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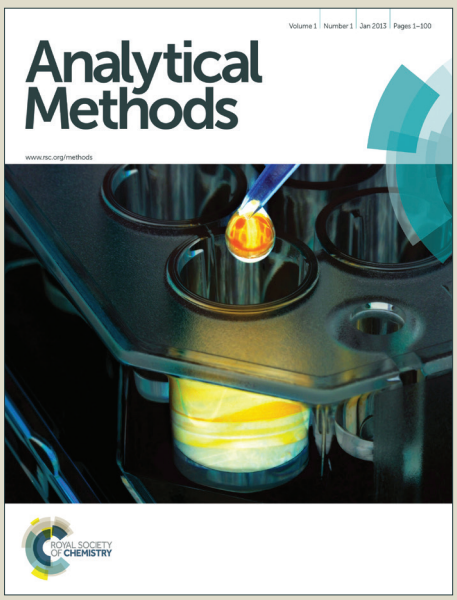

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Dispersive Micro-Solid-Phase Extraction of hormones in liquid cosmetics with Metal-organic Framework

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Abstract

The dispersive micro-solid-phase extraction based on -metal-organic framework MIL-101（Cr） was developed and applied to the extraction of hormones from cosmetics. The hormones were separated and determined by high performance liquid chromatography. Several experimental parameters, including extraction method, extraction time, pH value of sample solution, amount of MIL-101 (Cr), concentration of NaCl, volume of elution solvent and elution time, were investigated. The limits of detection and quantification for the analytes ranged from 0.36 to 0.91 μg/L and from 1.20 to 3.04 μg/L, respectively. The precision for determining hormones was lower than 6.1%.

Key words: cosmetics, hormones, MIL-101(Cr), dispersive micro-solid-phase extraction

1 Introduction

Cosmetics are used in the treatment of various skin disorders over the world and usually used daily in the long term. Some cosmetics are also exposed to infants and kids. In recent years, the cosmetics appear to be gaining in popularity. However, many chemicals can be illegally added in the cosmetics. Liu et al. reported that sulfapyridine at the concentration of 5.6 μg/kg in cosmetics was detectable [1]. Clobetasol propionate ranging from 32 to 96.4 mg/kg and 195.1 μg/kg of betamethasone dipropionate were found in some cosmetic products [2]. The potential accumulation hazard of chemicals used in daily lives has attracted more and more attention. Nowadays the European Union has established maximum residue limits for chemicals in cosmetics and the hormones in commercial cosmetic samples are prohibited. The hormones play an crucial role in reproductive system , bone health and neuroprotective processes. The hormones have extremely high biological activities at very low concentrations. The hormones are related to some alarming effects on reproduction and developmental processes, such as feminization, decreased fertility or hermaphroditism [3, 4].The sex hormones could also affect human immune system [5]. Unfortunately, some hormones have been detectable in cosmetics [6, 7]. Medroxyprogesterone at the concentration of 2.65 μg/L in cosmetics was reported by Kang et al.. So the determination of hormones in cosmetics is meaningful [6].

Some sample preparation methods have been developed for the determination of chemicals in cosmetics. The methods include liquid-liquid extraction (LLE) [7, 8], matrix solid phase dispersion (MSPD)[9], solid phase extraction (SPE) [10, 11], LLE-SPE [12], dispersive-SPE (DSPE) [13], stir-bar sorptive extraction (SBSE) [14] and supercritical fluid extraction [15]. Conventional LLE is a tedious procedure with the disadvantages in extractiontime, consumption of amounts of organic solvent, and tendency of emulsion formation. Recently, sample preparation trends for miniaturization and simplication has emerged.

