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Determination of anabolic steroids in human urine by automated in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry

Keita Saito, Katsuharu Yagi, Atsushi Ishizaki, Hiroyuki Kataoka*

School of Pharmacy, Shujitsu University, Nishigawara, Okayama 703-8516, Japan

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ABSTRACT

A simple, rapid and sensitive method was developed for determining the presence of seven anabolic steroids (boldenone, nandrolone, testosterone, methyltestosterone, epiandrosterone, androsterone, and atnozolol) in human urine. Glucuronide-conjugates of these compounds were hydrolyzed with β-glucuronidase. The anabolic steroids were analyzed by on-line in-tube solid-phase microextraction (SPME) coupled with liquid chromatography–mass spectrometry (LC–MS). The steroids were separated within 14 min by high performance liquid chromatography using a Chromolith RP-18e column and 5 mM ammonium formate/methanol (35/65, v/v) as a mobile phase at a flow rate of 1.0 mL/min. Electrospray ionization conditions in the positive ion mode were optimized for the MS detection of these compounds. The optimum in-tube SPME conditions were 20 draw/eject cycles with a sample size of 40 μL using a Supel-Q PLOT capillary column for the extraction. The extracted compounds could be desorbed readily from the capillary column by flow of the mobile phase, and no carryover was observed. Using the in-tube SPME LC–MS with SIM mode detection, good linearity of the calibration curve ($r > 0.995$) was obtained in the concentration range of 0.5–20 ng/mL, except for stanozolol. The detection limits (S/N = 3) of anabolic steroids were in the range 9–182 pg/mL and the proposed method showed 20–33-fold higher sensitivity than the direct injection method. The within-day and between-day precisions were below 4.0% and 7.3% ($n = 5$), respectively. This method was applied successfully to the analysis of urine samples without the interference peaks. The recovery rates of anabolic steroids spiked into urine samples were above 85%. This method is useful to analyze the urinary levels of these compounds in anti-doping tests.

1. Introduction

The misuse of drugs to enhance performance in human and animal sports, usually referred to as doping, is unfortunately widespread and has a long history. This unacceptable practice violates the spirit of fair play in sports, affects medical ethics and potentially puts the health of the athlete at risk. In particular, synthetic anabolic steroids, structurally related to testosterone, belong to a pharmacological group that has a great impact on sport due to its use in doping, and have been used to enhance anabolic effects, such as improving skeletal muscle performance and recovery by controlling catabolism after stress [1–3]. Other features of these compounds, often referred to as side effects, are their androgenic effects, such as cardiovascular and hepatic disorders. Although the use of anabolic steroids by athletes has been prohibited since 1976, doping using these substances remains a problem for sporting authorities. Therefore, the control of anabolic steroid abuse is a demanding task, and requires high speed, high sensitivity, and specific analytical methods [3–5].

Doping control analyses of anabolic steroids have been mainly carried out on urine because in general it contains relatively high concentrations of the drugs and/or their metabolites. Testing for anabolic steroids in urine samples is mainly carried out by gas chromatography with mass spectrometry (GC–MS) [5–11] and liquid chromatography with tandem mass spectrometry (LC–MS–MS) [12–17]. Although the GC–MS methods are robust and sensitive, they always require a laborious derivatization step and therefore sample throughput is quite low with long turn-around times. However, LC–MS–MS methods can provide a sensitive and selective way of comprehensively measuring anabolic steroid concentrations. Gas or liquid chromatography coupled with mass spectrometry has produced accurate and sensitive assays, but chromatographic separations require time. To avoid such tedious and lengthy procedures, vacuum matrix-assisted laser desorption ionization coupled with the linear ion trap mass spectrometry technique has been tested for its applicability as a rapid screening technique [18]. However, most

* Corresponding author. Tel.: +81 86 271 8342; fax: +81 86 271 8342.
E-mail address: hkataoka@shujitsu.ac.jp (H. Kataoka).

