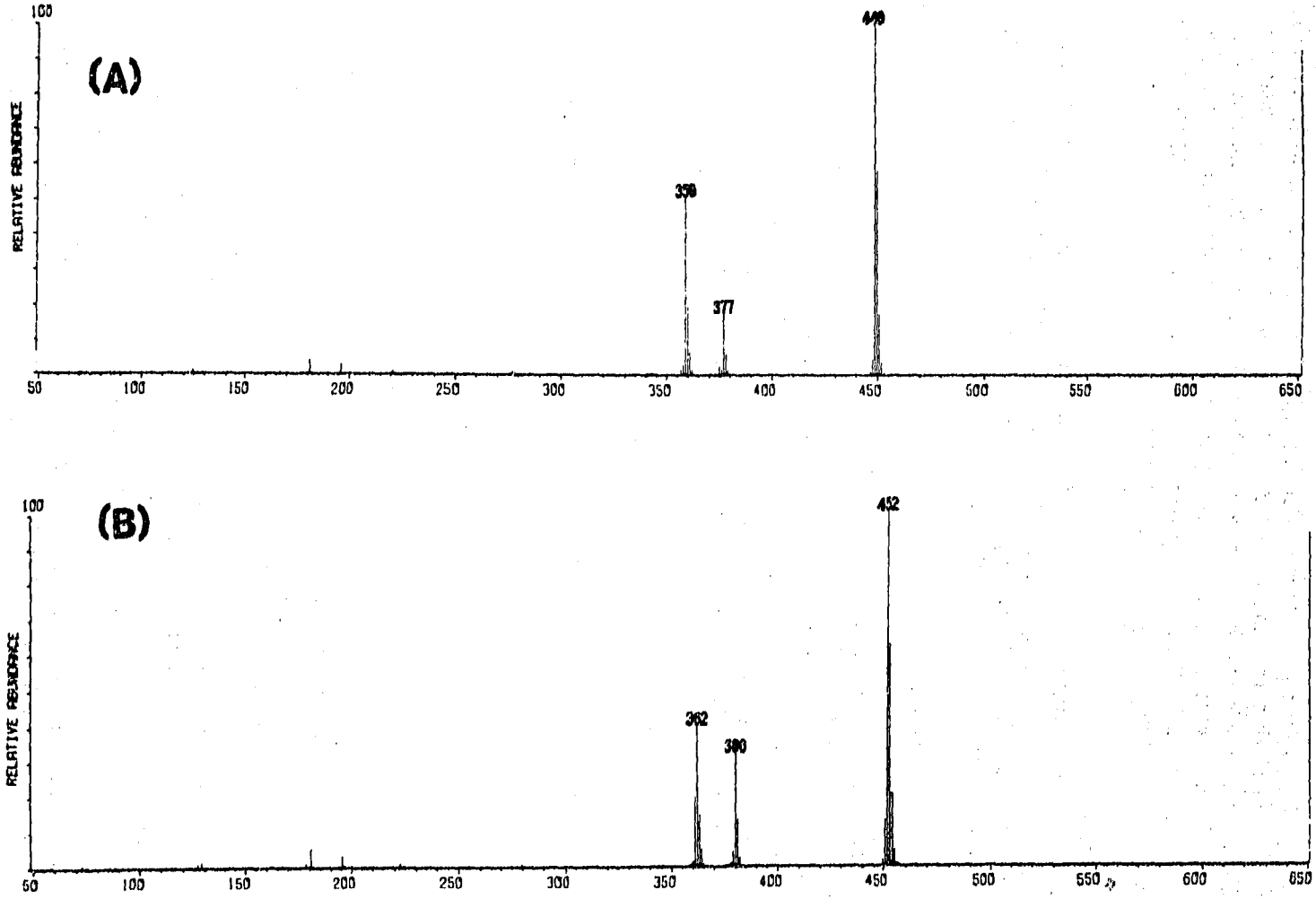


Fig. 1. Methane NCI mass spectra of TM-PGE$_2$ (A) and trideuterated TM-PGE$_2$ (B). Spectra were obtained under the GC—MS conditions described in the text.