Solid-phase microextraction (SPME) was applied to the extraction of parabens in cosmetics [16]. The polymer monolith microextraction (PMME) [17] and homogeneous ionic liquid microextraction (HILME) were applied to the extraction of hormones in cosmetics [6]. Lei et al. applied magnetically stirring extraction bar liquid–liquid microextraction (MSEBLLME) to the extraction of sexual hormones in cosmetics [18]. As a miniaturized alternative to DSPE, dispersive micro-solid-phase extraction (d-μ-SPE) has been developed and has the advantages of rapidity, simplicity, and consuming a small amount of sorbent and solvent [19, 20]. Various adsorbents or nanoparticles can be used in the dispersive mode to adsorb target analytes in aqueous samples [19-21]. The application of new sorbents promotes the development of this method. D-μ-SPE based on functionalized magnetite nanoparticle was successfully used to extract chemicals in cosmetics [13]. Metal-organic frameworks (MOFs) are hybrid inorganic-organic microporous crystalline materials which are self-assembled straightforwardly from metal ions with organic linkers via coordination bonds [22]. The fascinating structures and unique properties, such as permanent nanoscale porosity, high surface area, good thermostability, and uniform structured cavities, make MOFs attractive for analytical applications, especially in chromatography separation [23-28] and sample pretreatment [23, 24, 29-34]. MOFs have been explored as stationary phases for gas chromatography [25, 26] and liquid chromatography [27, 28]. In sample pretreatment, MOFs have been successfully used as sorbents for sampling gaseous samples [29], MOFs also can be used in SPE [30-32] and SPME [33]. MIL-101 (Cr) was first reported by Férey et al. in 2005 and built up from a hybrid supertetrahedral building unit formed by terephthalate ligands and trimeric chromium octahedral clusters [22]. MIL-101 (Cr) has high surface area, large pore windows (1.2 and 1.47×1.6 nm.), mesoporous pores (2.9 and 3.4nm), accessible coordinative unsaturated sites, and excellent chemical and solvent stability. The MIL-101 (Cr) framework is hydrophobic. These outstanding properties make

MIL-101 (Cr) attractive as a sorbent for extraction. MIL-101 (Cr) was used to extract naproxen and clofibric acid from aqueous solution [34]. MIL-101 (Cr) was used to extractnaproxen and 6-O-desmethylnaproxen in urine sample [35]. MIL-101(Cr) was used for magnetic solid-phase extraction of polycyclic aromatic hydrocarbons in environmental water samples [36]. To the best of our knowledge, there is no report on the extraction of hormones with MIL-101 (Cr).

In present method, d-μ-SPE based on MIL-101 (Cr) was developed and applied to the extraction of hormones from liquid cosmetics. A small amount of sorbent was added into the diluted sample to adsorb the analytes. The operation is simple and rapid. The hormones were determined by high performance liquid chromatography.

2 Materials and methods

2.1 Reagents and chemicals

The hormone standards (purity, 96.8–99.5%) including prednisone, meprednisone, boldenone, nandrolone, testosterone, dexamethasone acetate, 17-hydroxyprogesterone, norgestrel, medroxyprogesterone, megestrol acetate, progesterone and testosterone 17-propionateand were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The physical-chemical properties of the hormones are listed in Table 1. Each hormone was dissolved in acetonitrile to obtain the stock solution at the concentration of 100 μg /mL and the stock solutions were stored at 4 °C. Working and mixed working standard solutions were prepared by diluting stock standard solutions withacetonitrile. Chromatographic grade acetonitrile was purchased from Fisher Scientific (NJ, USA). $Cr(NO_3)_3{\cdot}9H_2O$(＞99.0%) was purchased from XLong chemical Co., Ltd (Guangdong, China). 1,4-Benzene dicarboxylic acid (H_2BDC, ≥98.5%) was purchased from Tianjin Guangfu Fine Chemical Research Institute(Tianjin, China). Hydrofluoric acid (HF, ≥40%) was purchased from Nanjing

Chemical Reagent Co., Ltd (Nanjing, China). Pure water was obtained with a Milli-Q water purification system (Millipore Co., USA). All other reagents were of analytical-reagent grade and purchased from Beijing Chemical Factory (Beijing, China).

2.2 Instruments

Chromatographic separation and determination of the hormones were carried out on the 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with diode-array detector (DAD) and quaternary gradient pump. Eclipse XDB-C18 column (3.5 μm, 4.6 mm × 150 mm, Agilent, USA) was used. The KQ3200E ultrasonic cleaner was purchased from Kunshan Ultrasonic Instrument Co., Ltd. (Kunshan, China). The frequency and output power of the ultrasonic cleaner are 40 kHz and 150 W, respectively. The HC-2006 high speed centrifuge was purchased from AnHui USTC Zonkia Scientific Instruments Co., Ltd (Anhui, China).

The X-ray diffraction (XRD) patterns were recorded on a Rigaku D/max-2550 diffractometer equipped with a graphite monochromator (Rigaku, Japan) and Cu Kα radiator (λ = 1.5418 Å). Transmission electron microscopic (TEM) characterization was performed on a Tecnai G2 F20 S-Twin (FEI,America). BET surface area was measured on an ASAP 2020 micropore physisorption analyzer (Micromeritics, Norcross, GA) using nitrogen adsorption at 77 K.

