— **Regular Article** —

Determination of Selected Corticosteroids in Sewage-treatment-plant Samples by Liquid Chromatography-mass Spectrometry

Yuko Kitaichi,[a] Aoi Miyamoto,[b] and Kazuo Uchikura*,[a]

[a]*Graduate School of Pharmacy and* [b]*School of Pharmacy, Nihon University, 7–7–1 Narashinodai, Funabashi 274–8555, Japan*

(Received April 19, 2010; Accepted June 12, 2010; Published online June 21, 2010)

An analytical method was developed for simultaneous determination of trace amounts of nine corticosteroids in sewage-treatment-plant (STP) samples. The compounds studied were prednisolone, dexamethasone, betamethasone, triamcinolone acetonide, fluocinolone acetonide, prednisolone acetate, hydrocortisone acetate, betamethasone valerate, and betamethasone dipropionate. The method involves concentration and purification of analytes by solid-phase extraction, subsequent separation by liquid chromatography, and detection by mass spectrometry. Quantitative analysis was performed by the standard addition method. Instrument detection limits were in the range 1.6–9.4 pg for the target compounds. Recoveries were 57.2–106.7% in the STP influent sample and 59.1–112.5% in the STP effluent sample with relative standard deviations of 8.7–19.2% and 7.3–17.1%, respectively. This method was used for the determination of these chemicals in STP samples from Takase (Funabashi, Japan); some corticosteroids were determined at levels as low as single nanograms per liter.

Key words —— pharmaceuticals and personal care products, corticosteroid, sewage-treatment-plant, solid-phase extraction, liquid chromatography-mass spectrum

INTRODUCTION

A new category of environmental pollution materials—pharmaceuticals and personal care products (PPCPs)—has been of concern since the mid-1990s.[1)] Recently, PPCPs have been detected in surface water, ground water, and sewage treatment water.[2–8)] Research is now ongoing on the toxic effects of PPCPs to aquatic organisms.[2, 9, 10)]

Pharmaceuticals are obviously designed to have particular physiological activating functions. Until now, the presence of pharmaceuticals like antipyretic analgesic, lipid-lowering drug and antibiotic of high consumption are analyzed and researched in water environment.[3–8)] However, in Japan about 1500 prescription pharmaceuticals exist[11)] and the presence of only a few of them has been studied.

Among pharmaceuticals not yet sufficiently studied are corticosteroids. These are widely used as anti-inflammatory and immunosuppressive agents against rheumatism, collagenosis, malignant tumors, skin disease, *etc*. They are either taken orally, injected, or applied externally. When applied externally, they remain on the skin surface and adhere to clothing, and are eventually are washed into the environment without metabolism. Corticosteroids synthesized for external use are thought to have particularly high residual rates in the environment, because they were developed for higher oil dissolubility and stability in acid than natural cortisone in order to limit their effect on the skin. In addition, corticosteroids are hormonal drugs, so even the smallest amount released into the environment can affect aquatic organisms. Because synthetic corticosteroids have several times stronger effects than do natural corticosteroids, even their trace amounts can affect aquatic organisms. Their effects on aquatic organisms are in fact unknown, but their reproductive toxicity against mammals has been reported.[12, 13)] Only a few reports exist about contamination levels of corticosteroids in environmental water. For example, Piram and coworkers detected

*To whom correspondence should be addressed: Graduate School of Pharmacy, Nihon University, 7–7–1 Narashinodai, Funabashi 274–8555, Japan. Tel. & Fax: +81-47-465-6573; E-mail: uchikura.kazuo@nihon-u.ac.jp

a few nanograms per liter of synthetic steroids in sewage-treatment-plant (STP) influent and effluent in France.[14] Hence, the aim of this study was to analyse synthetic corticosteroids in environment.

Numerous analytical methods for determining the concentrations of pharmaceuticals and their metabolites in environmental water have been described in the literature. After solid-phase extraction (SPE), the analysis of pharmaceuticals in environmental water has been performed largely by liquid chromatography (LC)-MS, LC-MS-MS, or GC-MS. Because most pharmaceuticals are high-polarity compounds, analysis by GC-MS requires sample derivatization.[3, 6, 7] Analysis by LC-MS and particularly LC-MS-MS is more suitable for determining ultra-trace concentrations of many pharmaceuticals that are polar and thermolabile in environmental water,[4, 5, 10, 14] but the latter of these requires expensive high-performance apparatus. Hence, we chose to investigate the possibility of sample concentration and purification by SPE followed by quantification by LC-MS. In this paper, we report the optimization of an analytical method that combines SPE concentration and purification with LC-electrospray ionization (ESI)-MS for the quantification of corticosteroids with the standard addition method.

MATERIALS AND METHODS

Chemicals, Reagents, and SPE Cartridges —— Prednisolone (P), betamethasone (B), dexamethasone (D), triamcinolone acetonide (Tc), fluocinolone acetonide (Fc), prednisolone acetate (Pa), hydrocortisone acetate (Ha), betamethasone valerate (Bv), and betamethasone dipropionate (Bd) were purchased from Wako Pure Chemical Industry (Osaka, Japan). These target compounds are listed in Table 1 (> 98% chemical purity).

HPLC-grade methanol, HPLC-grade acetonitrile, dichloromethane super grade, *n*-hexane super grade, acetone super grade, ethyl acetate super grade, and 2-propanol super grade were purchased from Kanto Kagaku (Tokyo, Japan). Water (18 MΩ/cm) was purified using a Milli-Q Direct system (Millipore, Tokyo, Japan).

