Journal of Chromatography, 289 (1984) 249–258
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 232

MICRODETERMINATION OF PROSTAGLANDINS AND THROMBOXANE B_2 BY CAPILLARY GAS CHROMATOGRAPHY AND NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

HIROSHI MIYAZAKI*
Research and Development Administration of Pharmaceutical Division Nippon Kayaku Co., 1-11-2 Fujimi, Chiyoda-ku, Tokyo 102 (Japan)
MASATAKA ISHIBASHI, HIDEKI TAKAYAMA, KOUWA YAMASHITA and IZUMI SUWA
Research Laboratories, Research and Development Administration of Pharmaceutical Division, Nippon Kayaku Co., 3-31-12 Shimo, Kita-ku, Tokyo 115 (Japan)
and
MAKOTO KATORI
Department of Pharmacology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228 (Japan)

SUMMARY

Application of the dimethyl-*n*-propylsilyl (DMnPS) ether derivatives of prostaglandins (PGs) and thromboxane (TX) B_2 pentafluorobenzyl (PFB) esters to negative ion chemical ionization mass spectrometry (NICIMS) was investigated. These derivatives were separated completely within 10 min by the use of a fused-silica capillary column coated with methyl silicone. In NICIMS, all of the DMnPS ether derivatives of PGs and TXB_2 PFB esters yielded the characteristic negative ion $[M - 181]^-$ which was produced by elimination of PFB from the molecule. The detection limit of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was found to be 200 fg with a signal-to-noise ratio of 5 when monitoring the ion of m/z 653 ($[M - 181]^-$) in the high-resolution mode ($R = 2500$) using ammonia as a reagent gas. The method was applied to the quantitation of PGE_2 and $PGF_{2\alpha}$ in an extract obtained from the plasma of a lung–heart preparation from a dog.

INTRODUCTION

In the biomedical field, much attention has been focused on the elucidation of the relationship between the tissue or organ distribution of individual prostaglandins (PGs) and thromboxanes (TXs) and their physiological rôles because of their potent biological activities. In gas chromatography–mass spectrometry (GC–MS) of PGs, gas chromatographic resolution must be carefully considered and it is also very important to select derivatives suitable for separation. As PGs have very similar chemical structures, it had been thought that the use of the conventional trimethylsilyl (TMS) ether derivatives for determining their metabolic profiles would be unsuitable due to incomplete separation.

0021-9673/84/$03.00

Previously, we reported that the dimethylethylsilyl (DMES), dimethyl-*n*-propylsilyl (DMnPS) and dimethylisopropylsilyl (DMiPS) ether derivatives of PGs and TXB_2 provided excellent GC–MS properties[1–4]. Of these silyl ether derivatives, the DMnPS ether derivatives of PGs and TXB_2 pentafluorobenzyl (PFB) esters gave the most favourable GC separation using a fused-silica capillary column, and also extremely good sensitivity when using electron capture detection[3]. It has been reported[5–7] that the introduction of a PFB moiety having high electron affinity into the molecule greatly enhances the sensitivity of PGs in the negative ion chemical ionization (NICI) mode. This paper deals with the development of a more sensitive and specific method for the trace analysis of PGs and TXB_2 based on a combination of the PFB ester–DMnPS ether derivative and capillary gas chromatography–NICI–selected ion monitoring (SIM).

EXPERIMENTAL

Materials

PGD_2, PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$ and TXB_2 were purchased from Funakoshi Yakuhin (Tokyo, Japan). Bis(trimethylsilyl)-acetamide (BSTFA), DMES-imidazole, DMnPS-imidazole, DMiPS-imidazole, methoxyamine hydrochloride, and PFB bromide were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Sephadex LH-20 and silica gel (Kiesel Gel 60, 70–230 mesh) were obtained from Pharmacia (Uppsala, Sweden) and E. Merck (Darmstadt, F.R.G.), respectively. Clin Elut® was purchased from Analytichem International (CA, U.S.A.). Other reagents and solvents used in this study were of the highest purity available.