2.3 Synthesis of MIL-101 (Cr)

MIL-101 (Cr) was synthesized according to the method reported by Férey et al. [22]. Cr(NO3)3•9H2O (800 mg), terephthalic acid (322 mg) and HF (0.1 mL) were mixed with ultrapure water (9.6 mL) in a Teflon autoclave. The Teflon autoclave was then sealed and placed in an oven at 220℃ for 8 h. Teflon autoclave was then cooled down to room temperature. The resulting green crystalline solid was washed

thoroughly with dimethyl formamide and hot ethanol, and collected by centrifugation at 10000 rpm for 5 min. The washing was repeated at least three times to remove the unreacted terephthalic acid from MIL-101 (Cr) pores. Finally, the obtained solid was dried in an oven at 150 ℃ overnight.

2.4 Sample preparation

The cosmetic samples were purchased from local large-scale commercial market and stored at room temperature. In the study, the liquid cosmetics (Sample 1-4) were analyzed. The spiked samples containing hormones were prepared by spiking the working solutions into cosmetic samples and shaking for 10 min. All results were obtained with sample 1 except for those mentioned in Section 3.3.3.

2.5 Extraction procedure

5 mL of cosmetic sample was transferred into a 10 mL polyterafluoroethylene (PTFE) centrifuge tube. 4 mL of water and NaCl were added and the concentration of NaCl was 15% (w/v). The pH value of sample was adjusted with 1 mol/L NaOH and 1 mol/L HCl. Then 7 mg of MIL-101 (Cr) was added into the tube and the tube was placed in ultrasonic bath for 30 s for the dispersion of MIL-101 (Cr). The mixture was shaken for 10 min and then centrifuged for 3 min at 10000 rpm. The supernatant was removed. The residue was washed by 0.5 mL of water. 1.5 mL of methanol was added into the tube and ultrasonic elution of the analytes was carried out. After centrifugation, the obtained eluate was dried under nitrogen stream and dissolved in 250 μL of methanol. The obtained solution filtered with 0.22 μm PTFE filter was referred to as analytical solution and 20 μL of analytical solution was injected into the HPLC system.

2.6 HPLC conditions

The HPLC analysis was conducted in gradient mode. Mobile phases A and B are water and acetonitrile,

respectively. The gradient program was as follows: 0-6 min, 35-50 % B; 6-8 min, 50-55% B; 8-14 min, 55-65% B; 14-18 min, 65-85% B; 18-20 min, 85-95% B; 20-25 min, 95% B; 25-27 min, 95-35% B. The flow rate of mobile phase was set at 0.5 mL min^{-1} and column temperature was kept at 30 ℃. The injection volume of analytical solution was 20 μL. The monitoring wavelengths were 290 nm for megestrol acetate and 242 nm for the others. The reference wavelength and bandwidth were 360 and 16 nm, respectively.

3 Results and discussion

3.1 Characterization of the synthesized MIL-101 (Cr)

The experimental XRD pattern of the synthesized MIL-101 (Cr) crystals is shown in Fig. 1A. The XRD pattern of the as-synthesized MIL-101 (Cr) was in good agreement with the simulated XRD pattern of MIL-101(Cr) reported previously [22, 25], showing the successful preparation of MIL-101(Cr). The N_2 sorption-desorption isotherm is shown in Fig. 1B. P is pressure and P^0 is saturation pressure of N_2. The N_2 sorption isotherm of the prepared MIL-101(Cr) is of type I. The BET surface area of MIL-101 (Cr) is 3023 m^2/g. The TEM image is shown in Fig. 1C and exhibits cubic shaped crystals of MIL-101 (Cr).

3.2 Optimization of d-μ-SPE

3.2.1 Effect of extraction method and extraction time

In the present work, the MIL-101 (Cr) was first dispersed in the sample for 30s. The effect of extraction methods including ultrasonic and shake methods was then investigated. The results are shown in Fig. 2. The recoveries obtained by the two methods are similar. When the ultrasonic method was applied, the noise was produced, which is not beneficial to human health. Therefore, the shake method was selected for

further studies.