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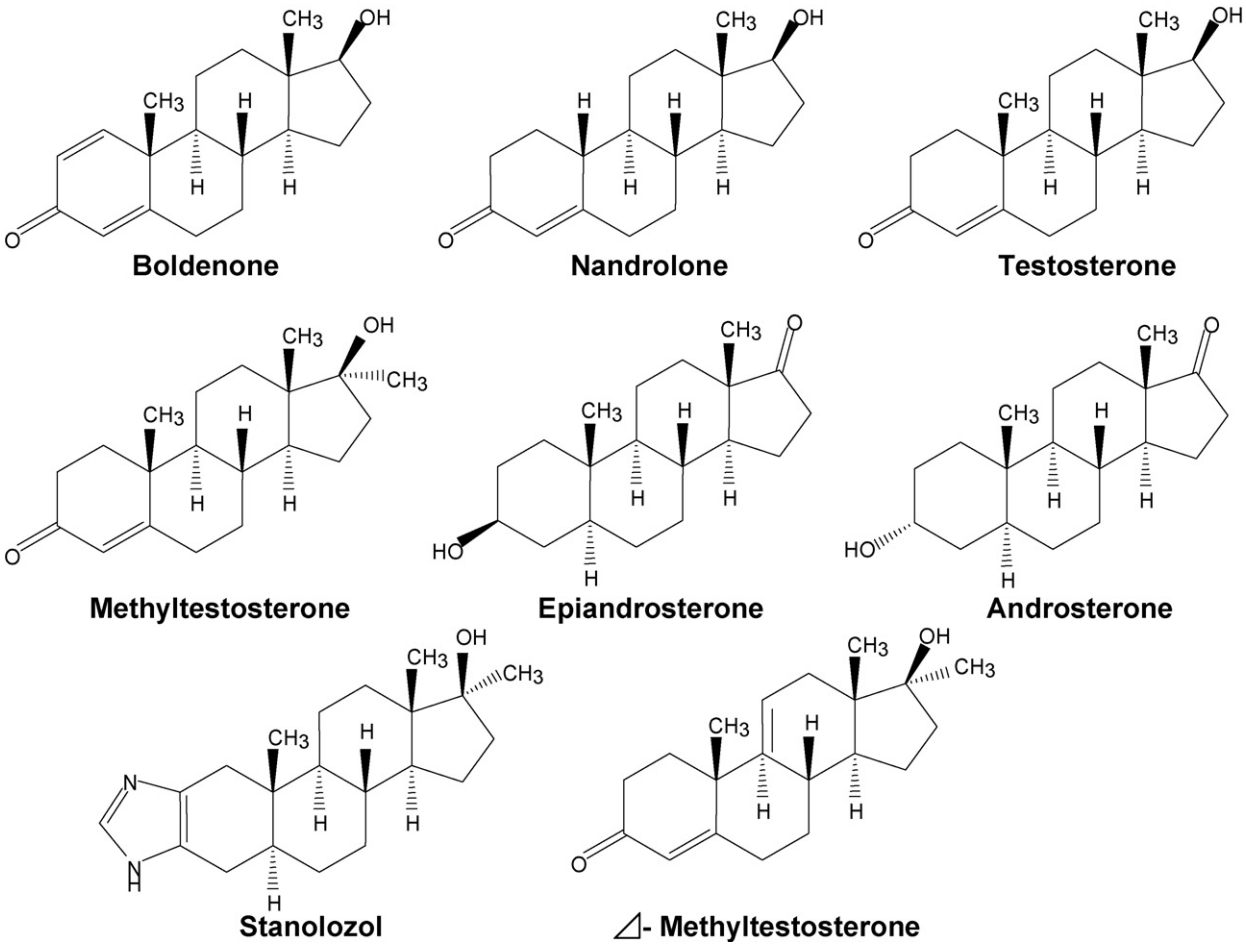

Fig. 1. Structures of anabolic steroids.

of the above methods generally require time-consuming sample preparation procedures, such as liquid–liquid extraction or solid-phase extraction, to remove coexisting substances in urine samples prior to analysis.

In-tube solid-phase microextraction (SPME), using an open tubular fused-silica capillary with an inner surface coating as the SPME device, is simple and can be coupled easily on-line with HPLC and LC–MS. In-tube SPME allows convenient automation of the extraction process, which not only reduces analysis time, but also provides better precision and sensitivity than manual off-line techniques. We recently developed an in-tube SPME method for the determination of urinary drugs [19], cortisol [20], and nicotine and cotinine [21] by coupling the methods with LC–MS. The details of the in-tube SPME technique and its applications have also been summarized in a number of reviews [22–25]. Here we report an automated on-line in-tube SPME LC–MS method for the simultaneous determination of anabolic steroids in urine samples.

2. Experimental

2.1. Materials

Boldenone, nandrolone, testosterone, methyltestosterone, epiandrosterone, androsterone, and stanozolol were purchased from Sigma–Aldrich, Japan (Tokyo, Japan). Δ-Methyltestosterone as an internal standard (IS) was purchased from Sigma–Aldrich. The structures of these compounds are shown in Fig. 1. Each compound was dissolved in methanol to make a stock solution at a concentration of 1 mg/mL. Each solution was stored at 4 °C and diluted to the required concentrations with pure water prior to use. The β-glucuronidase (*E. coli*, type IX-A) was purchased from Sigma–Aldrich, and dissolved in 0.1 M potassium phosphate buffer (pH 7.0). LC–MS grade methanol and distilled water used as mobile phases were purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were of analytical grade.

2.2. Instrument and analytical conditions

A Model 1100 series LC-MSD system (Agilent Technologies, Boeblingen, Germany) was used. It consisted of a binary pump, an on-line degasser, an autosampler, a column compartment, an atmospheric pressure electrospray ionization (ESI) MS, and an HP ChemStation. A Chromolith RP-18e column (150 mm × 4.6 mm i.d.) from Merck, Japan (Tokyo, Japan) was used for LC separation. LC conditions were as follows: column temperature, 30 °C; mobile phase, 5 mM ammonium formate/methanol (35/65, v/v); and flow rate, 1.0 mL/min (during the in-tube SPME treatment, the flow rate was set to 0.2 mL/min to save mobile phase solution). ESI-MS conditions were as follows: nebulizer gas, N_2 (50 psi); drying gas, N_2 (11 L/min, 350 °C); fragmenter voltage, 120 V; capillary voltage, 2000 V; ionization mode, positive mode; mass scan range, 100–400 amu; selected ion monitoring (SIM), *m*/*z* 287 (boldenone), *m*/*z* 275 (nandrolone), *m*/*z* 289 (testosterone), *m*/*z* 303 (methyltestosterone), *m*/*z* 308 (epiandrosterone and androsterone), *m*/*z* 329 (stanozolol), and *m*/*z* 301 (Δ-methyltestosterone); and SIM ion dwell times, 144 ms. LC–MS data were processed using an HP ChemStation (Hewlett-Packard, Palo Alto, CA, USA).