Gas chromatography–mass spectrometry

Capillary GC–MS was carried out using a VG Analytical MM ZAB-HF mass spectrometer equipped with a Shimadzu GC-9A gas chromatograph. For NICIMS, methane, isobutane and ammonia were used as reagent gases. A 25-m Ultra No. 1 fused-silica capillary column coated with methyl silicone (Hewlett-Packard) was used throughout this study. Solventless injection was achieved using a Van den Berg solventless injector with an inlet pressure of 0.4 kg/cm^2. The carrier gas was helium at a linear velocity of 25 cm/sec. The temperatures of the injection port and transfer line were kept at 300°C and that of the ion source was at 260°C. The ionization energy and emission current were 50 eV and 1 mA, respectively. The accelerating voltage was −8 kV. Computer-controlled SIM was carried out in the high-resolution mode (R = 2500) using perfluorokerosene as a reference compound.

Extraction of PGs from biological fluid

After addition of $[^2H_5]PGF_{2\alpha}$ (40 ng) and $[^2H_7]PGE_2$ (40 ng) to plasma (1 ml) from a dog, the plasma was acidified to pH 3 with 0.2 M hydrochloric acid and the resulting solution was transferred onto a Clin Elut No. 1003 column. The column was allowed to stand for 5 min, then the PGs were eluted with ethyl acetate–benzene (9:1) (24 ml). The solvent was evaporated under reduced pressure below 40°C and the residue was used for derivatization.

Derivatization of standard PGs and biological extract

Standard $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were treated with PFB bromide and diisopropyl-

ethylamine in acetonitrile at 40°C for 1 h to give the corresponding PFB esters. After evaporation of the excess of reagent, the residue was purified by silica gel column chromatography (5 × 0.8 cm I.D.) with ethyl acetate–methanol (99:1) (20 ml) as eluent. PGs with carbonyl groups were converted into the methoxime (MO) derivatives by treatment with methoxyamine hydrochloride in pyridine solution at 60°C for 1 h prior to esterification. The PFB esters or MO–PFB esters were treated with BSTFA, DMES-, DMnPS- or DMiPS-imidazole at room temperature for 1 h. The excess of reagent, except for BSTFA, was removed by means of Sephadex LH-20 column chromatography (5 × 0.8 cm I.D.) using chloroform–*n*-hexane–methanol (10:10:1) (2.8 ml). After evaporation of the solvent, the residue was dissolved in *n*-hexane solution containing 0.5% pyridine and subjected to NICIMS. The extract obtained from the biological samples was treated in the same manner as for PGs having carbonyl groups.

RESULTS AND DISCUSSION

The DMnPS ether derivatives permitted the PGs and TXB_2 PFB esters to be classified into two distinct groups of di- and trihydroxy compounds which greatly enhance the separation of individual PGs and TXB_2 in comparison with the corresponding TMS, DMES and DMiPS ether derivatives, as shown in Fig. 1. The DMnPS ether derivatives of PGs and TXB_2 PFB esters or methoxime (MO)–PFB esters gave excellent GC separation and high sensitivity when electron capture detection was employed as previously reported[3]. However, the sensitivity of these derivatives in the electron impact ionization (EI) mode was very poor. With hydroxysteroids, the EI mass spectra of the DMnPS ether derivatives were characterized by their inherent ions $[M - 43]^+$ with high relative intensity[8]. The corresponding DMnPS ether derivatives of PGs and TXB_2 gave only low intensity ions in the high mass region. However, it was possible to identify the structures of derivatives obtained by the present derivatization procedure as described above under EI mode on the basis of their molecular ions.