The effect of extraction time in the range of 0–20 min was examined under the same experimental conditions. The results were shown in Fig. 3. The recoveries of the analytes increase with the increase of extraction time from 0 to 5 min and changed slightly when the extraction time is longer than 5 min. To ensure complete extraction, the extraction time of 10 min was selected.

3.2.2 Effect of the pH value

The pH value of solutions plays an important role in extraction process, because it can affect the species of the target analytes and thus the recoveries of target analytes. The results are shown in Fig. 4. It can be seen that the recoveries of the analytes are highest when the value of pH is 5. When the value of pH is higher than 7, the recoveries of analytes decrease obviously. MIL-101 (Cr) surface was positively charged when the pH value was below the point of zero charge (9.6). The zeta potential of MIL-101 (Cr) was negative and MIL-101 (Cr) was unstable when the pH value exceeded 9.6 [36]. The hormones are planar and the molecular dimension is smaller than the diameter of MIL-101 (Cr) pores, so the hormones can enter the pores of MIL-101 (Cr). Because of large octanol-water partitioning coefficients of the hormones and the hydrophobic MIL-101 (Cr) framework, the adsorption of analytes on MIL-101 (Cr) should be mainly due to hydrophobic interaction. The analytes are weak acid compounds. When the pH value was excessively low, the analytes can be protonated which is not beneficial to the extraction. In basic solution, the analytes can be ionized which can promote the dissolvent of analytes in sample solution, MIL-101 (Cr) is unstable [36] and thus the recoveries of analytes decrease. Therefore, the pH value of 5 was selected.

3.2.3 Effect of the amount of MIL-101 (Cr)

The effect of the amount of MIL-101 (Cr) ranging from 0.5 to 9 mg was investigated. The results are shown in Fig. 5. It can be seen that the recoveries increase with the increase of the amount of MIL-101 (Cr)

from 0.5 to 5 mg and change slightly with the further increase of MIL-101 (Cr). To ensure complete extraction, 7 mg of MIL-101 (Cr) was selected.

3.2.4 Effect of the concentration of NaCl

Ionic strength of the sample solution was adjusted by addition of NaCl from 0% to 25% (w/v). The effect of ionic strength on the extraction efficiency is shown in Fig. 6. It is observed that the recoveries of the analytes increase with the increase of the concentration of NaCl from 0% to 15% and change slightly with further increase of NaCl concentration. The addition of the NaCl can decrease the solubility of hormones in the aqueous phase and enhance the hydrophobic interaction between hormones and the sorbent and thus the recoveries increase. Therefore, 15 % NaCl was selected for further studies.

3.2.5 Effect of elution condition

In the work, methanol was used as elution solvent in terms of its strong dissolvent of hormones. The effect of volume of methanol ranging from 0.5 to 2 mL was investigated. The results are shown in Fig. 7. It can be seen that the recoveries increase with the increase of methanol from 0.5 to 1.5 mL and change slightly with a further increase of methanol volume. So 1.5 mL of methanol was used as elution solvent.

The elution was carried out in ultrasonic bath, because ultrasonic irradiation can facilitate the elution of analytes. The effect of elution time ranging from 0.5 to 5 min was investigated. The recoveries increase slightly with the increase of elution time from 0.5 to 1 min and change slightly from 1 to 5 min. Therefore, the elution was performed under ultrasonic irradiation for 1 min.

3.3 Evaluation of the method

3.3.1 Limits of detection and quantification

The working curves were constructed by plotting the peak areas measured versus the concentrations of the analytes in spiked sample. The working curves were also evaluated by using correlation coefficient. The

linear regression equations and correlation coefficients are listed in Table 2. Good linearity was achieved for all of the analytes as indicated by the equations.

The limit of detection (LOD) and quantification (LOQ) for each analyte was determined as the concentrations which yields a signal-to-noise (S/N) ratio of 3 and 10, respectively. The LODs for analytes ranged from 0.36 to 0.91 μg/L. The LOQs of all analytes ranged from 1.20 to 3.04 μg/L.

3.3.2 Precision and Recovery

The intra- and inter-day precision was obtained by analyzing spiked cosmetic samples at the three spiked concentrations of 5, 50 and 100 μg/L, respectively. The intra-day precision was obtained by analyzing a sample five times in one day. The inter-day precision was obtained by analyzing a sample once a day over five consecutive days. The intra- and inter-day precision is expressed as the relative standard deviations (RSDs). The results are listed in Table 3. The RSDs for intra- and inter-day are lower than 6.1% and the recoveries for intra- and inter-day ranged from 92.7 to 102.0%. The precision and recovery of the present method are satisfactory.

