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Microdetermination of 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ in human urine using gas chromatography–high-resolution selected-ion monitoring

Michinao Mizugaki*[,a], Takanori Hishinuma[a], Grace S.P. Yu[a], Kitae Ito[a], Masazumi Nishikawa[a], Yoshiharu Ohyama[a], Masataka Ishibashi[b], Yumiko Nakagawa[b], Noriaki Harima[c]

[a]*Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan*
[b]*Research Laboratories, Pharmaceuticals Group, Nippon Kayaku Co., 3-31 Shimo, Kita-ku, Tokyo 115, Japan*
[c]*Application Laboratory, JASCO International Co. Ltd., 2-4-21 Sennin, Hachioji, Tokyo 193, Japan*

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Abstract

The microdetermination of 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (**I**) in human urine is described. Samples to which the [$^2H_4$]-analogue was added as an internal standard were extracted by chromatographic sample preparation using a Bond Elut C_{18} cartridge and a silica gel column. Conversion of the extracted **I** into the 1-methyl ester-6-methoxime-9,11,15-trisdimethylisopropylsilyl ether derivative was followed by gas chromatography–high-resolution selected-ion monitoring (GC–HR-SIM). Interfering substances from the urine matrix were eliminated during GC–HR-SIM analysis using a DB-1 column. A good linear response over the range 10 pg–100 ng per tube was demonstrated. Compound **I** could be detected in the range 26–375 pg/ml of human urine. The proposed method can be applied to the determination of **I** in human urine.

1. Introduction

2,3-Dinor-6-ketoprostaglandin $F_{1\alpha}$ (**I**) is one of the major urinary metabolites of prostaglandin I_2 (PGI_2) and has been the subject of much interest in understanding the physiological role of the parent compound [1,2]. The plasma levels of 6-keto-$PGF_{1\alpha}$, another metabolite of PGI_2, is readily confounded by sampling artifacts. In response to advances in PGI_2 metabolism, microanalytical methods for the determination of **I** have been developed using gas chromatography–selected-ion monitoring (GC–SIM) [3–5]. Compound **I** has the hemiketal hydroxyl group in a position favourable for the formation of a γ-lactone and is thus prone to γ-lactonization. In fact, a major part of **I** exists in the spirolactone form in acidic aqueous solution and is extracted as such with organic solvents. Therefore, when this compound was treated sequentially with diazomethane, O-methylhydroxylamine and a silylating agent, the carboxyl group was not initially available to react with diazomethane. Some workers have been successful in obtaining

* Corresponding author.

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I in an acyclic form and in the formation of an O-methoxime derivative at C-6 with a free terminal carboxyl group without the induction of γ-lactonization. The revealed carboxyl group was esterified with N,O-bis(trimethylsilyl)trifluoroacetamide [3], diazomethane [4] or pentafluorobenzyl bromide [5,6], and then I was determined as the trimethylsilyl (TMS) ether derivative. Chiabrando *et al.* [7] overcame this serious problem of derivatization by extraction of I as its free acid form followed by immunoaffinity column chromatography.

However, the GC–MS properties of dimethylisopropylsilyl (DMIPS) ether derivatives have been found to be superior to those of TMS ether derivatives in enhancing GC separation and improving the chemical stability for purification by column chromatography [8,9]. In addition, they improve the intensity of characteristic ions for quantification by GC–SIM. With prostanoid derivatives, the 6-keto-$PGF_{1\alpha}$ derivative is dominated by the base peak ion $[M - C_3H_7]^+$. This indicates that the choice of the DMIPS ether derivative may make it a suitable candidate for specific and sensitive microdetermination of I by GC–SIM.

It was found from our previous work on the determination of urinary TXA_2 metabolites, that chromatographic sample preparation using silica gel led to the efficient elimination of interfering substances in the extract from a human urine matrix [10,11].

