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Simultaneous determination of androstenediol 3-sulfate and dehydroepiandrosterone sulfate in human serum using isotope diluted liquid chromatography–electrospray ionization-mass spectrometry

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Abstract

A simple method for simultaneous determination of androstenediol 3-sulfate (Adiol-3S) and dehydroepiandrosterone sulfate (DHEA-S) in human serum using isotope diluted liquid chromatography–electrospray ionization-ion trap-mass spectrometry (LC–ESI-ion trap-MS) was developed. After addition of deuterated internal standards ([$^2H_5$]Adiol-3S and [$^2H_4$]DHEA-S), human serum (100 μl) was deproteinized with acetonitrile and then applied to a solid-phase extraction cartridge, Oasis HLB. The obtained steroid sulfates fraction was washed with hexane and then analyzed by LC–ESI-MS operated in the negative ion mode. The quantification ranges of Adiol-3S and DHEA-S were 10–400 ng/ml and 0.05–8 μg/ml, respectively. The method does not require the chemical or enzymatic hydrolysis of the conjugates and purification with high-performance liquid chromatography, and shows satisfactory reproducibility and accuracy. The concentrations of these sulfates in the sera of healthy male volunteers ($n = 14$) were 19.2–245.3 mg/ml (Adiol-3S) and 0.175–5.16 μg/ml (DHEA-S), and those of patients with prostate cancer ($n = 19$) were 15.3–182.7 ng/ml (Adiol-3S; four samples, not detectable) and 0.110–2.421 μg/ml (DHEA-S).

Keywords: Androstenediol 3-sulfate; Dehydroepiandrosterone sulfate

1. Introduction

During the last decade, prostate cancer has become a commonly diagnosed malignancy in aged males. Prostate cancer is an androgen-dependent cancer, the growth of which is promoted by androgen. The major circulating active androgen in the blood of male is

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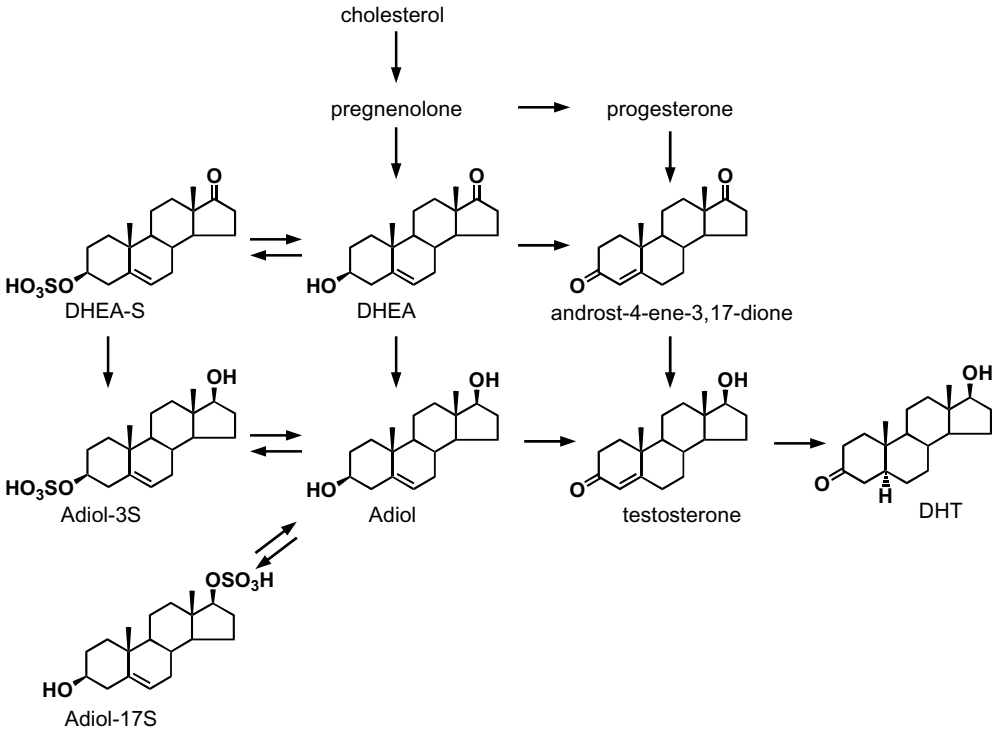