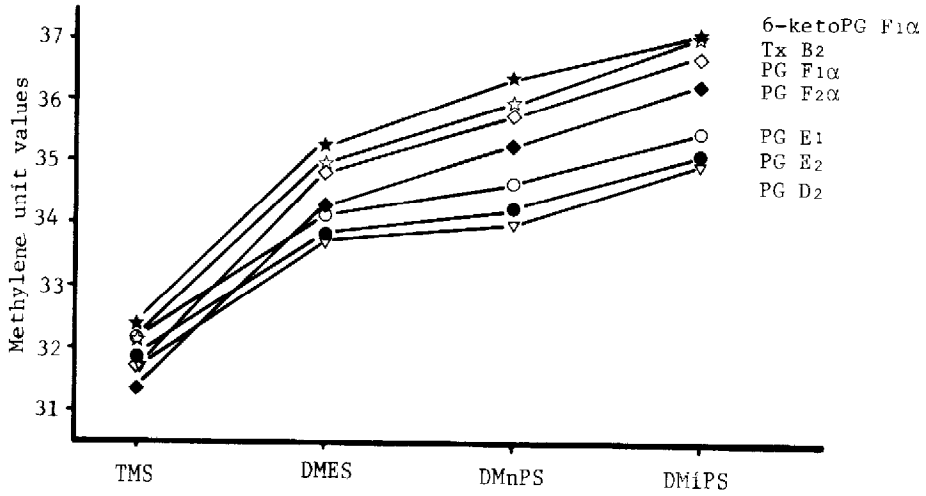


Fig. 1. Gas chromatographic data for the TMS, DMES, DMnPS and DMiPS ether derivatives of six types of PGs and TXB_2 PFB esters or MO-PFB esters.

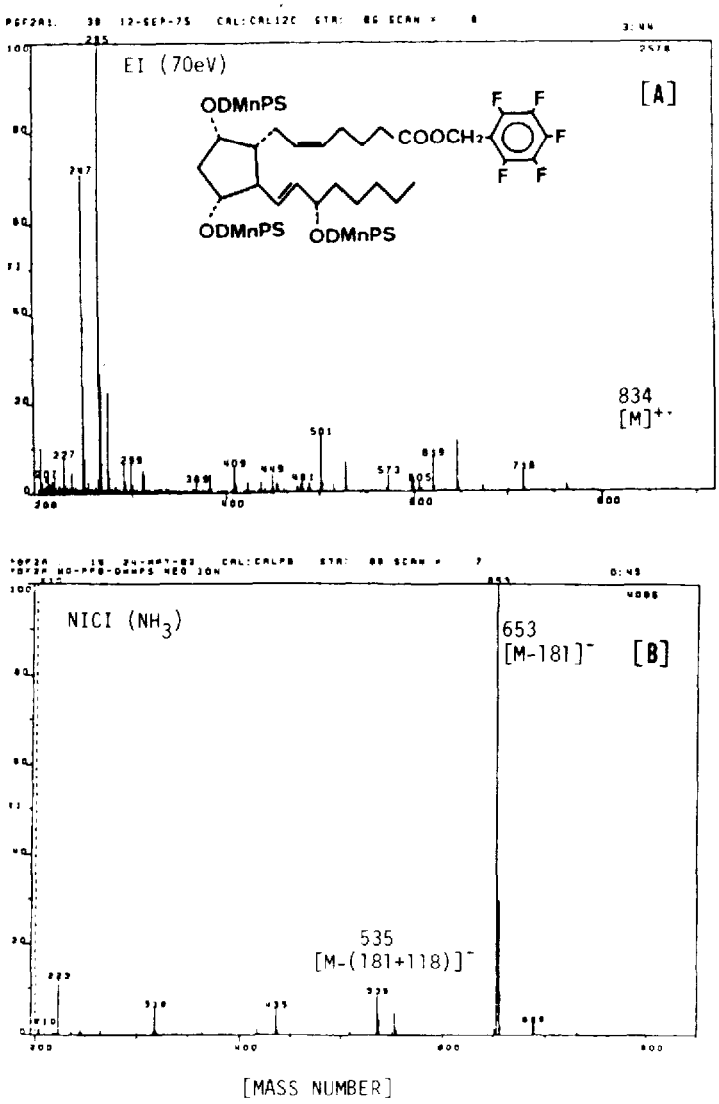
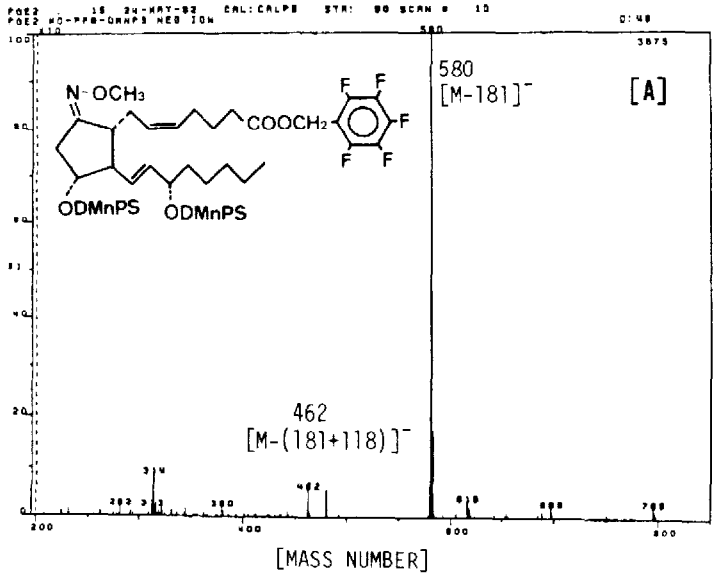