GC–high resolution (HR) SIM has been recognized as the most specific and reliable method for the microdetermination of prostanoids in biological samples, because the technique of monitoring characteristic ions of exact mass permits the selective detection of the compound of interest without serious interference from other endogenous substances [9]. This paper deals with the GC–MS properties of I methyl ester-methoxime-DMIPS ether derivative in combination with the use of the chemically stable DMIPS ether, using a column chromatographic purification procedure and GC–HR-SIM for the determination of I in human urine.

2. Experimental

2.1. Samples and reagents

2,3-Dinor-6-keto-$PGF_{1\alpha}$ (I) and [3,3,4,4-$^2H_4$]-6-ketoprostaglandin $F_{1\alpha}$ were purchased from Cayman Chemicals (Ann Arbor, MI, USA). [19,19,20,20-$^2H_4$]-I [internal standard (I.S.)] was kindly donated from Upjohn Pharmaceuticals (Ibaragi, Japan). DMIPS-imidazole was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Bond Elut C_{18} and Bond Elut silica cartridges were obtained from Analytichem International (Harbor City, CA, USA). Diazomethane was prepared from N-methyl-N-nitroso-*p*-toluenesulphonamide. Other solvents and reagents were of analytical-reagent grade.

2.2. Gas chromatography

A Shimadzu (Kyoto, Japan) GC-9A gas chromatograph equipped with a flame ionization detector, an all-glass VandenBerg-type solventless injector and a data processing system was employed. The column was a 25 m × 0.25 mm I.D. fused-silica capillary cross-linked with methylsilicone (DB-1; J & W Scientific, Folsom, CA, USA). Helium was used as the carrier gas and make-up gas. The temperatures of the injection port and detector were maintained at 320°C and that of the column oven at 280°C.

2.3. Gas chromatography–mass spectrometry

A VG AutoSpec mass spectrometer (VG Analytical, Manchester, UK) interfaced to a Hewlett-Packard (Avondale, PA, USA) Model 5890II gas chromatograph was employed. The column was a 25 m × 0.25 mm I.D. fused-silica capillary cross-linked with methylsilicone (DB-1; J & W Scientific). The temperature of the column oven was maintained at 200°C for 1 min and then programmed to 310°C at 10°C/min. The carrier gas was helium with a linear velocity of about 25 cm/s. The temperatures of the injection port and the transfer line were kept at 320 and 280°C, respectively, and that of the ion source at 250°C.

The ionization energy and the trap current were 70 eV and 100 μA, respectively. The accelerating voltage was 8 kV. Mass spectra were recorded by repeated scanning (1 s per decade) over the mass range *m/z* 800–90 (cycle time *ca.* 1.5 s) with a dynamic resolution of 1000.

2.4. GC–high-resolution selected-ion monitoring

GC–HR-SIM was performed under the same conditions as used in GC–MS but with a static resolution of 10 000 and the trap current of 250 μA. The ions at *m/z* 642.4041 and 646.4239 were monitored together with the ion at *m/z* 630.9601 from perfluorokerosene as a lock mass to ensure stability.