In order to further evaluate the performance of the present method, the enrichment factor (EF) was investigated. To obtain the concentration of the analytes in analytical solution, the standard curve was prepared by direct introduction of the standard working solution into the HPLC system. EF was calculated based on the ratio of the concentration of analyte in analytical solution to the concentration of analytes in sample (5mL). In the present method, the EFs for prednisone, meprednisone, boldenone, nandrolone, testosterone, dexamethasone acetate, 17-hydroxyprogesterone, norgestrel, medroxyprogesterone, megestrol acetate, progesterone and testosterone 17-propionateand were 10.8, 13.7, 13.9, 16.7, 14.2, 16.1, 14.6, 17.8, 16.6, 15.8, 17.0, 17.1, 17.0 and 17.5, respectively.

3.3.3Analysis of samples

The analytes were undetectable in samples. The practical applicability of the present method was evaluated by determining the hormones from four spiked cosmetic samples. The recoveries and precision of analytes in spiked cosmetic samples are listed in Table 4. The recoveries range from 89.5 to 103.4%. The present method provides acceptable precision (≤6.7%) at two spiking concentration levels. The chromatograms of blank and spiked sample 1 are shown in Fig. 8.

3.3.4 Comparison of the present method with other methods

The performances of the present method were compared with those of other methods which were reported on determination of hormones in coesmetics. These methods include HILME [6], PMME [17], MSEBLLME [18], LLE-SPE) [12] and LLE [7]. The details are listed in Table 5. The LODs obtained by the present method are lower than those obtained by most listed methods. It can be seen that the present method is rapid, simple and solventless. It could be a potential method for the screening chemicals in cosmetics.

4. Conclusion

In the present method, a d-μ-SPE method based on MIL-101 (Cr) was developed for the extraction of hormones from liquid cosmetics. The consumption of sorbent and organic solvent is low. The operation is rapid and simple. The experimental results indicated that the present method is suitable for extraction of hormones in liquid cosmetics. It will be possible to extend this method to the extraction of hormones in cosmetic, biological and environmental samples, such as urine and water, by varying the extraction conditions.

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Figure captions

Fig. 1XRD pattern (A), N_2 adsorption-desorption isotherm (B), TEM image (C) of the preparaed MIL-101 (Cr)

Fig. 2 Effect of extraction method. Extraction time, 10 min; pH, 5; amount of MIL-101 (Cr), 7 mg; the concentration of NaCl, 15%; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.

Fig. 3 Effect of extraction time. Extraction method, shake; pH, 5; amount of MIL-101 (Cr), 7 mg; the concentration of NaCl, 15%; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.

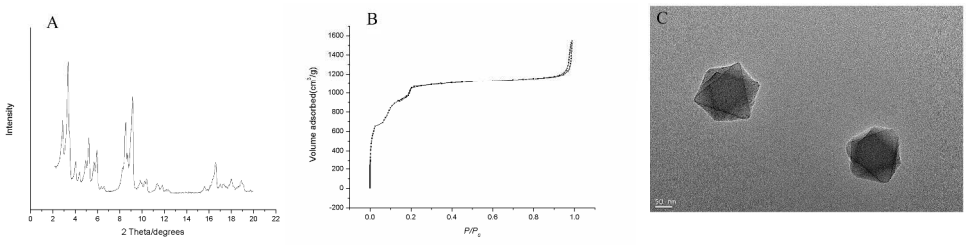
Fig. 4 Effect of pH of sample solution. Extraction, shake for 10 min; amount of MIL-101 (Cr), 7 mg; the concentration of NaCl, 15%; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.

Fig. 5 Effect of the amount of MIL-101 (Cr). Extraction, shake for 10 min; pH, 5; the concentration of NaCl, 15%; elution, 1.5 mLof methanol; spiked concentration, 50 μg/ L.

Fig. 6 Effect of concentration of NaCl. Extraction, shake for 10 min; pH, 5; amount of MIL-101 (Cr), 7 mg; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.

Fig. 7 Effect of the volume of elution solvent. Extraction, shake for 10 min; pH, 5; amount of MIL-101(Cr), 7 mg; the concentration of NaCl, 15%; spiked concentration, 50 μg/ L.