Fig. 2. EI (A) and NICI (NH_3) (B) mass spectra of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester.

Fig. 2A shows the EI mass spectrum of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester. The appearance of the molecular ion at *m/z* 834, with low relative intensity, was sufficient to confirm the structure of the derivatives. The mass spectrum was also characterized by the typical fragment ions $[M - 43]^+$, $[M - 71]^+$ and $[M - 118]^+$ due to the loss of n-C_3H_7, C_5H_{11} and $(CH_3)_2C_3H_7SiOH$ (DMnPSOH) from the molecular ion, as in the EI mass spectra of the DMiPS ether derivatives of PGs and TXB_2 methyl esters (ME)[2]. The ion at *m/z* 247 with its high relative intensity was indicative of the F prostaglandin ring system which was assigned as a structure corresponding to the ion of *m/z* 191 in the TMS ether derivative of $PGF_{2\alpha}$ ME.

Fig. 2B shows the mass spectrum of the DMnPS ether derivative of $PGF_{2\alpha}$

PFB ester in the NICI mode using ammonia as a reagent gas. In contrast to the EI mass spectrum, the NICI mass spectrum showed a simple fragmentation pattern. Although a quasi-molecular or adduct ion near the region of a molecular ion was not recognized, the characteristic negative ion $[M - 181]^-$ at m/z 653 which was produced by the elimination of PFB from the molecule was observed as a base peak. In addition, as in the EI mode, the ion $[M - 181]^-$ underwent further fragmentation to produce an ion $[M - (181 + 118)]^-$ of considerable intensity at m/z 535 duc to the loss of DMnPSOH. The NICI mass spectral fragmentation pattern of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was quite similar to that of the corresponding TMS ether derivatives as reported by Waddel and co-workers[9,10].



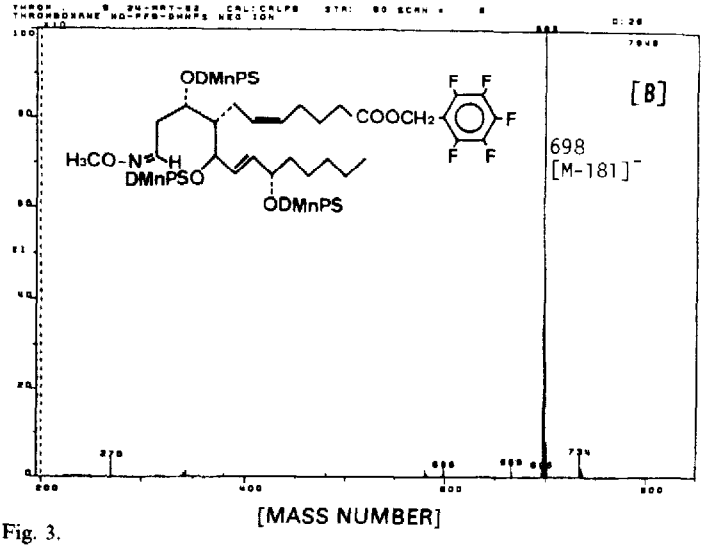


Fig. 3.