2.5. Sample preparation of 2,3-dinor-6-keto-$PGF_{1\alpha}$ from human urine

To human urine (5 ml) was added [$^2H_4$]-**I** (10 ng) as an I.S. The sample was acidified to pH 2.5 with 0.5 *M* HCl and allowed to stand for 1 h at room temperature and the resulting solution passed to a Bond Elut C_{18} cartridge. The cartridge was washed with water (10 ml) and *n*-hexane (10 ml). **I** and the I.S. were eluted with ethyl acetate (10 ml) and the eluate was evaporated to dryness. The residue was dissolved in *n*-hexane–ethyl acetate (2:1) (3 ml) and then transferred on to a silica gel column (5 × 0.5 cm I.D.). The column was washed with *n*-hexane–ethyl acetate (2:1) (8 ml) and *n*-hexane–ethyl acetate (1:1) (20 ml). The lactone form of **I** was eluted with *n*-hexane–ethyl acetate (1:2) (50 ml). After evaporation of the solvent under reduced pressure, the residue was dissolved in a 5% solution of O-methylhydroxylamine hydrochloride in pyridine (100 μl) and allowed to stand for 1 h at 60°C. The pyridine was evaporated under reduced pressure and saturated sodium chloride solution (1 ml) was added to the residue. The methoxime (MO) derivative was extracted twice with ethyl acetate (3 ml). The ethyl acetate solution was collected and dried under reduced pressure. The resulting MO derivative was treated with ethereal diazomethane (1 ml) for 30 min at room temperature. After evaporation, the residue was dissolved in *n*-hexane–ethyl acetate (1:1) (1 ml) and then transferred on to a silica gel column (5 × 0.5 cm I.D.). The column was washed with *n*-hexane–ethyl acetate (1:1) (20 ml) and the methyl ester (ME)-MO derivatives of **I** and the I.S. were eluted with ethyl acetate (20 ml). After evaporation, the residue was silylated with DMIPS-imidazole (50 μl) for 30 min at 60°C. The reaction product was dissolved in *n*-hexane (2 ml) and passed to a Bond Elut silica cartridge, which was washed with *n*-hexane (4 ml). The ME-MO-DMIPS ether derivative was eluted with *n*-hexane ethyl acetate (10:1) (6 ml). After evaporation of the solvent, the residue was dissolved in *n*-hexane–pyridine (99:1) (100 μl) and used for GC–HR-SIM.

Creatinine in human urine was determined using a creatinine test kit (Wako, Osaka, Japan).

3. Results and discussion

3.1. Equilibrium of ketone, hemiketal and spirolactone forms of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in acidic medium

2,3-Dinor-6-keto-$PGF_{1\alpha}$ (**I**) was dissolved in acidic aqueous solution at pH 2.5 and kept for 1 h at room temperature. After addition of [19,19,20,20-$^2H_4$]-6-keto-$PGF_{1\alpha}$ (which was used as an I.S. only for this equilibrium study), the ketone, hemiketal and spirolactone forms of **I** and [$^2H_4$]-6-keto-$PGF_{1\alpha}$ were passed to a Bond Elut C_{18} cartridge, which had previously been washed with water and *n*-hexane, and extracted with ethyl acetate. The extracts were then converted into the *n*-propylamide (PA)-methoxime (MO)-dimethylisopropylsilyl (DMIPS) ether derivative [12] for the spirolactone form of **I** or converted into the methyl ester (ME)-MO-DMIPS ether derivatives for the ketone and hemiketal forms of **I** and [$^2H_4$]-6-keto-$PGF_{1\alpha}$. GC–SIM analysis revealed indirectly that the recovery of the spirolactone form of **I** was about

90% because less than 10% ($n = 5$) of the total amount of the ketone and hemiketal forms of I were determined as the ME-MO-DMIPS ether derivative. Nakagawa *et al.* [13] observed by NMR that three conformations of ketone, hemiketal and spiro lactone forms exist in equilibrium in aqueous solution at pH 3.1. This result may support the above finding that I does not exist exclusively in the lactone form under acidic conditions at pH 3.0.

3.2. Gas chromatography

The spirolactone form of I was extracted from acidic aqueous solution and was readily converted into its ME-MO-DMIPS ether derivative by treating it with O-methylhydroxylamine hydrochloride, diazomethane and DMIPS-imidazole. The resulting derivative gave resolved doublet peaks on GC analysis. This finding is most likely attributable to the formation of *syn*- and *anti*-isomers of the oxime, as was observed with the ketonic prostanoids series. The methylene unit value of this derivative was 30.93 and 31.02, being about 4.1 units higher than that of the corresponding TMS ether derivative (26.82 units). Under these conditions the isomers of TMS ether derivative could not be separated.