Fig. 8 The chromatograms of (A) blank and (B) spiked sample 1 at 50μg/L of hormones obtained at 242 nm and 290 nm. 1, prednisone; 2, meprednisone; 3, boldenone; 4, nandrolone; 5, testosterone; 6, dexamethasone acetate; 7, 17-hydroxyprogesterone; 8, norgestrel; 9, Medroxyprogesterone; 10, megestrol acetate; 11, progesterone and 12, testosterone 17-propionateand.



XRD pattern (A), N2 adsorption-desorption isotherm (B), TEM image (C) of the preparaed MIL-101 (Cr)
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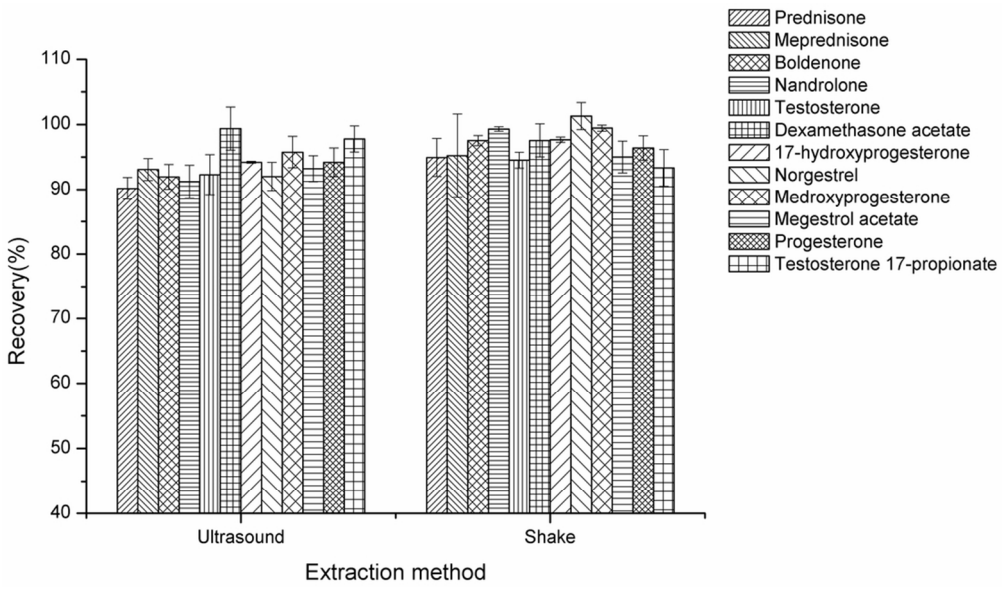

Effect of extraction method. Extraction time, 10 min; pH, 5; amount of MIL-101 (Cr), 7 mg; the concentration of NaCl, 15%; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.
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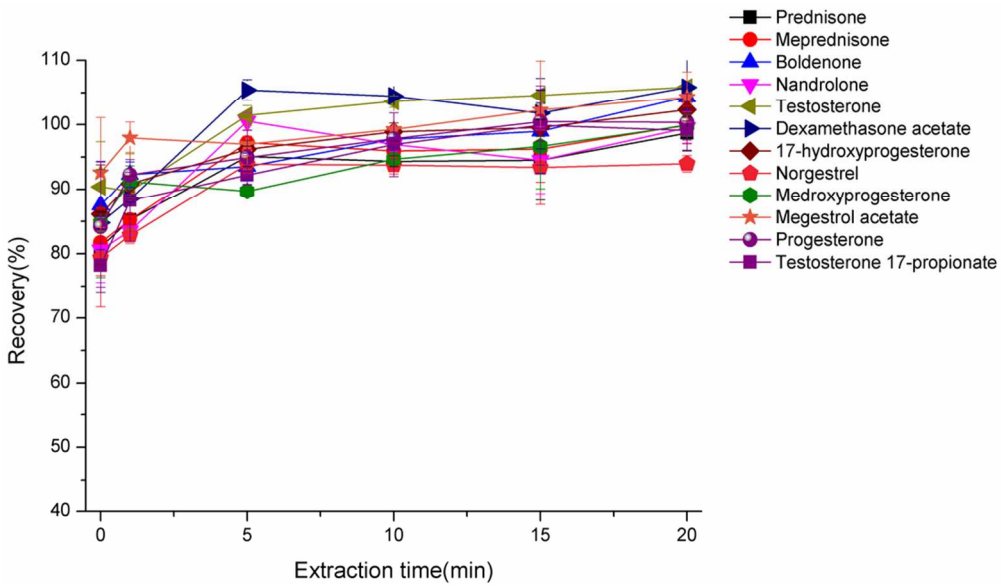