Fig. 1. Biosynthetic pathway of androgens.

testosterone secreted from the testis and it is converted into the most potent androgen, 5α-dihydrotestosterone (DHT), in the prostate. In human, the adrenal gland secrets a large amount of dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), but whose androgenic activity is low. However, it is recognized that DHEA is converted into testosterone via androst-4-ene-3,17-dione or androst-5-ene-3β,17β-diol (androstenediol, Adiol), and further metabolized into DHT in the peripheral tissues (Fig. 1) [1–3]; it has been pointed out that DHEA and its metabolites are in part responsible for the growth and recurrence of prostate cancer. Recently, it was demonstrated that Adiol could activate the androgen receptor target gene, and that some anti-androgens failed to block completely the androgenic activity of Adiol in human prostate cancer cells [4,5]. In our previous paper, we identified Adiol as the major metabolite of DHEA formed from human prostate homogenate [6]. In human serum, DHEA-S is the most abundant androgen, and whose concentration is several hundred folds higher than that of non-conjugated DHEA. Therefore, Adiol 3-sulfate (Adiol-3S) is one of the abundant steroid sulfates normally occurring in human peripheral circulation. Although the biological function of Adiol-3S in prostate cancer has not been clarified, the presence of steroid sulfatase activity has long been known to be widespread in the body including prostate tissue [7]; it has been proposed that Adiol-3S shows its androgenic activity after deconjugation. Therefore, significant interest is focused on the prostate and serum concentrations of Adiol-3S together with those of DHEA-S in order to investigate the mechanism of the recurrence of prostate cancer.

Several determination methods for Adiol-S in biological fluids using immunoassay [8–14] or gas chromatography–mass spectrometry (MS) [15–17] have been developed. Most of these were indirect methods that required the chemical or enzymatic hydrolysis of conjugates, purification with high-performance liquid chromatography (HPLC) and/or a derivatization procedure. Accordingly these were time consuming

and furthermore could not identify the conjugated position, though hydroxysteroid sulfotransferase is capable of producing the 17-sulfate (Adiol-17S) and Adiol 3,17-disulfate as well as Adiol-3S [18,19]. Because of existence of the isomeric sulfate and the relatively high concentration of DHEA-S, assay for Adiol-3S in biological fluids should be highly specific and sensitive. Although many determination methods for DHEA-S in biological fluids have been reported [2], most of them have the similar disadvantage as described earlier and the practical profile analysis of androgen sulfate in biological fluids has not been done. LC–MS has been used to overcome these problems; for example, Shackleton et al. reported a method for determination of DHEA-S using LC–thermospray ionization-MS without the chemical or enzymatic hydrolysis [20]. It was demonstrated that the other androgen sulfates, including Adiol-3S, could be measured simultaneously. But [7,7-$^2H_2$]DHEA-S was used as an internal standard (IS) and the same monitoring ion (*m*/*z* 369 $[M–H]^-$) was used for the detection of Adiol-3S and IS. Even if these compounds were chromatographically separated, ambiguity remained in its quantification value because the level of Adiol-3S was much lower than that of IS used. Also the matrix effect should be considered in case of using IS having a different retention time (t_R).

In this paper we describe the simultaneous determination method of Adiol-3S and DHEA-S using [7,7,16,16,17-$^2H_5$]Adiol-3S (IS_1) and [7,7,16,16-$^2H_4$]DHEA-S (IS_2) as the appropriate ISs, and using LC–ion trap-MS with electrospray ionization (ESI) in the negative-ion mode, which is useful for the detection of anionic compounds such as sulfates.

2. Experimental

2.1. Materials and reagents

DHEA-S and IS_2 were prepared from DHEA (Tokyo Kasei Kogyo, Tokyo, Japan) and [7,7,16,16-$^2H_4$]DHEA [21,22], respectively, with chlorosulfonic acid–pyridine complex. Adiol-3S and IS_1 were prepared by reduction of DHEA-S using $NaBH_4$ and that of IS_2 using NaB^2H_4, respectively. Adiol-17S was synthesized from DHEA acetate with reduction followed by sulfation and alkaline hydrolysis of the 3-acetate. These structures were confirmed by LC–ESI-MS, and [$^2H_{0–4}$] and [$^2H_{0–3}$] contents of IS_1 and IS_2, respectively, were determined to be less than 0.5%. The standard solutions of mixture containing Adiol-3S and Adiol-17S (100 and 20 ng/ml in ethanol, respectively), and DHEA-S (0.5 or 2 μg/ml in ethanol) together with IS solutions (IS_1, 1 μg/ml in ethanol; IS_2, 10 μg/ml in ethanol) were prepared in the usual manner.