A stock standard solution (1000 mg/l) of each target compound was prepared in methanol and stored in dark bottles at 4°C until use. Working standard solutions were prepared from individual stock solutions. Each stock solution was mixed and diluted with methanol. A series of mixed working standard solutions was prepared containing all nine target compounds.

The following SPE cartridges were used: Oasis® hydrophilic lipophilic balance (HLB, 225 mg), Sep-Pak® Plus C18 (500 mg), Sep-Pak Plus Florisil® (500 mg), Sep-Pak Plus NH2 (500 mg), and Sep-Pak Plus Silica (500 mg), purchased from Waters (Tokyo, Japan), and GL-Pak PLS2 (200 mg), purchased from GL Science (Tokyo, Japan).

Sampling —— Environmental samples were collected from Takase STP (Funabashi, Japan). This plant serves a population of about 52500 and receives domestic wastewater. The STP uses anaerobic/anoxic/oxide processing, and the maximum daily treatment capacity is 68200 m^3. An outline of the procedure used for sewage treatment is shown in Fig. 1.

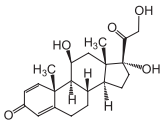
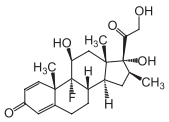
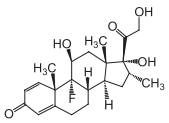
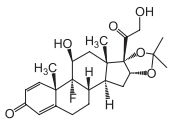
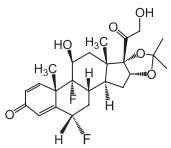
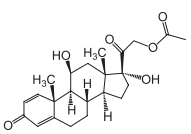
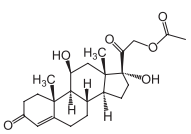
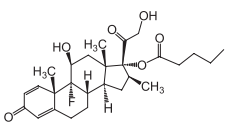
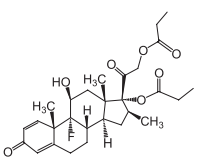
Four samples were obtained from different points that included STP influent wastewater (S1), effluent of treatment primary sedimentation (S2), final sedimentation (S3), and final effluent from the plant (S4). The samples were stored in glass bottles until extraction and analysis, with standard precautions against contamination. They were then filtered through a glass microfiber filter (GF/B) within 24 hr of collection and continuous analyses were performed within the day.

Sample Preparation —— Both reverse- and normal-phase SPE cartridges were tested. The reverse-phase cartridges were GL-Pak PLS2 (polymer-base), Oasis HLB and Sep-Pak Plus C18 (silica-base). The normal-phase cartridges were Sep-Pak Plus Florisil, Sep-Pak Plus NH2, and Sep-Pak Plus Silica. SPE was performed using a vacuum manifold (GL Sciences) for 12 columns connected to a vacuum tank equipped with a vacuum pump. A schematic representation of the final method is shown in Fig. 2.

Instrumentation —— Analyses were performed using a high-performance liquid chromatograph (Agilent Technologies 1100 series, Tokyo, Japan) equipped with a quaternary pump, vacuum membrane degasser, thermostated column compartment, autosampler, variable-wavelength detector, and SL quadrupole mass spectrometer system that can use ESI interfaces. Acquisition modes [selected ion monitoring (SIM) and full scan monitoring] can be performed simultaneously.

Liquid chromatographic columns of Develosil

Table 1. Structures, Target Ions and Retention Time of the Target Compounds
Quantitation ions in bold; confirmation ions in normal font. *a*) $[M\text{-}H]^-$, *b*) $[M+CH_3COO]^-$.

Molecule	Structure	Ion assignments (m/z)	Retention time (min)
Prednisolone (P)		359[a)] **419**[b)]	3.82
Betamethasone (B)		391[a] **451**[b)]	5.64
Dexamethasone (D)		391[a)] **451**[b)]	5.64
Triamcinolone acetonide (Tc)		433[a)] **493**[b)]	6.54
Fluocinolone acetonide (Fc)		451[a)] **511**[b)]	7.10
Prednisolone acetate (Pa)		401[a)] **461**[b)]	7.85
Hydrocortisone acetate (Ha)		403[a)] **463**[b)]	8.31
Betamethasone valerate (Bv)		476[a)] **535**[b)]	12.46
Betamethasone dipropionate (Bd)		504[a)] **564**[b)]	13.83

C30-UG-3 (2.0 × 150 mm, 3 μm particle size; Nomura Chemistry, Seto, Japan), TSKgel ODS-100Z (2.0 × 150 mm, 5 μm particle size; Tosoh, Tokyo, Japan), and Capcell Pak C8 (2.0 × 150 mm, 5 μm particle size; Shiseido, Tokyo, Japan) were tested.

LC-MS Conditions —— Chromatographic separation of compounds was performed on a Develosil C30-UG-3 column. Standards and samples were separated using a gradient mobile phase consisting of 5 mM acetic acid solution (solvent A) and acetonitrile (solvent B). The gradient elution program started with 40% B. A linear gradient was run to 90% B over 10 min and held for 5 min. After each run, the column was re-equilibrated for 10 min at the initial conditions before the next injection. The flow rate was 0.2 ml/min, the injection volume was 5 μl, and the column temperature was maintained at 40°C.

The ESI interface in the negative mode was chosen for compound identification and quantification.