Effect of extraction time. Extraction method, shake; pH, 5; amount of MIL-101 (Cr), 7 mg; the concentration of NaCl, 15%; elution, 1.5 mL of methanol; spiked concentration, 50 µg/ L.
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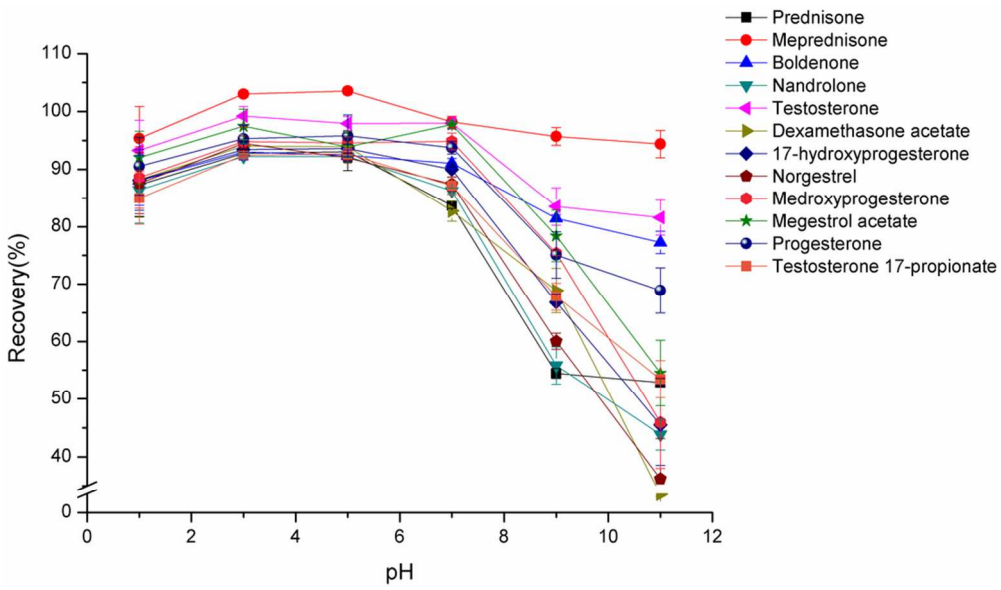