Table 1
Program for in-tube SPME process.

Sequence	Event	Switching valve	Vial	Draw/ejection		
				Cycle[a]	Volume (μL)	Speed (μL/min)
1	Conditioning of the capillary	Load	MeOH	D/E (2)	40	200
2	Drawing of air into the capillary	Load	Empty	D (1)	50	200
3	Conditioning of the capillary	Load	Water	D/E (2)	40	200
4	Extraction of analytes into the capillary	Load	Sample	D/E (20)	40	150
5	Needle washing	Load	MeOH	D/E (1)	2	200
6	Desorption of analytes from the capillary	Inject	–	–	–	–
7	HPLC separation of analytes and return to sequence 1	Load	–	–	–	–

[a] D: draw, E: ejection.

2.3. In-tube solid-phase microextraction

A Supel-Q PLOT capillary column (60 cm × 0.32 mm i.d., 17 μm film thickness; Varian Inc., Lake Forest, CA, USA) was used as the in-tube SPME device. The column was placed between the injection loop and injection needle of the autosampler, and the injection loop was retained in the system to avoid fouling of the metering pump. Capillary connections were facilitated by use of a 2.5-cm sleeve of 1/16-in. polyetheretherketone (PEEK) tubing at each end of the capillary (1 in. ≈ 2.54 cm), and 330 μm i.d. PEEK tubing was found to be suitable to accommodate the capillary used. Normal 1/16-in. stainless steel nuts, ferrules, and connectors were then used to complete the connections. The autosampler software was programmed to control the in-tube SPME extraction, desorption, and injection. Vials (2 mL) were filled with 1.0 mL of sample for extraction, and set into the autosampler programmed to control the SPME extraction and desorption technique. In addition, 1.5-mL aliquots of methanol and water in 2-mL autosampler vials with a septum were set on the autosampler. The capillary column was washed and conditioned by 2 repeated draw/eject cycles (40 μL each) of these solvents, and then a 50-μL air plug was drawn prior to the extraction step. The extraction of cortisol onto the capillary coating was performed by 20 repeated draw/eject cycles of 40 μL of sample at a flow rate of 150 μL/min with the six-port valve in the LOAD position. After washing the tip of the injection needle by one draw/eject cycle of 2 μL of methanol, the extracted compounds were desorbed from the capillary coating with mobile phase flow. Then, the compounds were transported to the LC column by switching the six-port valve to the INJECT position, and detected by the MS system in SIM mode. During the analysis, the SPME capillary was washed and conditioned with the mobile phase for the next extraction. The extraction procedure is shown in Table 1. An outline of the in-tube SPME/LC–MS system can be seen in previous papers [22–25].

2.4. Sample preparation

The aim of the experiment was explained to the subjects beforehand and consent was obtained after confirmation that they fully understood the experiment. Urine samples from male healthy volunteers and an anabolic steroid user were collected in glass bottles, and processed immediately or stored at −20 °C until use. Anabolic steroid user took a drug containing methyltestosterone (5 mg), and urine was sampled just before use of drug and after 5 h. For the analysis of free (unconjugated) anabolic steroids, 0.1 mL of urine was added to 0.05 mL of 100 ng/mL internal standard (IS) solution and the total volume was made up to 1.0 mL with distilled water. The mixtures were used for the following in-tube SPME LC–MS analysis. For the analysis of conjugated anabolic steroids, 0.3 mL of urine was hydrolyzed during 90 min at 50 °C after the addition of 0.2 mL of 0.4 M potassium phosphate buffer (pH 7.0) and 0.1 mL of 5 units/mL β-glucuronidase solution according to the previous method [17]. After centrifugation at 3000 × *g* for 1 min, 0.2 mL of the supernatant was performed as described for the free anabolic steroids. A standard mixture was added to control urine samples (which did not include anabolic steroids) at concentrations of 0.5, 1.0, 2.0, 5.0, 10, and 20 ng/mL of each compound, and calibration curves were constructed from the peak height ratios against the IS.

3. Results and discussion

3.1. LC–MS analysis of anabolic steroids

For MS operation, ESI positive ion mode was evaluated for the determination of anabolic steroids. To select the monitoring ion for these compounds, the ESI mass spectra were initially analyzed by LC–MS with direct liquid injection into the column. As shown in Fig. 2, each compound gave a very simple spectrum in scan mode for the mass range *m*/*z* 100–400. Most anabolic steroids had protonated molecules ($[M+H]^+$) as base ions. The ammonium adducts ($[M+NH_4]^+$) were also observed in epiandrosterone and androsterone (*m*/*z* = 308.3). Parameters, including nebulizer gas pressure, drying gas flow rate, fragmenter voltage, and capillary voltage, were optimized by flow injection analysis.

LC separation of anabolic steroids was performed using a Chromolith RP-18e column. As shown in Fig. 3, these compounds were eluted within 14 min using 5 mM ammonium formate/methanol (35/65, v/v) as the mobile phase at a flow rate of 1.0 mL/min. Anabolic steroids could be detected selectively in SIM mode.