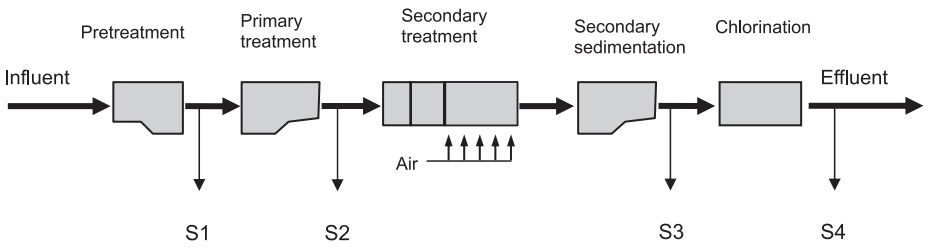


Fig. 1. Procedures Used for Sewage Treatment and STP Sampling Points (S1–S4)

Pretreatment: this process removes large garbage and sand from the raw wastewater. Primary treatment: this process removes small garbage that could not be removed in the pretreatment. Secondary treatment: this process degrade the organic matter of the sewage by obligate aerobic and bind much of the less soluble fractions into floc. Secondary sedimentation: this process is to settle out the biological floc and to produce sewage water containing low levels of organic material and suspended matter. Chlorination: this process is disinfection of treated water by sodium hypochlorite.

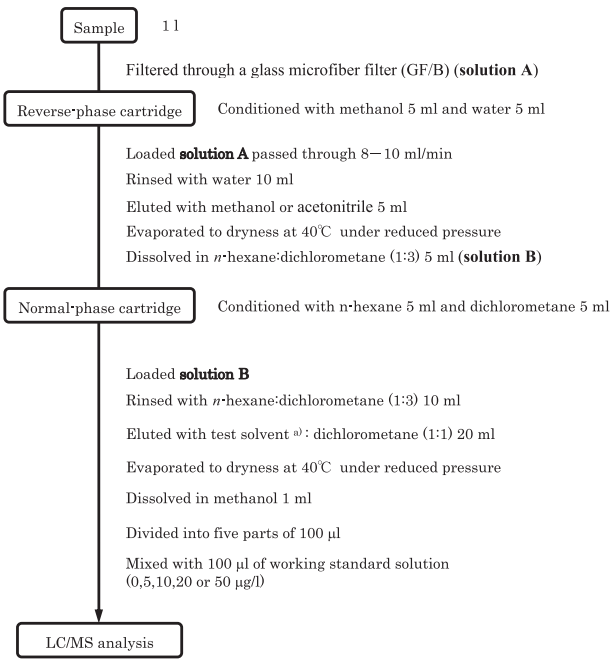


Fig. 2. Schematic Representation of the Analytical Method

a) Test solvent: acetonitrile, ethyl acetate, methanol, 2-propanol or acetone.

ESI-MS conditions were optimized by direct injection of working standard solutions of each compound (1 mg/l). The selected parameters are shown in Table 2.

RESULTS AND DISCUSSION

Preconcentration and Purification Procedure

Because of the complex nature of wastewater, an SPE method was selected that would be appropriate for extraction of target compounds from previously filtered samples. Several SPE procedures were assessed using 1 l of tap water spiked with 10 ng/l target compounds.

First, we tested the reverse-phase SPEs (GL-Pak PLS2, Oasis HLB, and Sep-Pak Plus C18) with methanol and acetonitrile as eluting solvent. These SPEs showed good recovery of target compounds. However, the Oasis HLB had slightly inferior recovery value. Both GL-Pak PLS2 and Sep-Pak Plus C18 had excellent relative standard deviation (RSD, $n = 3$) values, although the former had an inferior value as a whole when methanol was used as eluent. As a result, Sep-Pak Plus C18 was selected as the collector because of its high cost-effectiveness and numerous applications (Table 3). Both methanol and acetonitrile eluents showed the same recovery rates, but methanol was adequate for evaporation and further treatment during the next purification steps with normal-phase SPEs.

Next, we tested the normal-phase SPEs (Sep-Pak Plus Florisil, Sep-Pak Plus NH2, and Sep-Pak Plus Silica) for purification of target compounds and the elution efficiency of test solvents (acetonitrile, methanol, acetone, 2-propanol, and ethyl acetate). The target compounds (2 µg) were dissolved in 5 ml of *n*-hexane : dichloromethane (1 : 3) mixture. This mixture was passed through each cartridge and the target compounds were eluted with 10 ml of test solvent : dichloromethane (1 : 1) mixture. Recovery rates were lower for the high-polarity target compounds (such as P, B, D, Tc, and Fc) than for the low-polarity target compounds. The best combination for the target compounds, Sep-Pak Plus Silica and acetone, gave recovery rates of 73–107% (Table 4).

Finally, we examined the volume of elution solvent needed to total recovery of the target compounds (Fig. 3). Test solution was passed through a Sep-Pak Plus Silica cartridge and eluted five times with 5 ml of acetone : dichloromethane (1 : 1) mix-

Table 2. LC-MS Conditions

LC conditions					MS conditions	
Column	Develosil C30-UG-3 (2.0 × 150 mm)				Ionization	ESI Negative
Mobile phase	Solvent A: Acetonitrile				Nebulizer	N_2 (40 psig)
	Solvent B: 5 mM acetic acid				Drying gas	N_2 (10 l/min, 350°C)
Gradient program	Time (min)	0	10	15	V-Cap	3000 V
	A (%)	40	90	90	Fragmentor	125 V
Flow rate	0.2 ml/min					
Column temp.	40°C					
Injection volume	5 µl					

Table 3. Comparison of Recoveries (%) and RSD Using a Reverse-phase Cartridge and Solvent (n = 3)

See abbreviation in Table 1. 10 ng of target compounds was spiked 1 l of water and extracted by reverse-phase cartridge, after that eluted with 5 ml of acetonitrile or methanol.