3.3. Mass spectrometry

The mass spectra of the isomer pair of the I ME-MO-DMIPS ether derivative are shown in Fig. 1, exhibiting a series of ions characteristic of the expected derivative. The mass spectrum of the first-eluting component was considerably more complex than that of the second. Almost all fragment ions in the mass spectrum of the first were also observed as characteristic ions common in the second-eluting component. The molecular ion (m/z 685) and $[M - CH_3]^+$ ion were observed with low intensity. Loss of an isopropyl radical at the DMIPS group from the molecular ion gave rise to the $[M - C_3H_7]^+$ ion at m/z 642 as the base peak. The appearance of these ions in the spectrum, typical of the DMIPS ether derivatives [8], confirmed the formation of the expected derivative. Many other characteristic fragment ions reflecting I were also observed with moderate or low intensity. The molecular ion lost a methyloxy radical at the methoxime moiety to give $[M - OCH_3]^+$ ion at m/z 654 and

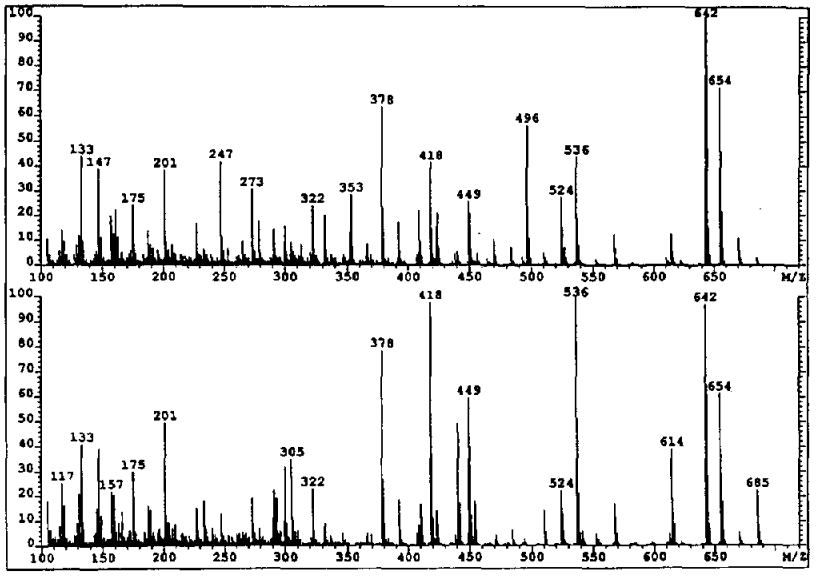

Fig. 1. Mass spectra of the structural isomers of the 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ methyl ester-methoxime-dimethylisopropylsilyl ether derivative. Top, the first-eluting component; bottom, the second-eluting component.

the C-16/C-20 hydrocarbon fragment to $[M - C_5H_{11}]^+$ ion at m/z 614. Successive losses of the dimethylisopropylsilanol ($C_5H_{13}SiOH$: 118 u) gave rise to ions at m/z 567 and 449 from the molecular ion, ions at m/z 536, 418 and 300 from $[M - OCH_3]^+$ ion, an ion at m/z 524 from $[M - C_3H_7]^+$ ion and ions of m/z 496 and 378 from $[M - C_5H_{11}]^+$ ion, respectively. The presence of the F-prostaglandin ring system, the 15-hydroxyl group and the C-13–C-14 double bond in the β-chain were confirmed by the ions at m/z 272, 247, 227 and 201. The ion at m/z 201 originated from the C-15/C-20 fragment, a characteristic of the prostanoid one and two series.

These results revealed that these mass spectra were similar to those of the corresponding 6-keto-$PGF_{1\alpha}$ derivative [8] except for the obvious shift produced by the lack of C-2/C-3 hydrocarbon units.