Oasis HLB cartridges (60 mg, 3 ml) were purchased from Waters Co. (Milford, MA, USA), and successively conditioned with methanol (2 ml) and water (2 ml) prior to use. All other reagents were analytical grade and commercially available.

2.2. Serum sample

Sera of patients with prostate cancer ($n = 19$; 62–88 years old) or with benign prostatic hypertrophy (BPH) ($n = 7$; 53–74 years old), who were not under hormone therapy, and those of healthy male volunteers ($n = 14$; 28–63 years old) were collected by our laboratories (the former: Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduated School of Medical Sciences; the latter: Teikoku Hormone Medical Research Center Co. Ltd.). These samples were obtained with the informed consent of patients or volunteers. Charcoal-stripped human plasma, which was used as a blank sample, was prepared from fresh and frozen plasma (the Japan Red Cross Service; Tokyo) by shaking with activated charcoal (48 h, room temperature) followed by centrifugation (1500 × *g*, 30 min, room temperature) and filtration (0.22 μm, cellulose acetate).

2.3. LC–MS (–MS)

An LC–MS system which consisted of a PU-980 chromatograph (JASCO, Tokyo) coupled with a LCQ ion trap-mass spectrometer (ThermoQuest, San Jose, CA, USA) was operated with ESI in the negative-ion mode. A semi-micro column, Develosil ODS-HG-5 (5 μm; 150 mm × 2.0 mm i.d.) (Nomura Chemical, Seto, Japan), was used at a flow rate of 0.15 ml/min at 40 °C and acetonitrile–5 mM HCO_2NH_4 (3:7) was used as the mobile phase. The ionization conditions were as follows: ion source voltage, 5 kV; capillary temperature, 280 °C; capillary voltage, −12 V; sheath

gas flow rate, 80 U; auxiliary gas flow rate, 20 U; tube lens offset voltage, −20 V. For the MS–MS, helium was used as the collision gas and relative collision energy was set at 5%. Precursor ions were as follows: *m/z* 369 and 374 (for Adiol-S and IS_1) (3.0–7.5 min) and *m/z* 367 and 371 (for DHEA-S and IS_2) (7.5–12 min). Monitoring ions were as follows: *m/z* 369 (Adiol-S), 372-375 (IS_1), 367 (DHEA-S) and 369-372 (IS_2).

2.4. Pretreatment procedure

IS_1 (20 ng/20 μl) and IS_2 (200 ng/20 μl) were pipetted into a borosilicate glass tube and the solvent was evaporated under a N_2 gas stream. Human serum (100 μl) was added to the tube, vortex-mixed for 10 s and then kept at room temperature for 10 min. Acetonitrile (250 μl) was added into the serum and vortex-mixed for 10 s. To remove the denatured protein, the suspension was centrifuged for 10 min at 1500 × *g*, and the supernatant was obtained. The precipitate was re-suspended with acetonitrile (250 μl), followed by centrifugation, the supernatant was combined with the above supernatant and then evaporated under a N_2 stream. The residue was dissolved with phosphate buffer (0.05 M, pH 7.4) (0.5 ml) and applied to a solid-phase extraction cartridge containing hydrophilic–lipophilic reversed-phase sorbent, Oasis HLB cartridge. The cartridge was washed with H_2O (2 ml) and then the non-conjugated steroids were removed with ethyl acetate (1 ml). The sulfates were eluted with methanol (1.5 ml) and evaporated under a N_2 gas stream. The obtained residue was re-dissolved with methanol (0.3 ml) and hexane (0.45 ml) was added and then vortex-mixed. After the hexane layer was removed, solvent was evaporated under a N_2 gas stream. The obtained residue was taken up with acetonitrile–5 mM HCO_2NH_4 (3:7) (30 μl), an aliquot of which was injected into the LC–MS.