	Acetonitrile						Methanol					
	Sep-Pak Plus C18		Oasis HLB		GL-Pak PLS2		Sep-Pak Plus C18		Oasis HLB		GL-Pak PLS2	
	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
P	92.6	3.5	87.5	12.5	102.0	6.8	101.0	1.7	81.7	6.6	89.9	17.4
B	97.1	4.8	85.7	10.8	101.0	7.3	97.2	2.7	85.1	5.4	88.0	16.5
D	94.6	3.8	85.1	8.9	94.0	6.5	90.1	3.4	92.9	3.3	117.7	38.7
Tc	96.0	0.7	89.3	10.2	103.0	7.4	96.8	3.0	77.8	6.9	82.6	16.2
Fc	125.0	4.6	107.0	7.9	103.0	8.8	101.9	2.7	83.0	7.2	83.4	8.7
Pa	99.5	1.4	91.7	11.1	103.0	6.9	98.2	3.6	75.2	5.8	82.5	16.1
Ha	99.5	1.5	86.8	11.0	104.0	7.1	96.7	4.2	76.0	5.9	78.6	13.9
Bv	89.5	2.6	85.2	5.2	99.2	6.7	72.1	10.2	79.5	7.5	82.3	14.0
Bd	82.4	1.3	79.4	5.8	107.0	9.2	81.1	3.9	76.7	9.3	90.8	9.7

Table 4. Comparison of Recoveries (%) Using a Normal-phase Cartridge and Solvent

See abbreviation in Table 1. FL: Sep-Pak Plus Florisil; SIL: Sep-Pak Plus Silica; NH2: Sep-Pak Plus NH2. 2 µg of target compounds was spiked 5 ml of n-hexane : dichlorometane (1 : 3) mixture and extracted by normal-phase cartridge, after that eluted with 10 ml of test solvent : dichloromethane (1 : 1) mixture.

	Acetonitrile			Ethyl acetate			Methanol			2-Propanol			Acetone		
	FL	SIL	NH2	FL	SIL	NH2	FL	SIL	NH2	FL	SIL	NH2	FL	SIL	NH2
P	0.0	8.6	51.4	43.0	23.6	20.9	87.4	63.9	62.2	39.1	56.1	58.0	11.1	73.1	50.4
B + D	18.4	16.2	54.0	0.0	68.6	31.9	54.9	75.1	72.9	57.4	59.7	63.4	33.5	85.7	64.9
Tc	34.5	68.0	76.2	17.3	71.2	62.9	56.4	90.9	82.7	65.4	75.7	81.0	44.3	89.2	60.2
Fc	34.4	70.0	70.6	13.8	66.2	36.6	71.4	91.8	80.4	68.4	74.4	76.7	43.0	92.8	62.0
Pa	67.3	106.3	78.1	103.6	110.3	91.7	66.7	96.9	81.4	76.9	97.0	64.2	69.9	107.3	60.9
Ha	67.3	112.3	57.3	108.1	94.5	42.7	66.6	100.8	44.8	96.4	100.0	27.7	71.0	95.8	31.4
Bv	78.9	111.6	111.6	43.4	92.9	107.0	92.7	120.3	108.5	69.0	68.4	27.0	79.1	90.0	100.0
Bd	73.3	92.1	39.1	70.3	86.6	46.2	71.9	96.7	78.6	82.3	76.7	24.5	54.6	98.0	14.9

ture. Almost all target compounds were eluted within the third elution. Thus, the optimum eluent volume was established at 20 ml.

Mass Spectrometry Analysis

A water : acetonitrile (1 : 1) mixture was used as the mobile phase. Formic acid, ammonium formate, acetic acid and ammonium acetate were tested as additives (Fig. 4). These additives influence the molecular-ion peak sensitivity in flow-injection-analysis (FIA) mode. Both positive and negative modes of ESI interfaces could be used for detection of all target compounds. Negative-mode analysis using acetic acid showed the best sensitivity with $[M+CH_3COO]^-$. Therefore, we selected the negative mode and acetic acid as the additive reagent. The SIM mode was used for quantitative analysis. $[M+CH_3COO]^-$ was measured as the quantification ion, and $[M–H]^-$ was measured at the same time as the confirmation ion.

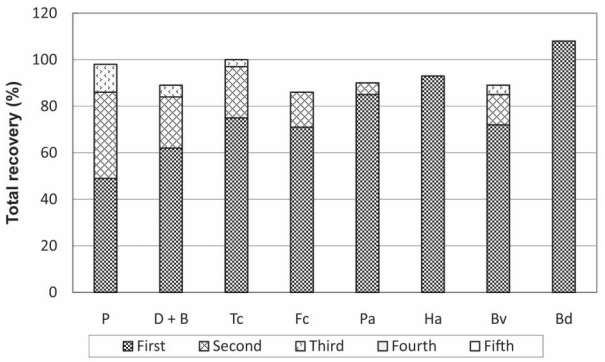


Fig. 3. Total Recovery from a Sep-Pak Plus Silica Eluted with Acetone : Dichloromethane (1 : 1) mixture
See abbreviation in Table 1.