3.2. Optimization of in-tube solid-phase microextraction and desorption

To optimize the extraction of anabolic steroids by in-tube SPME, several parameters, such as the stationary phase of the in-tube SPME capillary column and number and volume of draw/eject cycles, were investigated. Extraction efficiency in in-tube SPME was evaluated by comparison of peak height at each condition. Six different capillary columns, CP-Sil 5CB (Varian Inc., Lake Forest, CA, USA, 100% polydimethylsiloxane, 5 μm film thickness), CP-Sil 19CB (Varian, 14% cyanopropyl phenyl methylsiloxane, 1.2 μm film thickness), CP-Wax 52CB (Varian, polyethyleneglycol, 1.2 μm film thickness), and CP-Pora PLOT amine (Varian, basic modified stylene divinylbenzene polymer, 10 μm film thickness), Carboxen 1006 PLOT (Supelco, Bellefonte, PA, USA, carbon molecular sieves, 15 μm film thickness) and Supel-Q PLOT (Supelco, divinylbenzene polymer, 17 μm film thickness) were tested as extraction devices. With in-tube SPME, the amount of analyte extracted into the stationary phase of the capillary column depends on factors such as the surface area, film thickness and polarity of the capillary coatings. As shown in Fig. 4A, the extraction efficiency of the porous polymer-

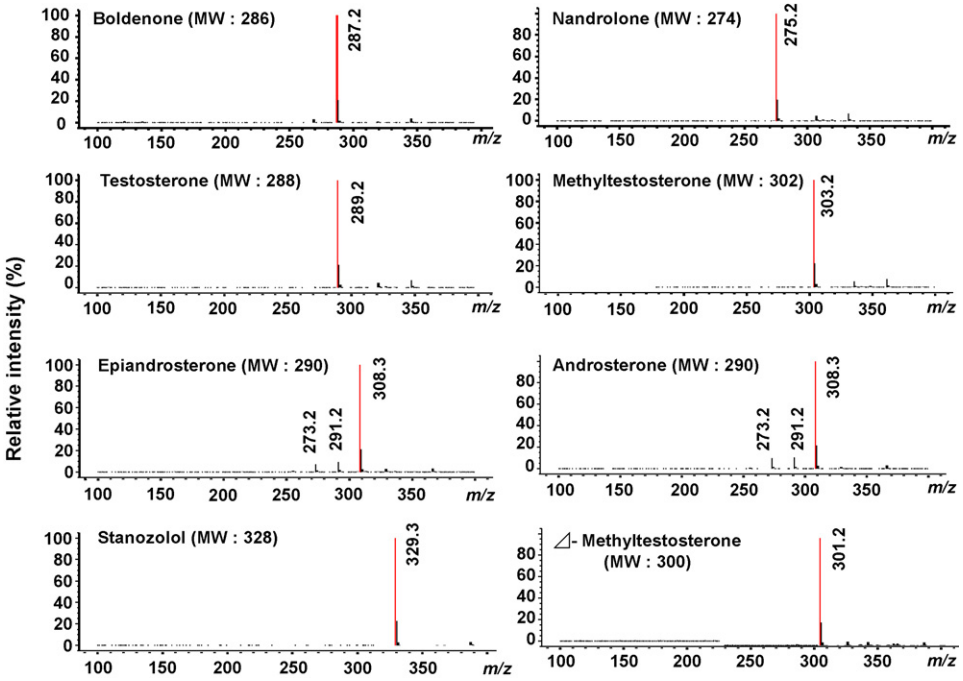

Fig. 2. Mass spectra of anabolic steroids.

type capillary column was higher than those of the other columns. As the PLOT column has a large adsorption surface area and thick film layer, the amount extracted was greater than that with liquid-phase type columns. Among the PLOT columns, a Supel-Q PLOT gave superior extraction efficiency.

With in-tube SPME, the extraction time, flow rate, and sample pH are related to the amount of a compound extracted. To monitor the extraction time profile of anabolic steroids by in-tube SPME, the number of draw/eject cycles was varied from 5 to 25 using a Supel-Q PLOT capillary column. As shown in Fig. 4B, the extraction equilibrium of these compounds was not reached with 25 draw/eject cycles of 40 μL of sample. It is possible to stop extraction even before extraction equilibrium is reached, in order to reduce the analysis time, because quantitative reproducibility is obtained by fixing SPME conditions using an autosampler. Therefore, 20 draw/eject cycles were used in this method. The draw/eject rate in in-tube SPME was tested at 50, 100, 150 and 200 μL/min, but there was little difference in extraction efficiency between these. In the method employed here, a draw/eject rate of 150 μL/min was used as it was the optimal flow rate. The effect of the pH of the sample matrix on the extraction of anabolic steroids was examined using several buffer solutions at pH 3, 4, 5, 6, 7, and 8. However, there was almost no change in extraction efficiency. The absolute amounts of these compounds extracted by the SPME cap-