3.4. Sensitivity

Fig. 2 shows the selected-ion recording (SIR) trace at resolution 10 000 of this ME-MO-DMIPS ether derivative of **I** obtained by monitoring the ion at m/z 642.4041 corresponding to the exact mass for the atomic composition of $[M - C_3H_7]^+$. Injection of 10 pg of this **I** derivative gave rise to a doublet with a signal-to-noise ratio of more than 100:1 in HR-SIM. This suggests that this derivative makes it possible to detect picogram levels of **I** with high reliability. Although the first-eluting component of the present derivative concentrated only about 3.6% of the total ion current (above m/z 100) into the $[M-C_3H_7]^+$ ion at m/z 642, use of GC–HR-SIM allowed the highly sensitive detection of the $[M - C_3H_7]^+$ ion appearing in high-mass region. On the other hand, when 10 pg of the **I** derivative were injected, the low-resolution SIM result showed doublet peaks with a signal-to-noise ratio of about 10:1. This was a ten times lower response factor than the HR-SIM result shown in Fig. 2. This discrepancy may be explained by the fact that GC–HR-SIM is less susceptible to interference from co-existing impurities. Consequently, the sensitivity of detection in the high-resolution mode was considered to be more reliable.

3.5. Sample preparation

The crude extract from the octadecylsilica cartridge was chromatographed over silica gel by stepwise elution using a mixture of *n*-hexane and ethyl acetate, and **I** was recovered as its spirolactone form. However, when the lactonized **I** was treated sequentially with diazomethane and O-methylhydroxylamine, the carboxyl group was not initially available to react with diazomethane. In response to this problem, the sequence of the derivatization reaction was reversed by first carrying out methyloximation, as reported by Falardeau *et al.* [3]. The ME-MO derivative obtained was further purified by silica gel column chromatography. It was essential to include the additional purification step for the urine extract, because contaminants can over-

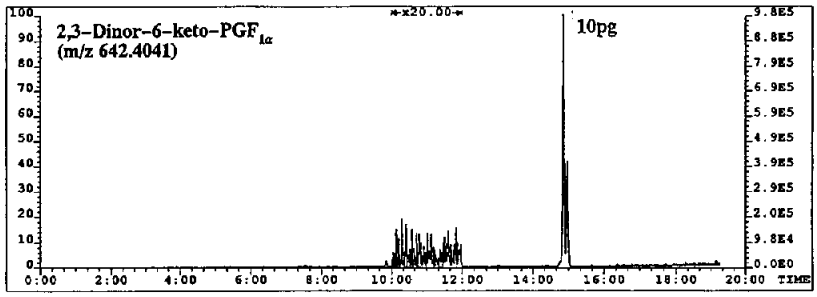

Fig. 2. Selected-ion recording from 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ methyl ester-methoxime-dimethylisopropylsilyl ether derivative at a resolution of 10 000 over a GC retention time range of 0–20 min. The retention time range of 10–12 min was magnified 20-fold to show the noise level.

shadow the relatively low concentrations of **I**. As mentioned earlier, the DMIPS ether derivative exhibited excellent hydrolytic stability on silica gel column chromatography, it being possible to achieve a high degree of purification without hydrolysis of the derivative. Consequently, as impurities from the urine matrix in addition to excess silylating reagent could be removed, GC–HR-SIM analysis permitted the detection of low levels of **I** in human urine. Thus, after extraction using an octadecylsilica cartridge, further purification by silica gel column chromatography was carried out to eliminate possible interferences from endogenous substances with the greatest circumspection from the unfavourable isotope effect of an internal standard (I.S.) of the $[^2H_4]$-analogue.

3.6. Calibration graph

The calibration graph for **I** was obtained by plotting the peak-area ratio of **I** to the I.S. against their mass ratios. Good linearity was observed in the range 10 pg–100 ng per tube (log $y = 0.6244528 \log x - 2.545012$, where x = peak-area ratio and y = amount in grams; $r = 0.9989$), which would cover the concentrations found in 2.5–20 ml of healthy human urine.