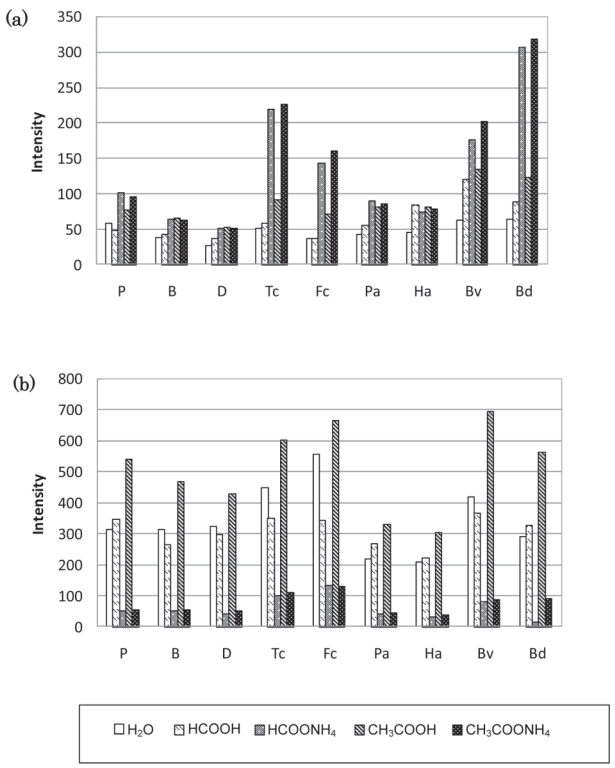


Fig. 4. Mass Spectrometric Responses of Target Compounds with Acetic Acid as the Additive Reagent
(a) Positive ion mode; (b) negative ion mode (see abbreviation in Table 1).

The sensitivity of each target compound was compared in 0, 5, 10, 20, and 50 mM concentrations of acetic acid in FIA mode (Fig. 5). The acetic acid could be determined with high sensitivity at concentrations of 5 and 10 mM. Therefore, we selected 5 mM acetic acid.

Fragmentor voltage was compared in the range 50–200 V. Each target compound was measured with high sensitivity within the range 100–150 V. Therefore, we selected 125 V as the fragmentor voltage.

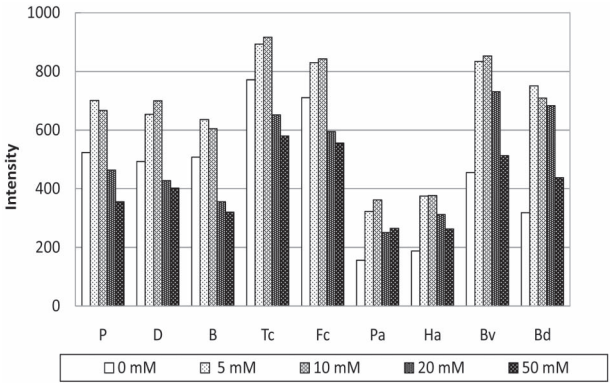


Fig. 5. Mass Spectrometric Responses of Target Compounds with Different Acetic Acid Concentrations by Negative Ion Mode
See abbreviation in Table 1.

Liquid Chromatographic Separation

The conditions for liquid chromatographic separation were optimized using a UV detector. In this report, the quantification by LC-MS used standard addition method. This method takes time, as one sample requires five concentrations points. Therefore, the analysis time for one sample was set as short as possible.

Column type and gradient conditions were both examined, with the objective that one analysis should take < 30 min, including column-equilibrating time. Three column types were examined: Capcell Pak C8, Develosil C30-UG-3, and TSKgel ODS-100Z. We selected Develosil C30-UG-3 because it provided good separations of and appropriate retention times for the target compounds (Fig. 6). Two organic solvents commonly used in reversed-phase liquid chromatography were evaluated: methanol and acetonitrile. Acetonitrile was selected because it provided better separations of target compounds than did methanol. The target compounds were separated within 15 min, except for B and D. In fact, separation of these two compounds required > 30 min because they are diastereoisomers and thus interact similarly with the chromatographic stationary phase. Therefore, these molecules had to be quantified together.

Analytical Performance

The detector response for all target compounds was linear in the studied concentration range 0.5–50 μg/l at six points under optimum SIM conditions; the correlation coefficients were better than 0.990 (Table 5). Instrumental detection limits (IDLs) were