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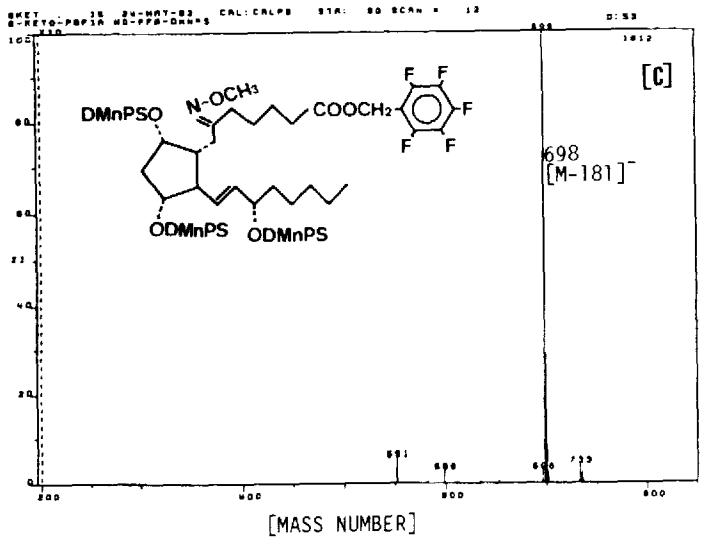

Fig. 3. NICI (NH_3) mass spectra of the DMnPS ether derivatives of the major isomer of PGE_2 (A), TXB_2 (B) and 6-keto $PGF_{1\alpha}$ (C) MO PFB esters.

Fig. 3 shows the NICI mass spectra of the DMnPS ether derivatives of the major geometric isomer of PGE_2 MO–PFB ester (A) formed during the methoxymation process, TXB_2 MO–PFB ester (B) and 6-keto $PGF_{1\alpha}$ MO–PFB ester (C). It is seen that the DMnPS ether derivatives of all PGs and TXB_2 PFB esters gave simple NICI mass spectra and their mass spectra were characterized by the inherent ion $[M - 181]^-$ observed as a base peak when using ammonia as a reagent gas.

Table I summarizes the mass spectral data of the DMnPS ether derivatives of PGs and TXB_2 PFB esters or MO–PFB esters in the EI and NICI modes. In the EI

TABLE I

EI AND NICI MASS SPECTROMETRIC DATA OF THE DMnPS ETHER DERIVATIVES OF PGs AND TXB_2 PFB ESTERS OF MO–PFB ESTERS

Relative intensities (%) are given in parentheses.

PGs	*EI (70 eV)***		*NICI (NH_3)*	
	$[M]^{+\cdot}$		*$[M - 181]^-$*	*$[M - (181 + 118)]^-$*
PGD_2*	761 (0.6)	718 (100)***	580 (100)	462 (5.2)
PGE_1*	763 (1.0)	491 (100)	582 (100)	464 (3.6)
PGE_2*	761 (0.8)	253 (100)	580 (100)	462 (6.1)
$PGF_{1\alpha}$	836 (0.2)	247 (100)	655 (100)	537 (2.8)
$PGF_{2\alpha}$	834 (0.4)	265 (100)	653 (100)	535 (9.1)
6-Keto $PGF_{1\alpha}$	879 (5.0)	572 (100)§	698 (100)	580 (0.4)
TXB_2	879 (0.2)	308 (100)	698 (100)	580 (2.5)

* The major isomer of the methoxime derivative.
** See ref. 3.
*** $[M - 43]^+$.
§ $[M - (71 + 2 \times 118)]^+$.