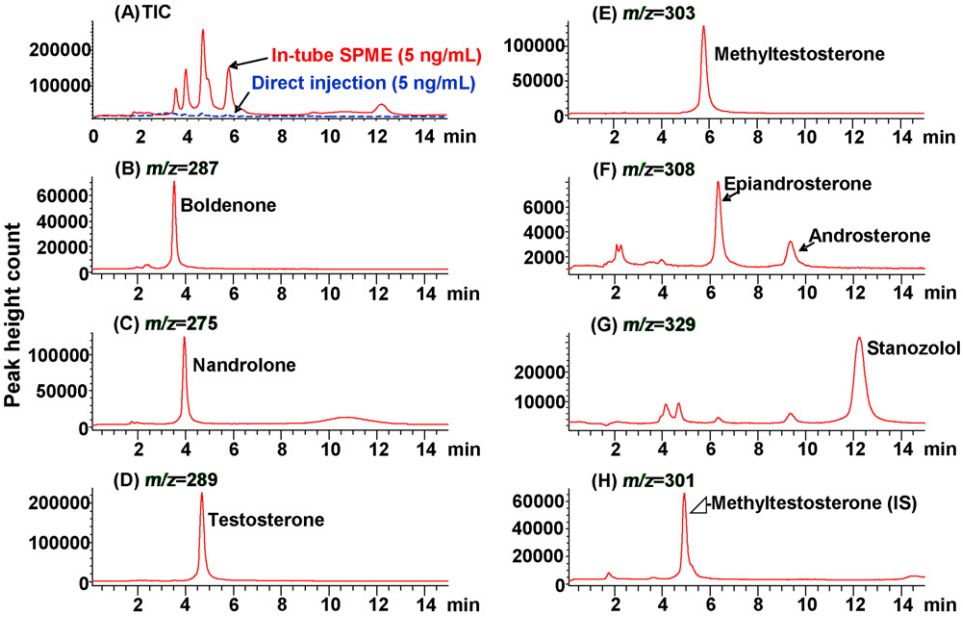

Fig. 3. Typical total ion and selected ion chromatograms obtained from standard anabolic steroids (5 ng/mL of each compound) by direct injection and in-tube SPME/LC–MS in positive ion mode. (A) Total ion chromatogram, (B)–(H) selected ion chromatograms. LC–MS conditions: see Section 2.

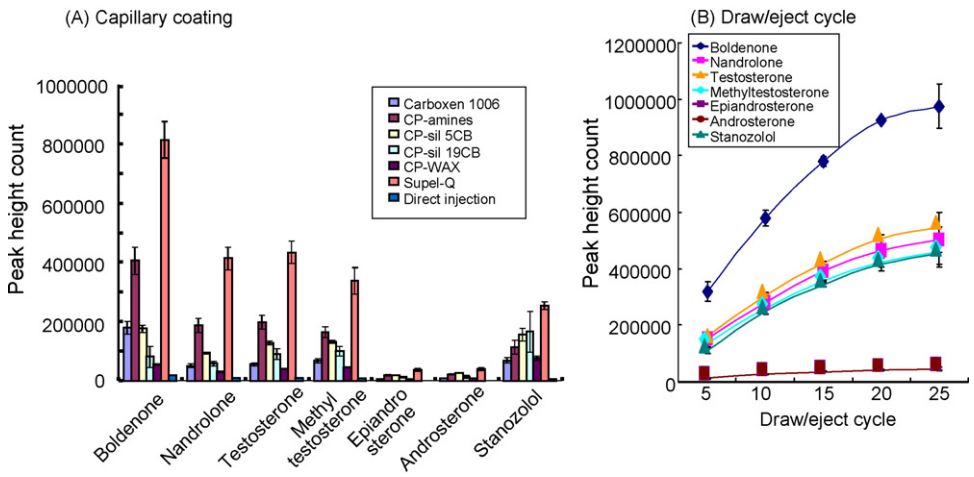

Fig. 4. Effects of (A) capillary coatings and (B) draw/eject cycle on the in-tube SPME of anabolic steroids. These compounds were extracted by draw/eject cycles of 40 μL of standard solution (5 ng/mL of each) at a flow rate of 150 μL/min.

Table 2
Linear regression data, detection limits and within-run and between-day precisions of anabolic steroids by in-tube SPME/LC–MS.

Compound	SIM, *m*/*z*	Regression line[a]		Correlation coefficient	Detection limit (ng/mL)[b]		*D*/*I* ratio[c]	Within-run RSD (%)[d]	Between-day RSD (%)[d]
		Slope	Intercept		Direct injection	In-tube SPME			
Boldenone	287	0.1815	0.0396	0.9997	0.36	0.011	32.7	2.2	6.8
Nandrolone	275	0.2136	0.0266	0.9998	0.23	0.009	25.6	2.2	7.2
Testosterone	289	0.1895	0.0107	0.9999	0.27	0.009	30.0	1.2	6.2
Methyltestosterone	303	0.1651	0.0120	0.9997	0.33	0.012	27.5	1.2	2.3
Epiandrosterone	308	0.0215	−0.0061	0.9995	2.62	0.106	24.7	3.4	5.9
Androsterone	308	0.0117	−0.0034	0.9992	4.72	0.182	25.9	1.4	7.3
Stanozolol	329	0.1190	−0.0244	0.9953	0.51	0.025	20.4	4.0	4.1

[a] Calibration range: 0.5–20 ng/mL, 6-point (n = 18).
[b] S/N = 3.
[c] Sensitivity rate of direct injection method against in-tube SPME method.
[d] n = 5.