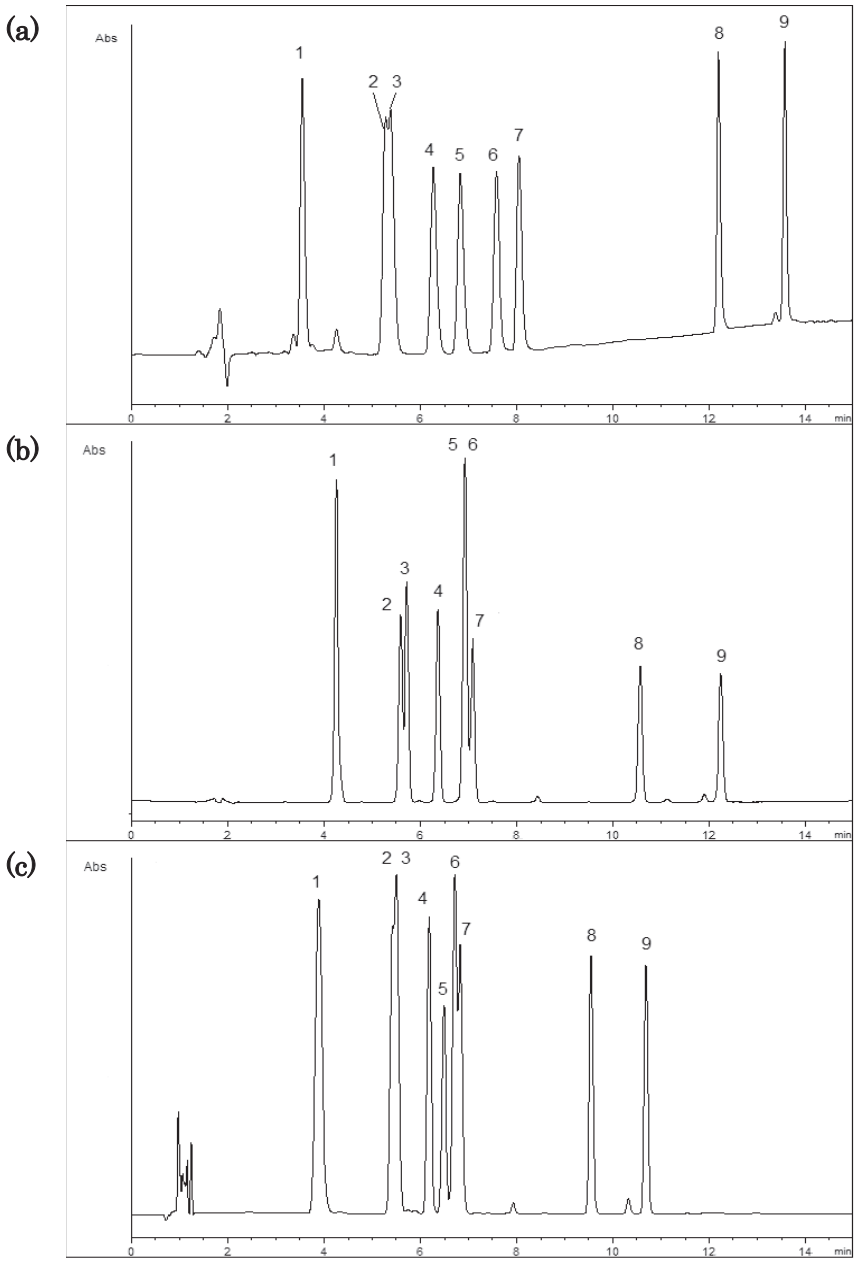


Fig. 6. LC-UV Chromatograms Using Different Columns and Gradient Conditions

(a) Develosil C30-UG-3, 40%A→linear 90% (10 min) const. (5 min); (b) TSKgel ODS-100Z, 30%A→linear 80% (15 min); (c) Capcell Pak C8, 30%A→linear 80% (15 min). Peak identifications: (1) P, (2) D, (3) B, (4) Tc, (5) Fc, (6) Pa, (7) Ha, (8) Bv, (9) Bd (see abbreviation in Table 1).

thus calculated with the optimized condition that the minimum injected mass gives a signal-to-noise ratio of S/N = 3. IDLs ranged from 1.6 to 9.4 pg for each target compound injected. Instrumental precision was assessed by replicate injection (n = 5) of the calibration solution on the same day. The intraday variance, expressed as the RSD (%) for each target compound's peak area, was usually < 10%. The accuracy of the method was determined by recovery studies conducted on spiked tap water at 10 ng/l. IDL, intraday variance RSD, recovery, and recovery RSD are shown in Table 6.

Quantification Method of Wastewater Samples

The LC-ESI-MS ionization process is susceptible to matrix signal suppression. The LC-MS response obtained from a standard can differ significantly from that obtained from a sample with matrices. Therefore, signal suppression presents a challenge in quantitative LC-MS applications. In gen-

Table 5. Slopes of Calibration Curve and Correlation Coefficients by the Standard Addition Method and External Standard Method
See abbreviation in Table 1.

Compound	Standard addition method								External standard method	
	S1		S2		S3		S4			
	slope	r	slope	r	slope	r	slope	r	slope	r
P	222	0.9910	204	0.9994	407	0.9968	364	0.9991	905	0.9995
B + D	209	0.9989	205	0.9991	410	0.9992	359	0.9997	865	0.9997
Tc	454	0.9994	486	0.9997	499	0.9953	500	0.9973	1052	0.9988
Fc	484	0.9991	500	0.9996	570	0.9971	509	0.9992	1132	0.9997
Pa	209	0.9954	211	0.9951	330	0.9960	258	0.9982	700	0.9993
Ha	265	0.9989	293	0.9998	401	0.9977	305	1.0000	778	0.9996
Bv	151	0.9997	190	0.9998	685	0.9999	563	1.0000	1464	0.9976
Bd	96	0.9906	102	0.9982	611	0.9997	531	0.9994	1331	0.9950

Table 6. Instrument Detection Limits (s/n = 3, pg), Intraday Variance Expressed as Relative Standard Deviation and Recovery Data (%, RSD) in Tap Water Spiked at 10 ng/l
See abbreviation in Table 1.

Compound	IDL (s/n = 3, pg)	Intraday variance RSD (10 pg, n = 5)	Recovery (%) (10 ng/l, n = 5)	RSD
P	6.3	7.3	83.8	10.9
D + B	5.0	6.7	100.4	14.6
Tc	5.1	6.5	72.9	15.8
Fc	7.8	4.0	88.8	6.6
Pa	9.3	7.8	93.0	16.1
Ha	9.4	6.1	90.0	6.8
Bv	2.3	7.2	89.9	6.0
Bd	1.6	7.1	85.5	6.1

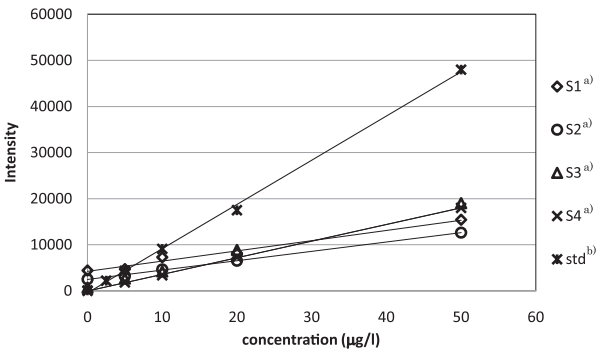


Fig. 7. The Calibration Curve of Prednisolone
a) The standard addition method (S1–S4); b) the external standard method.

eral, LC-MS analysis, an isotope of the analyte is used to correct a surrogate standard. However, isotope standards of our target compounds are not commercially available.