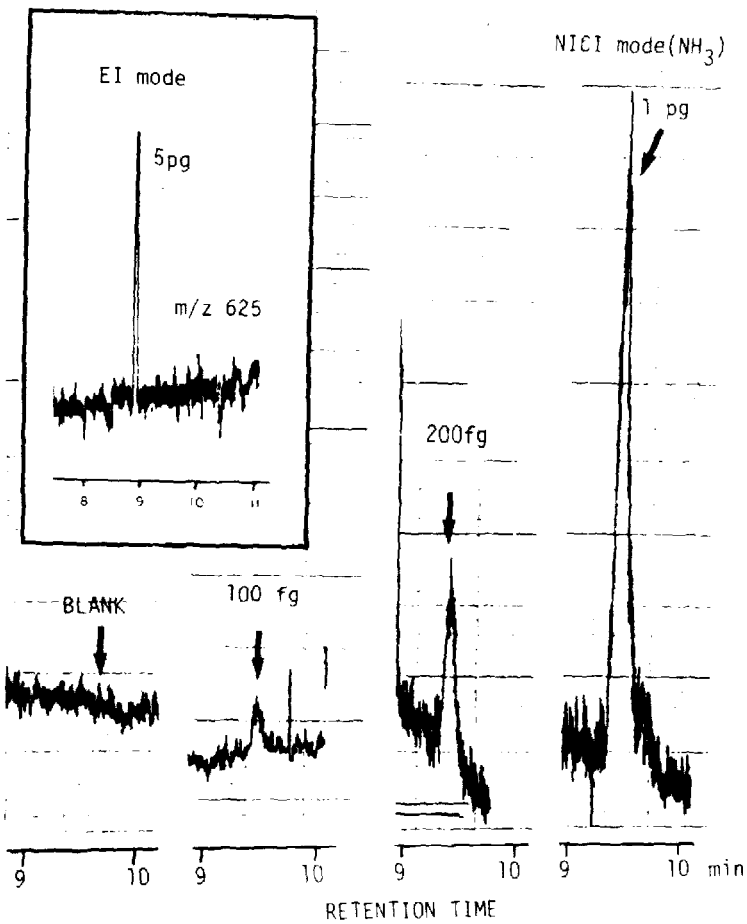

Fig. 4. Selected ion recordings of the ME-DMiPS ether derivative (EI mode, m/z 625 $[M - 43]^+$) and the PFB ester-DMnPS ether derivative (NICI mode, m/z 653 $[M - 181]^-$) of $PGF_{2\alpha}$.

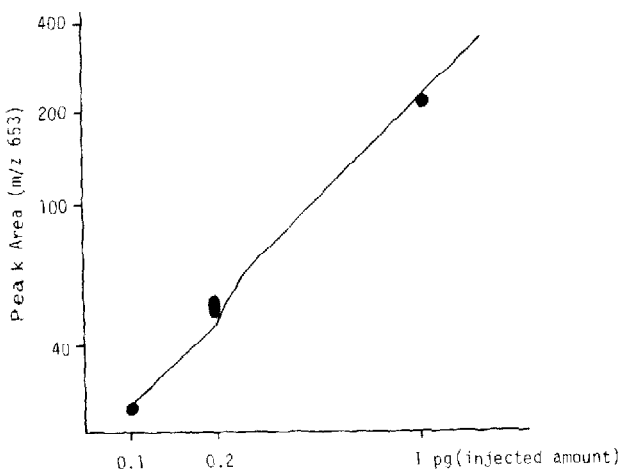

Fig. 5. Calibration graph for $PGF_{2\alpha}$ PFB ester DMnPS ether derivative.

mode, extensive fragmentation occurs for all the PGs and TXB_2, and the relative intensities of the fragment ions appearing in the high mass region were very low. Although, owing to their low intensities, these fragment ions were not suitable for the trace analysis of PGs and TXB_2 in biological fluids by SIM, they provided valuable information for the structural elucidation. On the other hand, all of the DMnPS ether derivatives of PG PFB esters provided the characteristic negative ions $[M - 181]^-$ in the high mass region as base peaks in the NICI mode as shown in Table I, suggesting that the combination of the present derivative and capillary GC–NICIMS will prove to be very useful for the trace analysis of PGs and TXB_2 based on SIM.