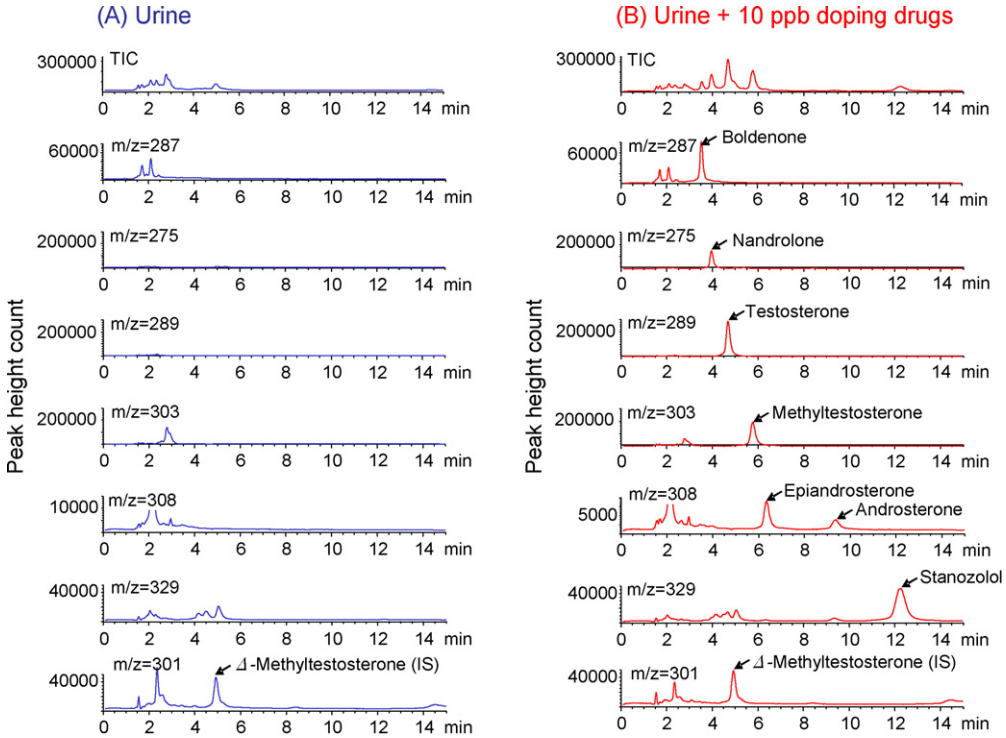

Fig. 5. Chromatograms obtained from urine samples. (A) Non-spiked urine and (B) spiked urine (10 ng/mL of each). For in-tube SPME/LC–MS conditions: see Section 2.

Table 3
Recoveries of anabolic steroids spiked into urine samples.

Compound	Spiked (ng/mL)	Recovery (%)/mean ± SD (n = 3)		Spiked (ng/mL)	Recovery (%)/mean ± SD (n = 3)	
		Average	RSD (%)		Average	RSD (%)
Boldenone	1.0	93.1 ± 1.0	1.1	10	93.6 ± 1.4	1.5
Nandrolone	1.0	85.7 ± 1.5	1.8	10	87.0 ± 3.3	3.8
Testosterone	1.0	99.3 ± 1.8	1.8	10	96.1 ± 0.5	0.5
Methyltestosterone	1.0	103.1 ± 1.8	1.7	10	104.7 ± 0.8	0.8
Epiandrosterone	1.0	112.0 ± 2.5	2.2	10	98.9 ± 3.6	3.6
Androsterone	1.0	92.2 ± 5.2	5.6	10	106.4 ± 2.0	1.9
Stanozolol	1.0	117.3 ± 9.7	8.3	10	99.7 ± 6.2	6.2

illary column were calculated by comparing peak area counts with the corresponding direct injection of the sample solution onto the LC column. At a sample concentration of 10 ng/mL, 2.41 ng (24.1%) of boldenone, 2.39 ng (23.9%) of nandrolone, 2.54 ng (25.4%) of testosterone, 2.51 ng (25.1%) of methyltestosterone, 2.65 ng (26.5%) of epiandrosterone, 2.52 ng (25.2%) of androsterone, and 2.76 ng (27.6%) of stanozolol were extracted onto the Supel-Q PLOT column by in-tube SPME. Although the extraction yields of these compounds were relatively low, they showed good reproducibility due to the autosampler.

The mobile phase was found to be suitable for the desorption of anabolic steroids extracted into the stationary phase of the capillary column. Dynamic desorption of these compounds from the capillary could be achieved readily by switching the six-port valve of LC–MS instrument. The desorbed compounds were transported to the LC column by mobile phase flow.

Air plugging before the extraction step was carried out to prevent not only sample mixing but also the desorption of analyte from the capillary coating by the mobile phase during the ejection step. No carryover was observed because the capillary column was washed and conditioned by draw/eject cycles of methanol and mobile phase prior to extraction. The extraction and desorption of anabolic steroids by the in-tube SPME method were accomplished automatically within 35 min, and automated analysis of about 40 samples per day was possible by overnight operation.

3.3. Sensitivity, linearity, and precision

Detection of anabolic steroids worked very well with ESI-MS. As shown in Table 2, the detection limits of these compounds were in the range 9–182 pg/mL with signal-to-noise ratio of 3:1 under the LC–MS conditions used. The in-tube SPME method was 20–33-fold more sensitive than the direct injection method (10 μL injection), because these compounds were concentrated in the capillary column during draw/eject cycles. Sensitivity of this method was above 10 times higher than that of the LC–MS–MS method reported previously [12–17]. The calibration curves for anabolic steroids were constructed from the peak height counts. As shown in Table 2, a linear relationship was obtained for each compound in the range 0.5–20 ng/mL urine (six-point calibration) and the correlation coefficients were above 0.999, except for with stanozolol. On the other hand, the within-day and between-day precisions (relative standard deviations, RSD) at a concentration of 5 ng/mL were below 4.0% (n = 5) and 7.3% (n = 5), respectively (Table 2).