2.5. Absolute recovery

Adiol-3S (5, 20 ng per tube), Adiol-17S (1, 4 ng per tube) and DHEA-S (100, 200 ng per tube) were added to the first set of blank plasma (100 μl), then treated the same as previously described and after that, IS_1 and IS_2 (20 and 200 ng, respectively) were added as the external standards. The second set of blank plasma (100 μl) was subjected to the pretreatment and after that, the same amount of Adiol-S, DHEA-S and ISs were added. To the third set of blank plasma (100 μl), IS_1 and IS_2 (20 and 200 ng, respectively) were added and subjected to the same pretreatment procedure, and then Adiol-3S (20 ng) and DHEA-S (200 ng) were added as the external standards. All sets of samples were dually prepared and analyzed with LC–MS. The absolute recoveries of Adiol-3S, Adiol-17S and DHEA-S were calculated by dividing the peak area ratios (Adiol-3S/IS_1, Adiol-17S/IS_1 and DHEA-S/IS_2) obtained from samples of the first set with those of the second set. The absolute recoveries of IS_1 and IS_2 were calculated by dividing the peak area ratios (IS_1/Adiol-3S, IS_2/DHEA-S) obtained from sample of the third set with those of the second set. The recovery rates are shown as the mean values.

2.6. Linearity and calibration curves

IS_1 (20 ng), IS_2 (200 ng) and graduated amounts of standard solutions (Adiol-3S, 2.5–40 ng; Adiol-17S, 0.4–8 ng; DHEA-S, 25–400 ng) were pipetted into the tubes and the solvent was evaporated under a N_2 gas stream. Human serum (100 μl) was added to the residue and then analyzed with LC–MS after pretreatment the same as described earlier. The peak area ratios (Adiol-3S/IS_1, Adiol-17S/IS_1 and DHEA-S/IS_2) (*y*) were plotted versus the amounts of sulfates (ng per tube) (*x*) to construct the regression lines.

The same amount of ISs and standard solution (Adiol-3S, 1–40 ng; Adiol-17S, 0.4–8 ng; DHEA-S, 5–800 ng) were pipetted into the tubes and the solvent was evaporated under a N_2 gas stream. The obtained residue was taken up with MeCN–5 mM HCO_2NH_4 (3:7) (30 μl), an aliquot of which was injected into the LC–MS. The peak area ratios were plotted versus the amount of sulfates, and the obtained regression lines were used as the calibration curves.

2.7. Analytical recovery test

The known amounts of Adiol-3S (0, 2, 4, 8 ng), DHEA-S (0, 100, 200 ng) and ISs (IS_1, 20 ng; IS_2, 200 ng) in solution were pipetted into a tube and human serum (100 μl) was added. After being left at room temperature for 10 min, the resulting samples were pretreated the same as the described method and determined by LC–MS using the calibration curves

described earlier. The concentrations of analyte found in sera were defined as F, and the analytical recoveries were calculated as follows.

$$\text{Analytical recovery (\%)} = \frac{F\ (\text{in added serum}) - F\ (\text{in non-added serum})}{\text{amount of added steroid}} \times 100$$

3. Results and discussion

3.1. LC–MS

A methanol–5 mM HCO_2NH_4 (2:3) was used initially to analyze Adiol-S and DHEA-S on a Develosil ODS-5-HG column, but gave poor results for the separation of Adiol-3S and Adiol-17S. The satisfactory separation of these isomers and DHEA-S was obtained by using acetonitrile–5 mM HCO_2NH_4 (3:7) (t_R: Adiol-17S, 4.7 min; Adiol-3S, 5.5 min; DHEA-S, 9.2 min) (Fig. 2a). These compounds were also separated from other steroid sulfates whose molecular weights were the same as Adiol-S or DHEA-S, such as androsterone sulfate (13.1 min), epiandrosterone sulfate (10.9 min) and epiethiocholanolone sulfate (10.6 min), ethiocholanolone sulfate (14.1 min), DHT sulfate (9.5 min), testosterone sulfate (6.3 min).