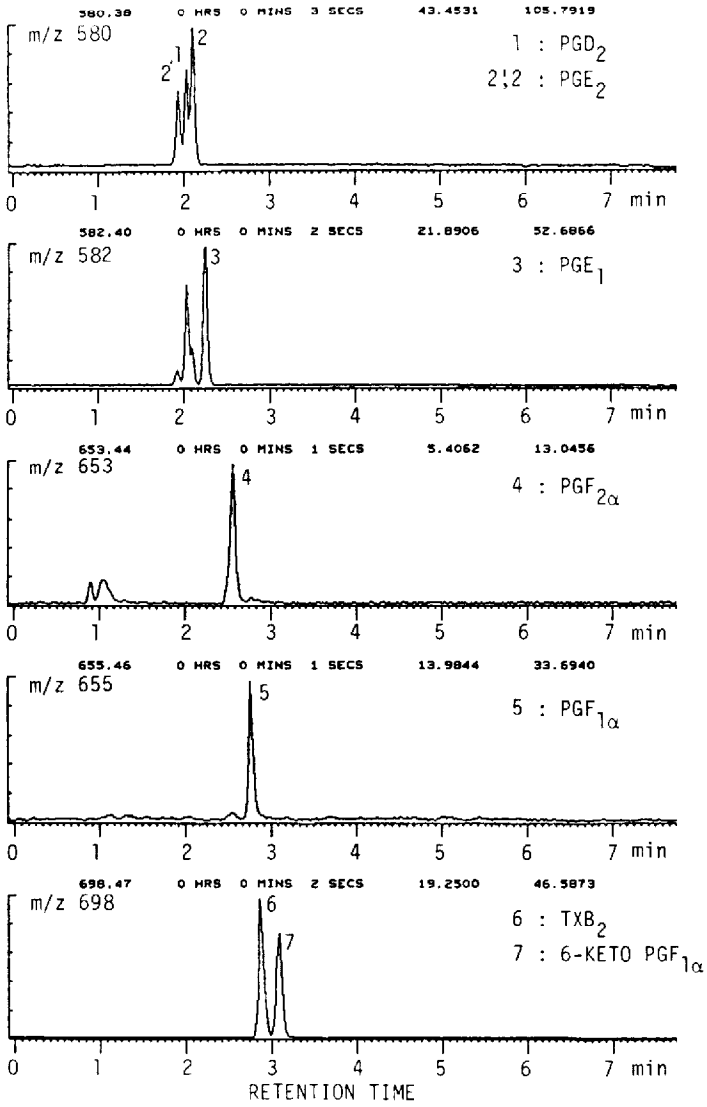

Fig. 6. NICI selected ion recordings of the DMnPS ether derivatives of six types of PGs and TXB_2 PFB esters or MO–PFB esters monitoring their characteristic ion $[M - 181]^-$.

The effect of the reagent gases for NICI mass spectral fragmentation was investigated using methane, isobutane and ammonia. When the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was used as a model compound, all reagent gases gave similar NICI mass spectra and the characteristic negative ion $[M - 181]^-$ was observed as a base peak. Subsequently, we attempted to investigate the effect of reagent gases on the sensitivity of SIM using the ion of *m/z* 653 derived from the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester. Although it had been thought to be difficult to measure the enhancement of absolute sensitivity in mass spectrometry, NICI–SIM using ammonia as reagent gas provided excellent sensitivity and was very useful for the analysis of PGs and TXB_2.