3.7. Purity of the peak corresponding to the 2,3-dinor-6-keto-$PGF_{1\alpha}$ derivative

Fig. 3 illustrates a typical SIM result from a urine sample, and shows that interfering substances from the urine matrix were eliminated during the microanalysis. Peaks appearing on the SIR traces of the m/z 642.4041 and 646.4239 ions from the urine extract sample corresponded to about 240 pg for **I** and 400 pg for the I.S. Peak matching was adopted to make certain that another urine constituent with the same retention time was not contributing to the recorded intensity of the conventional selected-ion recording (SIR). This method is based on the same concept as the intensity-matching technique [11,14]. Using this method, the two characteristic ions in the high-mass region of **I** were monitored under high-resolution conditions to eliminate possible interference from contaminant material and to maximize the specificity of detection. The

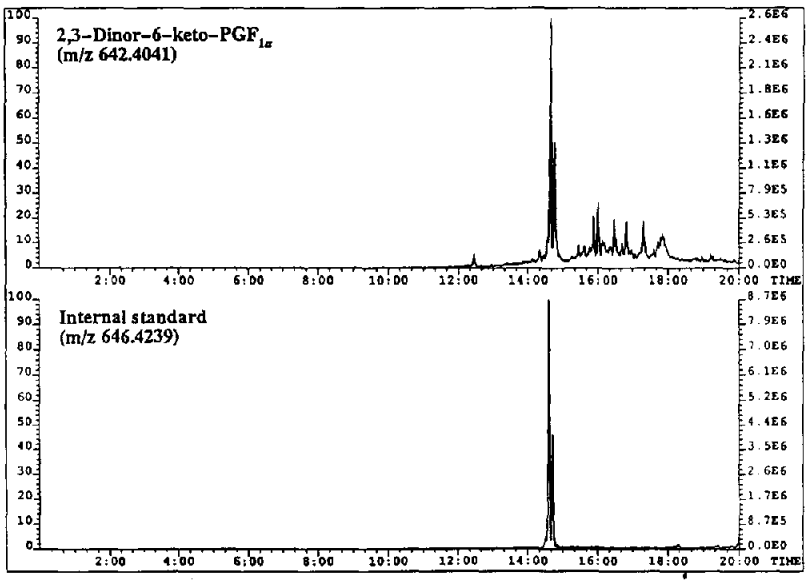

Fig. 3. Selected-ion recordings of the methyl ester-methoxime-dimethylisopropylsilyl ether derivative 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (m/z 642.4041) and its $[^2H_4]$-analogue (m/z 646.4239) from healthy adult urine over a GC retention time range of 0–20 min.

response ratio of the two ions selected, m/z 642.4041 and 654.4401, at 10 000 resolution was determined. The reconstructed ion profile for the first-eluting component was found to be a single peak indicating the absence of an impurity. Moreover, the ratio of the peak area of the ion at m/z 654.4401 to the base peak ion at m/z 642.4041 of the first-eluting component was found to be 0.362, which is in good agreement with the known value 0.353 from the authentic compound. These results give strong evidence that the component monitored was the derivative of **I** and that there was no interfering contaminant present. On the other hand, no peak was detected with the retention time of the $[^2H_4]$-analogue in the GC–SIM trace of the extract.

3.8. Reproducibility

After addition of the I.S. to the urine of a healthy human adult, three different samples were subjected to the sample preparation procedure and the experimental reproducibility was investigated by analysing these samples in triplicate. The results are given in Table 1. Statistical analysis was carried out according to a one-way analysis of variance [15] in order to separate the analytical errors arising from two sources: sample preparation and GC–SIM. The results shown in Table 2 indicate that there was no significant difference in sample preparation. The coefficient of variation in GC–SIM was 4.8%.