Negative-ion ESI-mass spectra of Adiol-S, DHEA-S, IS_1 and IS_2 showed the $[M–H]^-$ (*m/z* 369, 367, 374 and 371, respectively); other ions were not detected. However, there was a case that the base ion peak shifted 1 mass unit higher than the $[M–H]^-$, and this phenomenon was frequently observed when a large quantity of steroid (>50 ng) was injected into the ion trap-MS. It was speculated that the phenomenon was caused by ion saturation in the trap, and the detection using MS was not appropriate for the determination of these androgen sulfates. Then we attempted MS–MS analysis using $[M–H]^-$ as the precursor ions. When the relative collision energy was higher than 20%, some product ions were detected, but these were not distinctive ions and the intensities were not high enough to use as the monitoring ions. Using 5–15% of the relative collision energy, $[M–H]^-$ ions, which were the residual ions without fragmentation, were the only ions that appeared. However, when the MS–MS mode with 5% of the relative collision energy and these residual ions were used as the monitoring ions, the noise ions were reduced without decreasing the intensities of the monitoring ion. In addition, this ion did not shift even if a large quantity of steroid was injected. Therefore, this mode using low collision energy was used for the determination of these androgen sulfates.

Since the deuterated ISs were used, it was important to ascertain whether deuterium–hydrogen exchange occurred during pretreatment and the ionization stage. Although the completely exchanged ions ($[^2H_0]$Adiol-3S, *m/z* 369; $[^2H_0]$DHEA-S, *m/z* 367) were not observed, the ISs produced $[M–H–1]^-$ (ca. 20%) or $[M–H–2]^-$ (ca. 15%) and the intensity of the monitoring ion $[M–H]^-$ was reduced.

Based on these results, the mass chromatographic conditions were set as follows: precursor ion, *m/z* 369, 374, 367 and 371; monitoring ion, *m/z* 369, 371-375, 367 and 369–372 (for Adiol-S, IS_1, DHEA-S and IS_2, respectively); relative collision energy, 5%. Using this system, the limits of detection (signal/noise (S/N) = 5) of authentic Adiol-3S and Adiol-17S were 0.25 and 0.2 ng per injection, respectively, and the S/N of DHEA-S at 1 ng per injection was higher than 15.

3.2. Pretreatment of serum sample

Human serum (100 μl) was deproteinized with acetonitrile and purified with a solid-phase extraction cartridge containing hydrophilic–lipophilic reversed-phase sorbent, an Oasis HLB cartridge. The treatment of the cartridge followed by washing with hexane was useful for removing endogenous lipophilic substances including non-conjugated androgens. The representative mass chromatograms are shown in Fig. 2b. The peaks corresponding to Adiol-17S, Adiol-3S and DHEA-S were observed and their structures were confirmed by comparison with authentic samples in their chromatographic behavior and mass spectrum data. None of the interfering peaks with ISs were observed as shown in Fig. 2c.

Since Adiol-S and DHEA-S were endogenous androgen sulfates in human serum, charcoal-stripped plasma was used as a blank sample, and the absolute recovery rate of Adiol-S, DHEA-S and ISs extracted from standard added blank plasma were examined. At first, the blank plasma, which were added the known