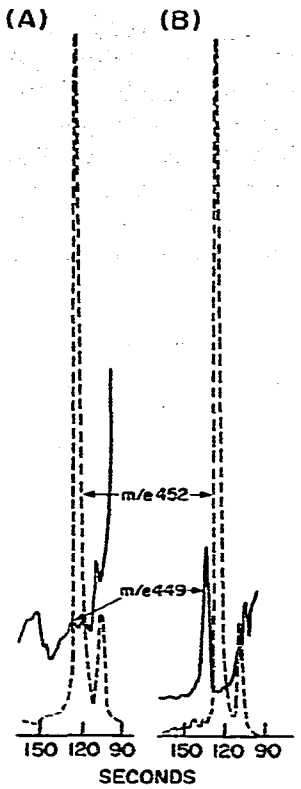


Fig. 2. Ion chromatograms from the analysis of 1 ml of control plasma spiked with either 0 ng (A) or 0.5 ng (B) of TM-PGE_2 (*m/e* 449), 2.5 ng of compound II (*m/e* 452) and 200 ng of compound III. For these ion chromatograms approximately 1 μl out of the available 100 μl was injected.

from the injection of known amounts of derivatized TM-PGE_2, was 56 ± 9%.

The effect of the blood collection container on assay accuracy was evaluated. A 25-ml sample of blood, freshly drawn into a glass syringe, was spiked with 62.5 ng of TM-PGE_2 (i.e. 2.5 ng ml^{-1}). The stoppers of six Vacutainers were removed. Aliquots (2 ml) of the spiked blood were transferred into the Vacutainers which were restoppered. Additional 2-ml aliquots of blood were transferred to six of the same 16-ml culture tubes which were used for plasma extractions. The culture tubes were capped and all twelve tubes were placed on a horizontal shaker and gently shaken at room temperature for 30 min. Three of the Vacutainer blood samples and three of the culture tube blood samples were centrifuged and the plasma collected. One milliliter each of the six plasma samples and 1 ml each of the six remaining whole blood samples were assayed for TM-PGE_2. The mean TM-PGE_2 concentration (± S.D.) measured was 2.30 ± 0.04 ng ml^{-1} for the blood samples exposed to the culture tubes and 2.18 ± 0.08 ng ml^{-1} for the blood samples exposed to the Vacutainers. The mean TM-PGE_2 concentration (± S.D.) measured was 3.26 ± 0.21 ng ml^{-1} for the plasma samples exposed to culture tubes and 3.51 ± 0.08 ng ml^{-1} for the plasma samples exposed to the Vacutainers. Thus, no adverse effect of the Vacutainer compared to the culture tube on assay accuracy could be established.

The stability of TM-PGE_2 in plasma and blood on prolonged storage was determined. To 50 ml of both control plasma and control blood were added 50 ng of TM-PGE_2. Duplicate 1-ml aliquots of the spiked blood were analyzed for TM-PGE_2 and the remaining blood and plasma were stored at −10°C. Duplicate 1-ml aliquots of the blood and plasma were analyzed on days 15, 21, and 133. The measured concentration of TM-PGE_2 for all these samples were within 6% of the initial blood concentration measured on day 1. Thus, given an assay precision of 5%, TM-PGE_2 seems to be stable in either frozen blood or plasma for at least four months.

Following these experiments, TM-PGE_2 was administered to two male volunteers. Ion chromatograms from the analysis of plasma from one of the volunteers obtained either before dosing (A), or 90 min post dosing (B), are shown in Fig. 3. The plasma concentration—time curves for the subjects can be seen in Fig. 4. Assuming that absorption is rapid and that distribution is complete after 40 min, the elimination half-lives were 26 and 20 min for subjects 1 and 2, respectively.