3.4. Application to the analysis of urine samples

Urine samples could be analyzed directly following sample dilution without any further pretreatment. As shown in Fig. 5, the urine samples from healthy volunteers were analyzed successfully without interference peaks by SIM mode detection. Free and

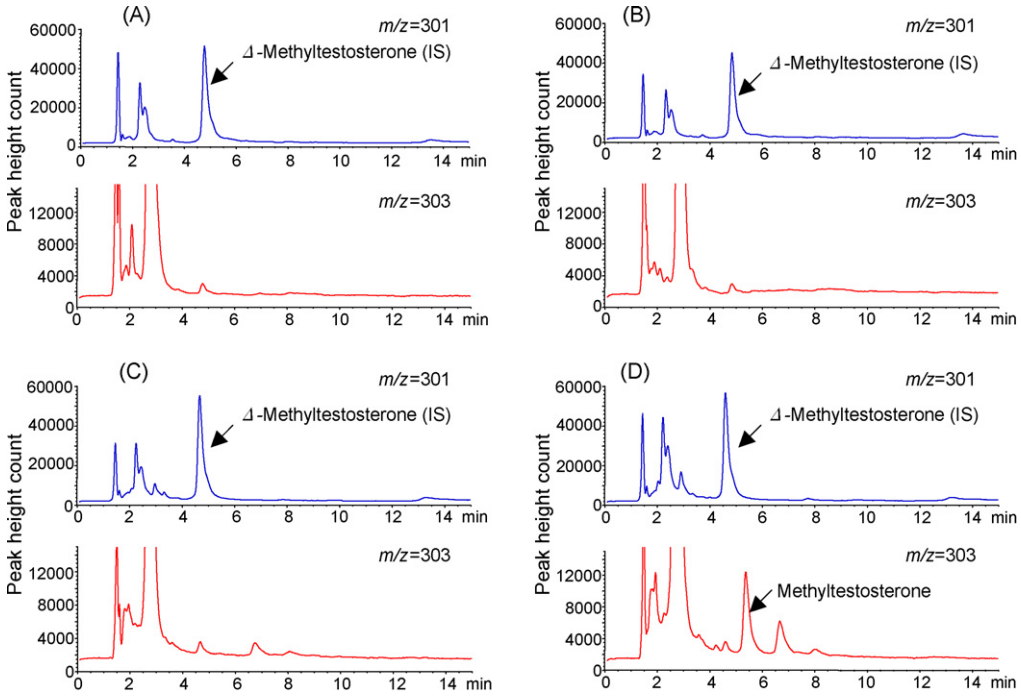

Fig. 6. Chromatograms obtained from urine samples of anabolic steroid user. (A) and (B): before use of drug; (C) and (D): after use of drug; (A) and (C): analysis without β-glucuronidase hydrolysis; (B) and (D): analysis with β-glucuronidase hydrolysis.

glucuronide-conjugates of anabolic steroids were not detected. To confirm the validity of this method, known amounts of anabolic steroids were spiked into 0.1 mL of pooled urine samples, and their recoveries were calculated. As shown in Table 3, the recoveries of these compounds were above 85% and relative standard deviations were below 8.3%. To evaluate the utility of the developed method, we analyzed the urine sample from a male anabolic steroid (methyltestosterone) user. As shown in Fig. 6A and B, free methyltestosterone and its glucuronide-conjugate were not detected in urine sample before use of drug. After use of drug, glucuronide-conjugate of methyltestosterone was detected at the concentration of 10.8 ng/mL in urine sample (Fig. 6D), but free form was not detected (Fig. 6C). Although urinary excretion of glucuronide-conjugate of methyltestosterone is less than 0.1% of dose, it sufficiently reflects doping by use of methyltestosterone. These results suggest that the developed method is applicable and reliable for routine doping control analysis.

4. Conclusions

The on-line in-tube SPME/LC–MS method developed in the present study can continuously perform extraction and concentration of anabolic steroids from urine samples, and then allow analysis by LC–MS. This method is automated, simple, rapid, selective, and sensitive, and can be readily applied to the analysis of urine samples. This method is a useful tool for anti-doping analysis.