Selected ion monitoring was carried out to examine the detection limit of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester in the high-resolution mode ($R = 2500$). As shown in Fig. 4, NICI–SIM provided high sensitivity and a good signal-to-noise ratio in comparison with EI–SIM using the ME-DMiPS ether derivative of PGF_2. When the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was monitored by its characteristic negative ion $[M - 181]^-$ using ammonia as a reagent gas, good linearity was obtained between the peak areas and the injected amount of the derivative. As judged from Fig. 4, the minimum detectable amount of the DMnPS ether derivative of $PGF_{2\alpha}$ PFB ester was estimated to be 200 fg with a signal-to-noise ratio of 5.

Fig. 6 shows a typical NICI selected ion recording (SIR) of the DMnPS ether derivatives of representative PGs and TXB_2 PFB esters or MO–PFB esters, monitoring their characteristic negative ions $[M - 181]^-$ when analyzed by means of a fused-silica capillary column. Complete separation of the DMnPS ether derivatives of PGs and TXB_2 PFB esters or MO–PFB esters could be achieved within 10 min.

The use of the present PFB ester–DMnPS ether derivative and the capillary GC–NICI–SIM system was applied to the analysis of PGs and TXB_2 in an extract obtained from the plasma sample of a dog lung–heart preparation. Fig. 7 shows a

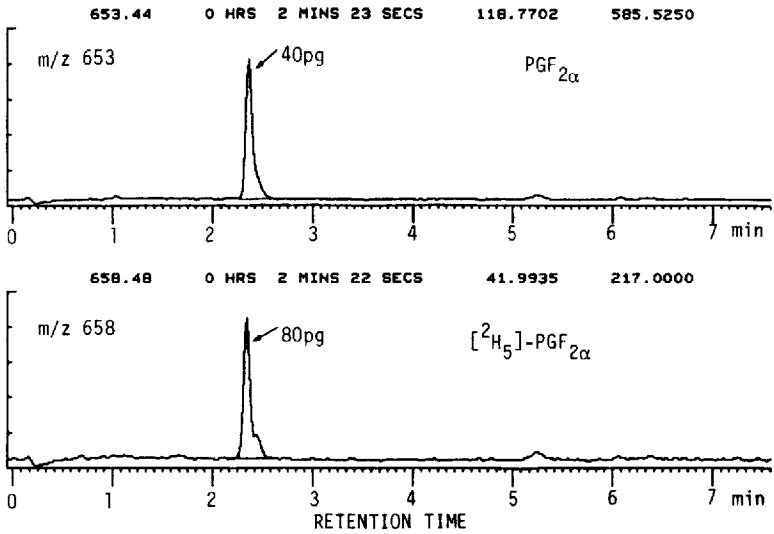

Fig. 7. NICI selected ion recordings of the DMnPS ether derivatives of $PGF_{2\alpha}$ and its $^2H_5$-labelled variant in an extract obtained from the plasma of a lung–heart preparation from a dog.

typical NICI–SIR obtained by analyzing a 1-μl aliquot of the plasma extract which contained 40 pg of $PGF_{2\alpha}$ and 80 pg of its deuterated variant used as an internal standard. The SIR was obtained with an extremely good signal-to-noise ratio when monitoring the ion $[M - 181]^-$ at m/z 653 for $PGF_{2\alpha}$ and m/z 658 for the internal standard, indicating that interfering substances co-existing in the extract obtained from the plasma sample were almost eliminated by the present clean-up procedure.

Further, the DMnPS ether derivatives of PGs exhibited excellent stability toward hydrolysis and silica gel chromatography[1,3]. This result provided the possibility of further purification of derivatives using silica gel column chromatography.

In conclusion, GC–SIM based on the combination of capillary GC–NICI–SIM and the present PFB esters–DMnPS ether derivative provided extremely high specificity and sensitivity for detection and quantitation of the very small amounts of PGs and TXB_2 in biological fluids without any disturbance from endogenous substances in comparison with that of EI–SIM. The present method may be useful for the elucidation of the relationship between the metabolic profiles of PGs and TXB_2 and their pharmacological activities.

ACKNOWLEDGEMENT

The authors are grateful to Dr. W. Tanaka, Institute of Microbial Chemistry, for his encouragement throughout this work.

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