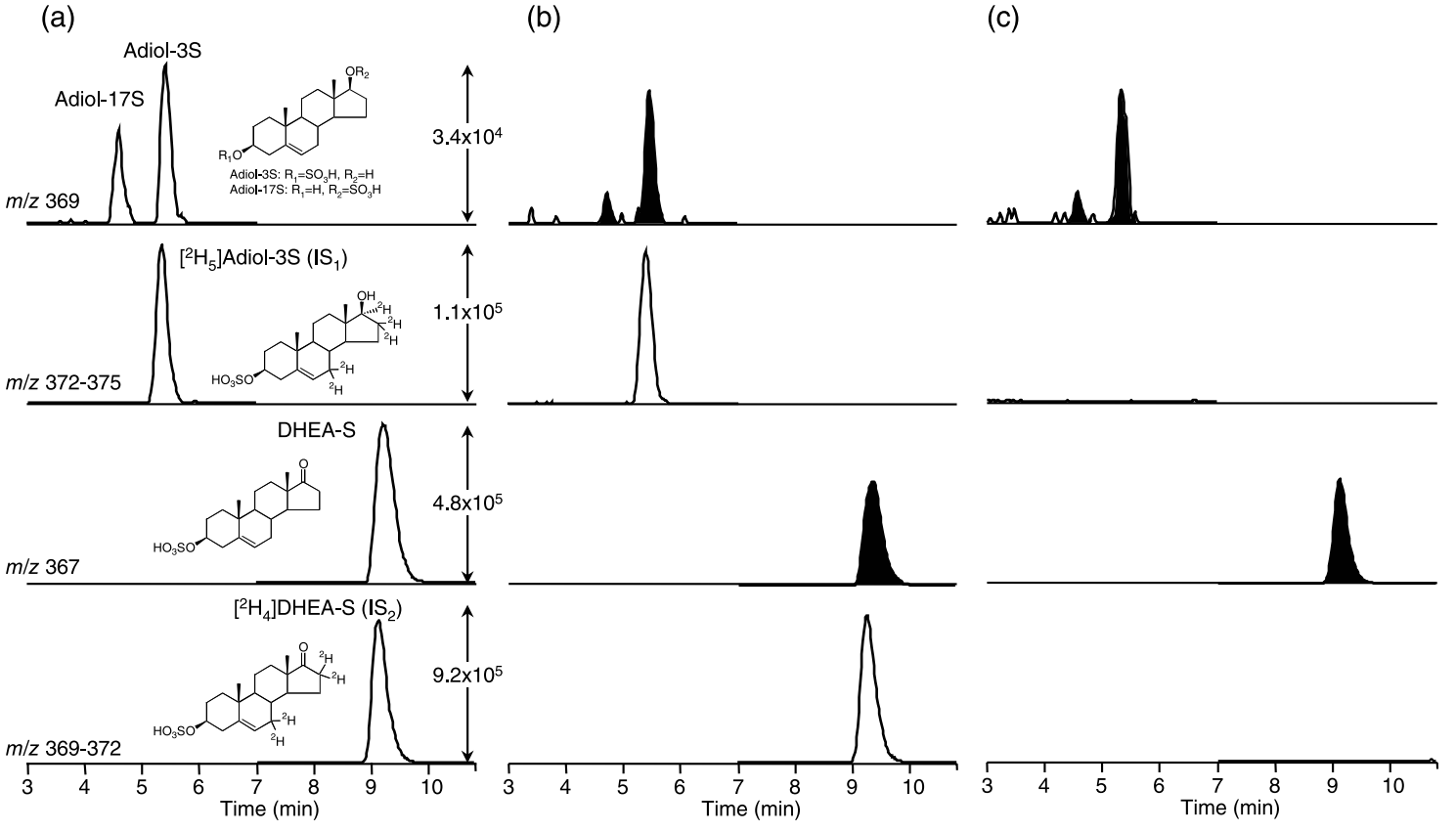

Fig. 2. Representative LC–ESI-MS chromatograms of Adiol-S, DHEA-S and ISs. (a) Authentic samples: Adiol-17S (0.5 ng), Adiol-3S (2.5 ng), DHEA-S (25 ng), IS_1 (5 ng), IS_2 (50 ng); (b) extract from human serum spiked with ISs; and (c) extract from human serum.

amount of these androgen sulfate previously, were pretreated and analyzed as described as earlier, and the obtained peak are compared to that of the same amount of authentic samples directly analyzed by LC–MS. However, the recovery rates exceed 100%. It was speculated that the discrepancy was caused as a consequence of so-called "matrix-effect"; that is, ionization efficiency is often affected by the matrix containing analyte, and enhancement or suppression of ionization occurs especially at using ESI-MS. To overcome this problem, the absolute recovery rates of the androgen sulfates and ISs were determined as follows. The peak area ratios of the analytes, which were added to the blank plasma and the extracted, were divided with the peak are ratios of the analyte, which were added to the pretreated blank plasma. Mean of recovery rates of every steroid sulfate was satisfactory: Adiol-3S, 83.8% (5 ng), 88.5% (20 ng); Adiol-17S, 84.7% (1 ng), 84.2% (4 ng); DHEA-S, 86.3% (100 ng), 90.9% (200 ng); IS_1, 83.7% (20 ng); IS_2, 85.3% (200 ng) ($n = 2$).

3.3. Linearity and calibration curves

In order to assess the linearity, graduated amounts of Adiol-3S, Adiol-17S and DHEA-S containing ISs were added to four different human sera and analyzed by LC–MS after the pretreatment. The regression lines showed satisfactory linearity with a regression coefficient values (r^2) of greater than 0.99; the reproducible slopes were obtained as shown in Table 1.