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References

[1] A.T. Kicman, D.B. Gower, Anabolic steroids in sport: biochemical, clinical and analytical perspectives, Ann. Clin. Biochem. 40 (2003) 321–356.
[2] L. Politi, A. Groppi, A. Polettini, Applications of liquid chromatography–mass spectrometry in doping control, J. Anal. Toxicol. 29 (2005) 1–14.
[3] O.J. Pozo, P.V. Eenoo, K. Deventer, F.T. Delbeke, Detection and characterization of anabolic steroids in doping analysis by LC–MS, Trends Anal. Chem. 27 (2008) 657–671.
[4] O.J. Pozo, P. Van Eenoo, K. Deventer, F.T. Delbeke, Development and validation of a qualitative screening method for the detection of exogenous anabolic steroids in urine by liquid chromatography–tandem mass spectrometry, Anal. Bioanal. Chem. 389 (2007) 1209–1224.
[5] C.G. Georgakopoulos, A. Vonaparti, M. Stamou, P. Kiousi, E. Lyris, Y.S. Angelis, G. Tsoupras, B. Wuest, M.W. Nielen, I. Panderi, M. Koupparis, Preventive doping control analysis: liquid and gas chromatography time-of-flight mass spectrometry for detection of designer steroids, Rapid Commun. Mass Spectrom. 21 (2007) 2439–2446.
[6] E. Haber, J.A. Muñoz-Guerra, C. Soriano, D. Carreras, C. Rodriguez, F.A. Rodriguez, Automated sample preparation and gas chromatographic-mass spectrometric analysis of urinary androgenic anabolic steroids, J. Chromatogr. B: Biomed. Sci. Appl. 755 (2001) 17–26.
[7] J. Marcos, J.A. Pascual, X. de la Torre, J. Segura, Fast screening of anabolic steroids and other banned doping substances in human urine by gas chromatography/tandem mass spectrometry, J. Mass Spectrom. 37 (2002) 1059–1073.
[8] M. Hebestreit, U. Flenker, G. Fusshöller, H. Geyer, U. Güntner, U. Mareck, T. Piper, M. Thevis, C. Ayotte, W. Schänzer, Determination of the origin of urinary norandrosterone traces by gas chromatography combustion isotope ratio mass spectrometry, Analyst 131 (2006) 1021–1026.
[9] M. Mazzarino, M. Orengia, F. Botrè, Application of fast gas chromatography/mass spectrometry for the rapid screening of synthetic anabolic steroids and other drugs in anti-doping analysis, Rapid Commun. Mass Spectrom. 21 (2007) 4117–4124.
[10] L. Lootens, P. Van Eenoo, P. Meuleman, P.G. Leroux-Roels, G.W. Van Thuyne, F.T. Delbeke, Development and validation of a quantitative gas chromatography–mass spectrometry method for the detection of endogenous androgens in mouse urine, J. Chromatogr. A 1178 (2008) 223–230.
[11] W.H. Kwok, D.K. Leung, G.N. Leung, F.P. Tang, T.S. Wan, C.H. Wong, J.K. Wong, Unusual observations during steroid analysis, Rapid Commun. Mass Spectrom. 22 (2008) 682–686.
[12] A. Leinonen, T. Kuuranne, R. Kostiainen, Liquid chromatography/mass spectrometry in anabolic steroid analysis—optimization and comparison of three ionization techniques: electrospray ionization atmospheric pressure chemical ionization and atmospheric pressure photoionization, J. Mass Spectrom. 37 (2002) 693–698.
[13] M. Thevis, H. Geyer, U. Mareck, W. Schanzer, Screening for unknown synthetic steroids in human urine by liquid chromatography–tandem mass spectrometry, J. Mass Spectrom. 40 (2005) 955–962.
[14] K. Deventer, P.V. Eenoo, F.T. Delbeke, Screening for anabolic steroids in doping analysis by liquid chromatography/electrospray ion trap mass spectrometry, Biomed. Chromatogr. 20 (2006) 429–433.
[15] A. Thomas, G. Sigmund, S. Guddat, W. Schänzer, M. Thevis, Determination of selected stimulants in urine for sports drug analysis by solid phase extraction via cation exchange and means of liquid chromatography–tandem mass spectrometry, Eur. J. Mass Spectrom. 14 (2008) 135–143.
[16] O.J. Pozo, K. Deventer, P.V. Eenoo, F.T. Delbeke, Efficient approach for the comprehensive detection of unknown anabolic steroids and metabolites in human urine by liquid chromatography–electrospray-tandem mass spectrometry, Anal. Chem. 80 (2008) 1709–1720.
[17] J.P. Danaceau, M.S. Morrison, M.H. Slawson, Quantitative confirmation of testosterone and epitestosterone in human urine by LC/Q-ToF mass spectrometry for doping control, J. Mass Spectrom. 43 (2008) 993–1000.
[18] H. Kosanam, P.K. Prakash, C.R. Yates, D.D. Miller, S. Ramagiri, Rapid screening of doping agents in human urine by vacuum MALDI-linear ion trap mass spectrometry, Anal. Chem. 79 (2007) 6020–6026.
[19] H. Kataoka, S. Yamamoto, S. Narimatsu, H.L. Lord, J. Pawliszyn, Development of automated in-tube SPME/LC/MS method for the drug analysis, J. Microcol. Sep. 12 (2000) 493–500.
[20] H. Kataoka, E. Matsuura, K. Mitani, Determination of cortisol in human saliva by automated in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry, J. Pharm. Biomed. Anal. 44 (2007) 160–165.
[21] H. Kataoka, R. Inoue, K. Yagi, K. Saito, Determination of nicotine, cotinine, and related alkaloids in human urine and saliva by automated in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry, J. Pharm. Biomed. Anal. 49 (2009) 108–114.
[22] H. Lord, J. Pawliszyn, Microextraction of drugs, J. Chromatogr. A 902 (2000) 17–63.
[23] H. Kataoka, Automated sample preparation using in-tube solid-phase microextraction and its application. A review, Anal. Bioanal. Chem. 373 (2002) 31–45.
[24] H. Kataoka, A. Ishuzaki, Y. Nonaka, K. Saito, Developments and applications of capillary microextraction techniques: a review, Anal. Chim. Acta 655 (2009) 8–29.
[25] H. Kataoka, Recent developments and applications of microextraction techniques in drug analysis, Anal. Bioanal. Chem. 396 (2010) 339–364.