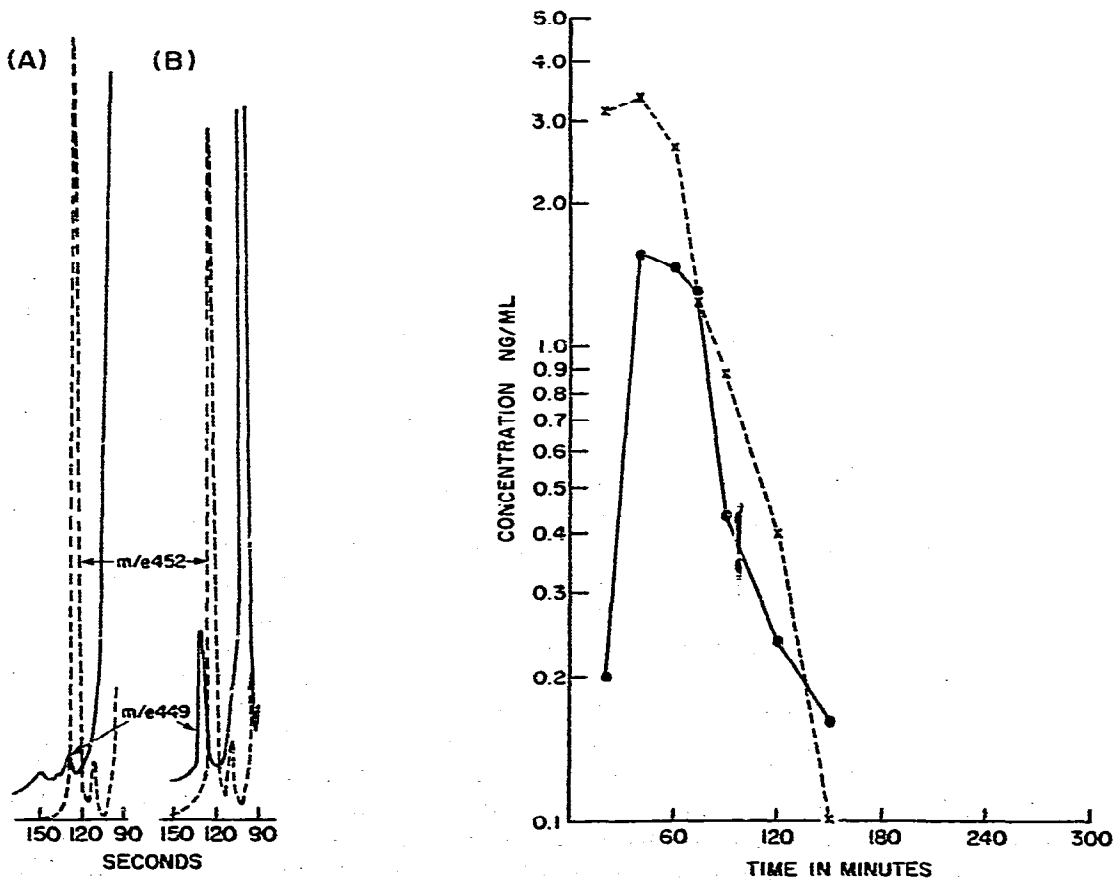


Fig. 3. Ion chromatograms from the analysis of 1 ml of plasma from a subject who had received a 10 $\mu g\ kg^{-1}$ oral dose of TM-PGE_2. (A) Plasma taken just prior to dosing; (B) plasma taken 90 min post dosing. The TM-PGE_2 concentration at 90 min was 0.4 $ng\ ml^{-1}$.

Fig. 4. Plasma concentration—time curve for two male volunteers who had received a 10 $\mu g/kg^{-1}$ oral dose of TM-PGE_2. (•) Subject 1, (×) subject 2.

CONCLUSIONS

(1) The pentafluorobenzyl ester—trimethylsilyl ether derivative most commonly used in the determination of prostaglandins by EC—GC can also be used for the analysis of these compounds by GC—NCIMS.

(2) Analysis by GC—NCIMS offers a significant increase in sensitivity over analysis by either positive ion GC—CIMS or EC—GC. The sensitivity limit of the GC—NCIMS assay for TM-PGE_2 is 100 pg ml^{-1}.

(3) The method was used successfully to determine TM-PGE_2 in plasma following a single 10 μg kg^{-1} oral dose of the drug in man.

(4) This analytical procedure should be useful in the measurement of other naturally occurring and xenobiotic prostaglandins.

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