Although the calibration curves should be drawn with the blank samples in order to avoid matrix effect, we examined the following calibration curves for convenience. Five sets of the graduated amount of standard solution containing ISs were individually measured and the regression lines were constructed. The lines obtained using a standard solution of Adiol-3S (1–40 ng per tube) and Adiol-17S (0.4–8 ng per tube) showed good linearity ($r^2 > 0.99$) and the slopes were reproducible. Because the concentration of DHEA-S was expected to be a wide range, we constructed the regression lines in two stages; low concentration (5–50 ng per tube) and high concentration (50–800 ng per tube). The slopes of these lines were different, however, these were reproducible and both lines showed satisfactory linearity. Concerning Adiol-3S and DHEA-S (50–800 ng per tube), there were no significant differences between the slopes of the lines obtained from standard solution and those obtained from standard-added human serum. Based on these data and the fact that the standard solutions did not require the pretreatment steps, the calibration curves were constructed using the standard solutions without biological matrix in the following studies. However the slope of the regression line obtained from serum spiked with Adiol-17S tended to be lower than the slope of the line obtained from standard Adiol-17S. These data suggested that the calibration curve of Adiol-17S should be constructed using the sample with same matrix or using deuterated Adiol-17S as an IS. But in our preliminary experiments, the levels of Adiol-17S in several human sera (especially from old persons and patients) were nearly or less than the detection limit (4 ng/ml). Therefore, the determination of Adiol-17S has not been done and left as a future work.

Table 1
Slopes and intercepts of regression lines

Steroid	Matrix	Range (ng per tube)	Slope[a]	Intercept[a]	n
Adiol-3S	Serum	2.5–40[b]	0.0460 ± 0.0044		4
	Standard solution	1–40	0.0429 ± 0.0014	−0.0445 ± 0.0184	5
DHEA-S	Serum	25–400[b]	0.0027 ± 0.0002		4
	Standard solution	50–800	0.0025 ± 0.0001	0.0850 ± 0.0153	5
	Standard solution	5–50	0.0039	−0.0030	2
Adiol-17S	Serum	0.4–8[b]	0.0925 ± 0.0097		4
	Standard solution	0.4–8	0.1125 ± 0.0144	−0.0305 ± 0.0167	5

[a] Mean (±S.D.).
[b] These ranges of standard compounds were added to the serum (100 μl).

Table 2
Reproducibility of the determination of Adiol-3S and DHEA-S

Steroid	Sample	Concentration[a]	
		Intra-assay[b]	Inter-assay[c]
Adiol-3S	Serum 1	47.8 ± 4.5 (9.37)	
	Serum 2	56.2 ± 2.9 (5.18)	55.8 ± 2.2 (3.96)
DHEA-S	Serum 1	1.425 ± 0.039 (2.74)	
	Serum 2	1.518 ± 0.085 (5.54)	1.478 ± 0.049 (3.32)

[a] Adiol-3S (ng/ml), DHEA-S (μg/ml), mean ± S.D. (R.S.D.%).
[b] $n = 5$.
[c] $n = 4$.

3.4. Reproducibility and accuracy

The relative standard deviation (R.S.D.) values of intra-assay determined in serum were lower than 10%, and satisfactory R.S.D. values of inter-assay (<4%) were obtained using two different sera as shown in Table 2. The analytical recoveries, which were obtained from two different sera spiked with known amounts of Adiol-3S and DHEA-S, were also

Table 3
Analytical recoveries of Adiol-3S and DHEA-S

Steroid	Sample	Concentration[a]		Recovery (%)[b]
		Added	Found	
Adiol-3S	Serum 3	0	41.6	–
		20	59.6	90.0
		40	81.0	98.5
	Serum 4	0	94.2	–
		40	135.5	103.2
		80	171.7	96.8
DHEA-S	Serum 3	0	1.660	–
		1.0	2.714	105.4
		2.0	3.635	98.7
	Serum 4	0	2.102	–
		1.0	3.105	100.4
		2.0	4.002	93.1

[a] Adiol-3S (ng/ml), DHEA-S (μg/ml), mean ($n = 2$).
[b] The analytical recoveries were calculated as follows: analytical recovery (%) = 100[F (in added serum) − F (in non-added serum)]/amount of added steroid, F: the concentration found in serum.