To address this situation, we considered using the standard addition method as an alternative to the external standard method. Table 6 shows the slopes of the calibration curve and the correlation coefficients (calibration curves were constructed using the working standard solutions) for each method and target compound. Because of ion suppression, the slopes are smaller for calibration curves obtained by the standard addition method than for those obtained by the external standard method. Therefore, quantitative values are underestimated by the external standard method. The calibration curve of P by the standard addition method and the external standard method are shown Fig. 7. There were no blank matrixes (that is, test samples that did not contain the target compounds), so it was not possible to evaluate the sensitivity of the method using environmental samples. Regarding quantification calculations, we calculated peak areas (S/N > 3) at points where additive concentration is zero, and calculated quantitative values as the absolute value of the point of x intercept on the calibration curve for the standard addition method. As an example, consider chromatograms for which the additive concentration points are 0 and 50 μg/l (Fig. 8). To validate our analytical method, we determined recovery rates for target compounds by adding 10 ng to 1 l of samples S1 and S4. The average recovery rates were in the range 56.5–106.7% for S1 and 59.1–112.5% for S4 for the target compounds (n = 3; Table 7). This result is similar to that reported by Piram *et al.*[14)]

Application to Wastewater Samples

Table 8 summarizes the concentrations of corticosteroids detected in the wastewater. In S1 (after pretreatment only), P, B+D, Ha, and Bv were detected. In S3 (after primary, biological, and secondary treatment) and S4 (after those treatments plus chlorination), Bv was detected but at lower

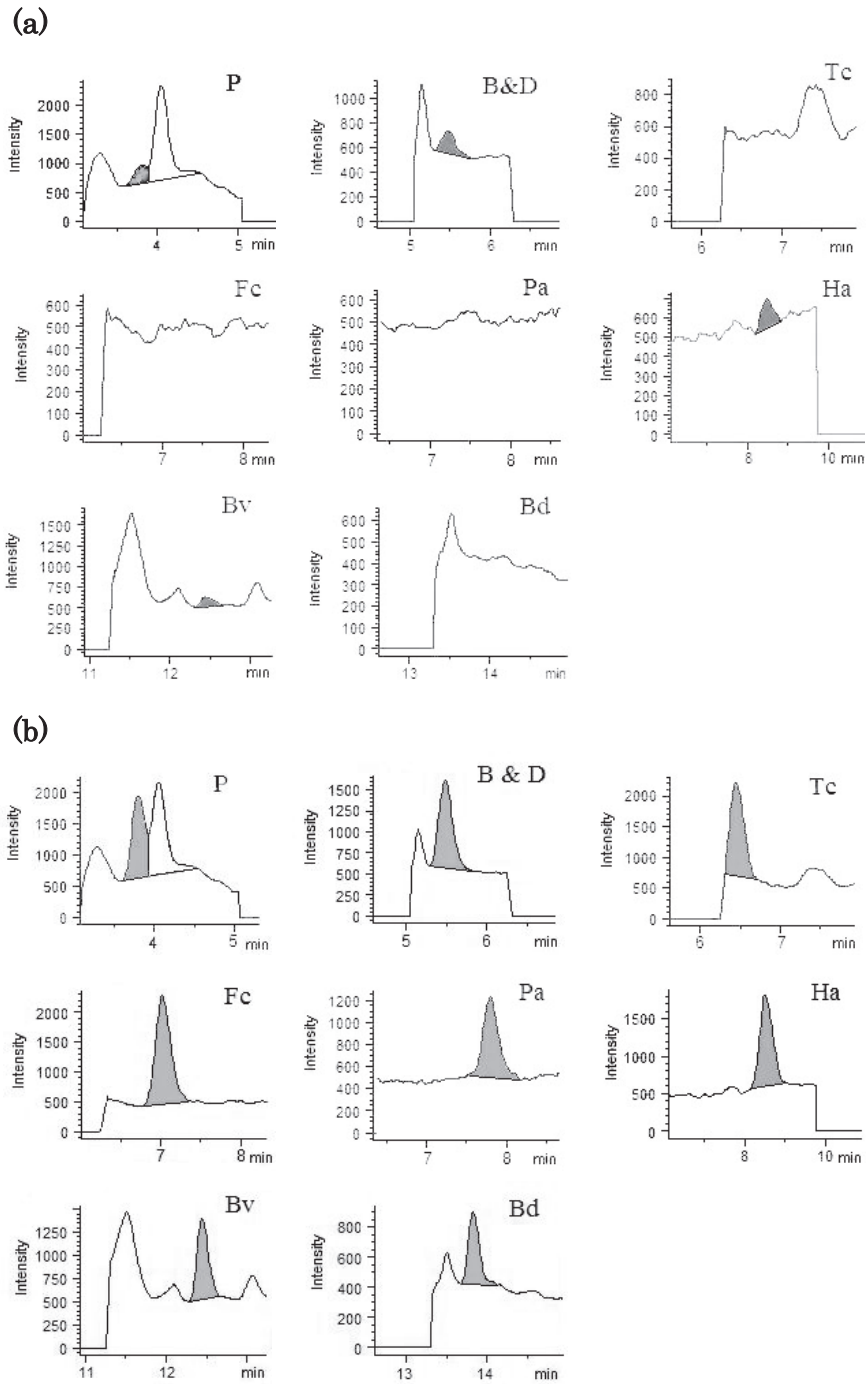


Fig. 8. LC-MS Chromatograms of S1 Sample at Two Additive Concentration Points
(a) 0; (b) 50 μg/l (see abbreviation in Table 1).