Table 2
Analysis of variance from the reproducibility test

Source	S	f	V	F_0
Sample preparation	0.404	2	0.202	3.497
Error (GC–SIM)	0.347	6	0.058	
Total	0.7515	8		

S = Residual sum of squares; f = number of degree of freedom ($f_1 = f_{\text{sample preparation}}$, $f_2 = f_{\text{error}}$); V = unbiased variance; F_0 = observed value following F distribution variance ratio ($V_{\text{sample preparation}}/V_{\text{error}}$); $F(f_2, f_1, \alpha)$ = density function of F distribution with f_1 and f_2 degrees of freedom. $F(2, 6, 0.05) = 5.14$.

In order to examine the accuracy and precision of the method, three urine samples spiked with **I** at concentrations of 0.4, 0.8 and 1.2 ng per 5 ml of urine were prepared. The **I** spiked concentrations correspond to *ca.* 10–400% of endogenous **I** found in healthy human urine [3,4,7]. These samples were analysed in duplicate. The results are given in Table 1. The recoveries of added **I** ranged from 96.4 to 115.9% with a mean ± standard deviation of 106.6 ± 7.4%. Statistical analysis was carried out according to a two-way analysis of variance [15] in order to

Table 1
Recovery of 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ added to human urine

Sample	Urinary levels (pg per 5 ml)	2,3-Dinor-6-keto-$PGF_{1\alpha}$ recovered	
		pg per ml	Recovery (%)
Non-spiked urine			
1	462, 500, 482		
2	574, 524, 510		
3	477, 520, 491		
Mean	506		
Spiked urine (5 ml)			
1 (+0.4 ng)	945, 970	439, 464	109.7, 115.9
2 (+0.4 ng)	892, 995	386, 489	96.4, 122.3
3 (+0.8 ng)	1304, 1380	798, 874	99.8, 109.3
4 (+0.8 ng)	1380, 1280	874, 774	109.3, 96.8
5 (+1.2 ng)	1832, 1760	1326, 1254	110.5, 104.5
6 (+1.2 ng)	1738, 1730	1232, 1224	102.7, 102.0
Mean ± S.D.			106.6 ± 7.4

separate analytical errors between three sources: I concentration, sample preparation and GC–SIM. The results indicates that there was no significant difference from I concentration and sample preparation (Table 3). Almost all of the error in this experiment was attributable to GC–SIM, because the errors from sample preparation and I concentration were negligible. The coefficient of variation of GC–SIM in this recovery test was 8.5%. The estimated urinary levels with the 95% confidence limit according to the orthogonal polynomial equation in this recovery test were calculated to be 5.36 ± 0.60, agreeing with the concentrations of endogenous I in non-spiked urine at the level of 506 pg per 5 ml of urine. These facts suggest that the present method makes it possible to determine picogram levels of I in urine with high reliability.

3.9. Application to the analysis of human urine

Twelve males (aged 23–50 years) and one female (aged 27 years) were studied. All had taken no medication for at least 2 weeks prior to urine collection. Four of them were smokers. Urine samples were collected after lunch. The results is shown in Table 4. Samples 1–12 were from healthy males and sample 13 was from a healthy female. The excretion of I was 103.2 ± 72 (range 64–338) pg/mg creatinine. Levels of urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ were reported to be 155 ± 23 ng/g creatinine in females ($n = 28$, 26.3 ± 9.1 years) and 78 ± 7.6 ng/g creatinine in males ($n = 28$, 31.9 ± 5.8 years) [16]. These data almost equal the result for 24 h urine (data not shown). It was also reported that the level ranged from 45 to 219 pg/mg creatinine in healthy males ($n = 54$, 18–75 years) [17]. Compared with these published data, the data obtained here seem fairly reasonable.

Table 3
Analysis of variance from the recovery test

Source	S	f	V	F_0
Concentration (A)	122.665	2	61.342	0.742
Sample preparation (B)	34.003	1	34.003	0.414
$A \cdot B$	6.672	2	3.336	0.040
Error (GC–SIM)	496.120	6	82.687	
Total	659.480	11		

Definitions as in Table 2.
$F(2, 6, 0.05) = 5.14$.
$F(1, 6, 0.05) = 5.99$.