Table 4
Concentrations of Adiol-3S and DHEA-S in human serum

Healthy males				Prostate cancer				BPH			
Entry	Age (*y*)	Adiol-3S (ng/ml)	DHEA-S (μg/ml)	Entry	Age (*y*)	Adiol-3S (ng/ml)	DHEA-S (μg/ml)	Entry	Age (*y*)	Adiol-3S (ng/ml)	DHEA-S (μg/ml)
1	28	83.0	2.945	15	62	36.3	0.497	34	53	31.3	1.370
2	28	176.9	3.379	16	62	15.7	0.362	35	61	41.5	0.937
3	30	133.0	2.422	17	64	22.1	0.429	36	63	45.6	0.719
4	32	164.3	2.483	18	64	78.6	1.349	37	64	27.7	0.486
5	33	228.4	5.160	19	65	15.3	0.309	38	70	ND	ND
6	47	245.3	4.812	20	68	ND[a]	0.110	39	71	41.5	0.937
7	48	182.9	2.806	21	70	54.7	1.066	40	74	41.4	1.094
8	52	87.8	2.286	22	71	54.4	0.236				
9	57	36.1	1.487	23	71	ND	0.150				
10	58	79.1	2.160	24	72	29.81	0.369				
11	59	38.8	0.758	25	73	ND	0.111				
12	62	19.2	0.175	26	73	35.6	0.885				
13	63	76.7	2.183	27	75	ND	0.449				
14	63	74.8	2.135	28	83	39.6	0.697				
				29	88	18.3	0.248				
				30	UK[b]	67.0	1.178				
				31	UK	52.1	1.228				
				32	UK	182.7	2.421				
				33	UK	69.3	1.696				

[a] ND, not detectable.
[b] UK, unknown.

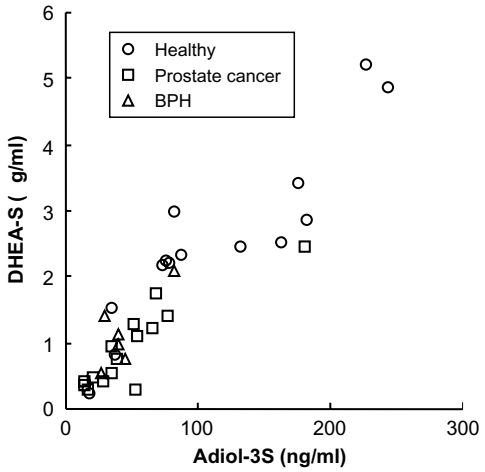

Fig. 3. Correlation between the concentrations of Adiol-3S and that of DHEA-S in human sera.

satisfactory (90–106%) (Table 3). These data show that the present method is highly reproducible and accurate.

3.5. Determination of Adiol-3S and DHEA-S in sera of healthy volunteers and patients with prostatic disease

The proposed method was applied to the determination of Adiol-3S and DHEA-S in the sera from healthy subjects ($n = 14$), patients with prostate cancer ($n = 19$) and patients with BPH ($n = 7$); the results are listed in Table 4 and Fig. 3. The serum concentrations of both Adiol-3S and DHEA-S in healthy subjects tended to decrease during aging and correlated with each other (Fig. 3). These results coincide with the previous reports [12–14]. Although both compounds in the sera of patients were shown to be a relatively low value, these were comparable with the serum levels in healthy males of their age. The relationship between these levels and prostatic disease has not been clarified owing to a shortage of sample numbers of healthy aged persons.

4. Conclusion

We developed a method for simultaneous determination of Adiol-3S and DHEA-S in human serum using LC–ESI-MS and appropriate deuterated internal standards. The proposed method does not require the chemical or enzymatic hydrolysis, or purification with HPLC, and proved to be satisfactory with respect to selectivity, accuracy and reproducibility. Using the method, we measured the serum concentration of Adiol-3S and DHEA-S in healthy male and patients with prostatic disease. Although additional investigation using more samples is necessary to make reference to the relation between the levels of these compounds and pathology of prostatic diseases, the practicability of the developed method for clinical research is demonstrated.

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