concentrations; B+D and Ha were not detected. Most of the target compounds were presumably processed by activated sludge in the reactive tank. However, the result suggests that some corticosteroids remain in STP effluent.

Piram *et al*. reported concentrations of corticosteroids in treated water that are higher than those reported herein.[14] However, it is inappropriate to compare these results, because to date, there have been only a few reports regarding the determination of corticosteroids. Piram *et al*. also reported that corticosteroid concentrations were the same in STP influent and effluent,[14] which is again contrary to our results. The differences between their results and ours are presumably due to the differences in the amounts used or in STP processing perfor-

Table 7. Recoveries (%) of Target Compounds in STP Water Samples
See abbreviation in Table 1.

compound	STP influent		STP effluent	
	Recovery (%) (10 ng/l, $n = 3$)	RSD	Recovery (%) (10 ng/l, $n = 3$)	RSD
P	57.2	10.8	112.5	17.1
B + D	106.7	8.7	59.1	12.7
Tc	104.1	19.2	74.6	17.0
Fc	97.9	10.7	69.1	12.6
Pa	67.4	16.4	78.8	12.4
Ha	67.3	13.5	83.2	10.9
Bv	56.5	13.7	84.9	10.6
Bd	72.8	19.2	87.9	7.3

Table 8. Measured Concentrations in Takase STP
See abbreviation in Table 1. nd: Not detected.

compound	Concentration (ng/l)			
	S1	S2	S3	S4
P	17.0	12.2	nd	nd
B + D	9.4	9.0	nd	nd
Tc	nd	nd	nd	nd
Fc	nd	nd	nd	nd
Pa	nd	nd	nd	nd
Ha	3.8	5.9	nd	nd
Bv	8.6	9.7	1.5	1.3
Bd	nd	nd	nd	nd

mance. In untreated layers S1 and S2, we detected Bv and B+D in nearly equal concentrations. In treated layers S3 and S4, we detected a tiny amount of Bv but no B+D. Activated sludge appears to degrade each corticosteroid differently, even though they have similar backbone structures.

The analytical performance of the proposed method was validated and the method was used successfully for the determination of these compounds in STP samples.

We believe that this method will be useful for monitoring corticosteroids in water samples.

Acknowledgements We thank Funabashi City Takase treatment plant for providing samples.

REFERENCES

1) Daughton, C. G. and Ternes, T. A. (1999) Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ. Health Perspect.*, **107**, 907–938.
2) Halling-Sørenesen, B., Nielsen, S. N., Lanzky, P. F., Ingerslev, F., Lützhøft, H. C. H. and Jørgensen, S. E. (1998) Occurrence, fate and effects of pharmaceutical substances in the environment—A review. *Chemosphere*, **36**, 357–393.
3) Ternes, T. A. (1998) Occurrence of drugs in german sewage treatment plants and rivers. *Water Res.*, **32**, 3245–3260.
4) Zuccato, E., Calamari, D., Natangelo, M. and Fanelli, R. (2000) Presence of therapeutic drugs in the environment. *Lancet*, **355**, 1789–1790.
5) Ternes, T., Bonerz, M. and Schmidt, T. (2001) Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. A*, **938**, 175–185.
6) Huggett, D. B., Khan, I. A., Foran, C. M. and Schlenk, D. (2003) Determination of beta-adrenergic receptor blocking pharmaceuticals in United States wastewater effluent. *Environ. Pollut.*, **121**, 199–205.
7) Nakada, N., Tanishima, T., Shinohara, H., Kiri, K. and Takada, H. (2006) Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Res.*, **40**, 3297–3303.
8) Gros, M., Petrović, M. and Barceló, D. (2006) Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta*, **70**, 678–690.
9) Fent, K., Weston, A. A. and Caminada, D. (2006) Ecotoxicology of human pharmareuticals. *Aquat. Toxicol.*, **76**, 122–159.
10) Gagné, F., Blaise, C. and André, C. (2006) Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Ecotoxicol. Environ. Saf.*, **64**, 329–336.
11) Yakugyo kenkyukai (04/2008) *Hokenyakujiten*, Jiho, Tokyo, Japan.
12) Andrew, F. D. and Zimmerman, E. F. (1971) Glucocorticoid induction of cleft palate in mice; No correlation with inhibition of mucopolysacharide synthesis. *Teratology*, **4**, 31–38.
13) Hagino, N. (1972) The effect of synthetic corticosteroids on ovarian function in the baboon. *J. Clin. Endocrinol. Metab.*, **35**, 716–721.
14) Piram, A., Salvador, A., Gauvrit, J.-Y., Lanteri, P. and Faure, R. (2008) Development and optimization of a single extraction procedure for the LC/MS/MS analysis of two pharmaceutical classes residues in sewage treatment plant. *Talanta*, **74**, 1463–1475.