Table 4
Determination of 2,3-dinor-6-keto-$PGF_{1\alpha}$ in urine of healthy human adults

Sample No.	2,3-Dinor-6-keto-$PGF_{1\alpha}$	
	pg/ml urine	pg/mg creatinine
1	86	79
2	203	110
3	26	64
4	61	68
5	277	338
6	182	69
7	375	110
8	178	77
9	145	103
10	167	64
11	208	83
12	194	98
13	74	78
Mean ± S.D.	167.4 ± 94	103.2 ± 72

4. Conclusion

The combination use of the column chromatographic sample preparation using silica gel and a chemically stable DMIPS ether derivative should be very useful in the microdetermination of I in human urine. In addition, selection of exact masses during GC–SIM allowed the selective detection of I without serious interference from the urine matrix.

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References

[1] B. Rosenkrantz, C. Fisher, E.K. Weimer and J.C. Frolich, *J. Biol. Chem.*, 255 (1980) 10194.
[2] G.A. FitzGerald, A.K. Pederson and C. Patrono, *Circulation*, 67 (1983) 1174.
[3] P. Falardeau, J.A. Oates and A.R. Brash, *Anal. Biochem.*, 115 (1981) 359.
[4] O. Vesterqvist and K. Green, *Prostaglandins*, 28 (1984) 139.
[5] A. Martineau and P. Falardeau, *J. Chromatogr.*, 417 (1987) 1.
[6] C. Weber, M. Holler, J. Beetens, F. De Clerck and F. Tegtmeier, *J. Chromatogr.*, 562 (1991) 599.
[7] C. Chiabrando, V. Pinciroli, A. Campoleoni, A. Benigni, A. Piccinelli and R. Fanelli, *J. Chromatogr.*, 495 (1989) 1.
[8] H. Miyazaki, M. Ishibashi, K. Yamashita, Y. Nishikawa and M. Katori, *Biomed. Mass Spectrom.*, 8 (1981) 521.
[9] M. Ishibashi, K. Yamashita, K. Watanabe and H. Miyazaki, in S.J. Gaskell (Editor), *Mass Spectrometry in Biomedical Research*, Wiley, Chichester, 1986, Ch. 23, p. 423.
[10] K. Watanabe, K. Yamashita, M. Ishibashi, Y. Hayashi, S. Yamamoto and H. Miyazaki, *J. Chromatogr.*, 468 (1989) 383.
[11] M. Ishibashi, K. Watanabe, F. Ishizaki, Y. Ohyama, M. Nishikawa, M. Mizugaki and N. Harima, *Biol. Mass Spectrom.*, 20 (1991) 399.
[12] M. Ishibashi, K. Watanabe, Y. Ohyama, M. Mizugaki and N. Harima, *Chem. Pharm. Bull.*, 37 (1989) 539.
[13] A. Nakagawa, N. Kobayashi, H. Haruyama, T. Takayama, S. Muramatsu and K. Nakamura, in Y. Seyama (Editor), *Proceedings of the 15th Annual Conference of the Japanese Society for Biomedical Mass Spectrometry, September 1990, Tokyo*, Japanese Society for Medical Mass Spectrometry, Tokyo, 1990, p. 123.
[14] M. Ishibashi, K. Watanabe, Y. Ohyama, M. Mizugaki, Y. Hayashi, W. Takasaki, *J. Chromatogr.*, 562 (1991) 613.
[15] G. Taguchi, *Experimental Designs*, Maruzen, Tokyo, 1962.
[16] S. Fischer, C. Bernutz, H. Meier and P.C. Weber, *Biochim. Biophys. Acta*, 876 (1986) 194.
[17] G.A. FitzGerald, B. Smith, A.K. Pedersen and A.R. Brash, *N. Engl. J. Med.*, 310 (1984) 1065.