Effect of pH of sample solution. Extraction, shake for 10 min; amount of MIL-101 (Cr), 7 mg; the concentration of NaCl, 15%; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.
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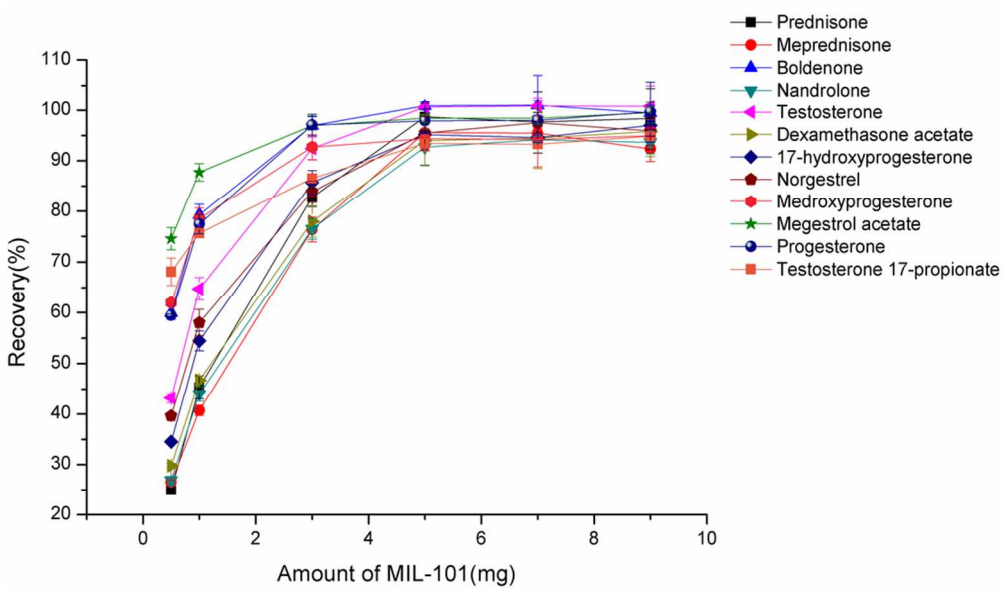
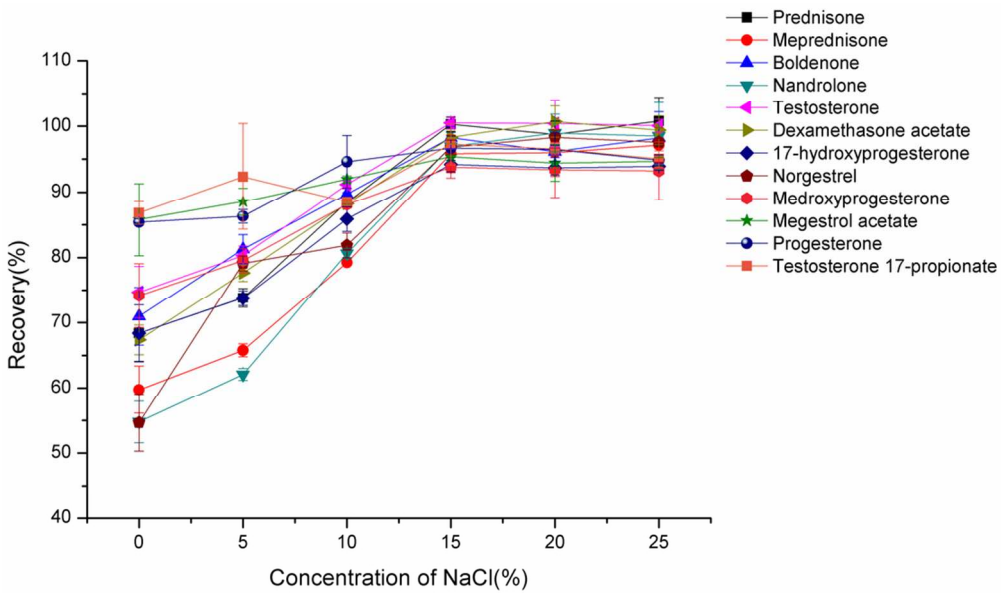
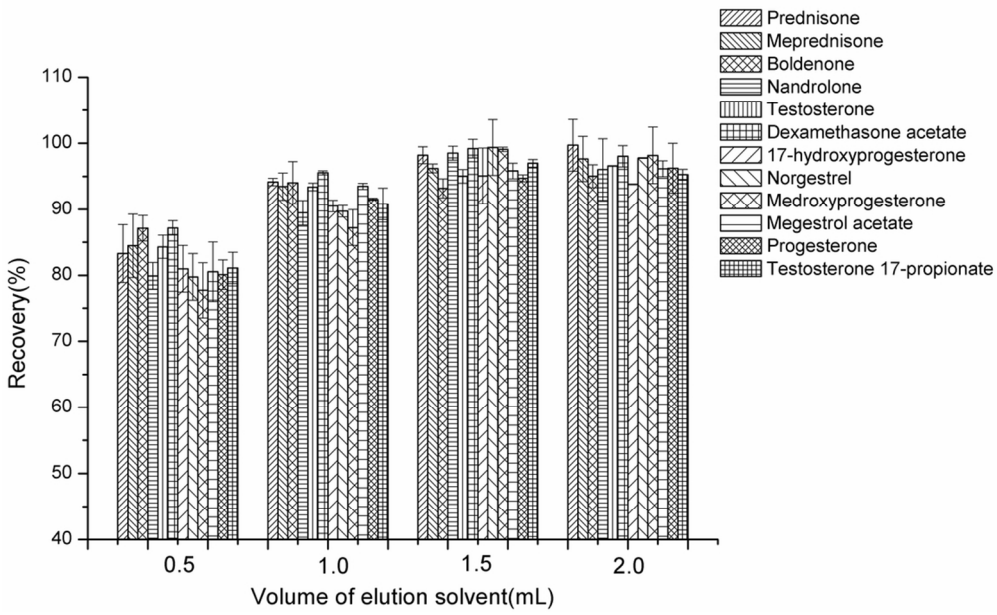


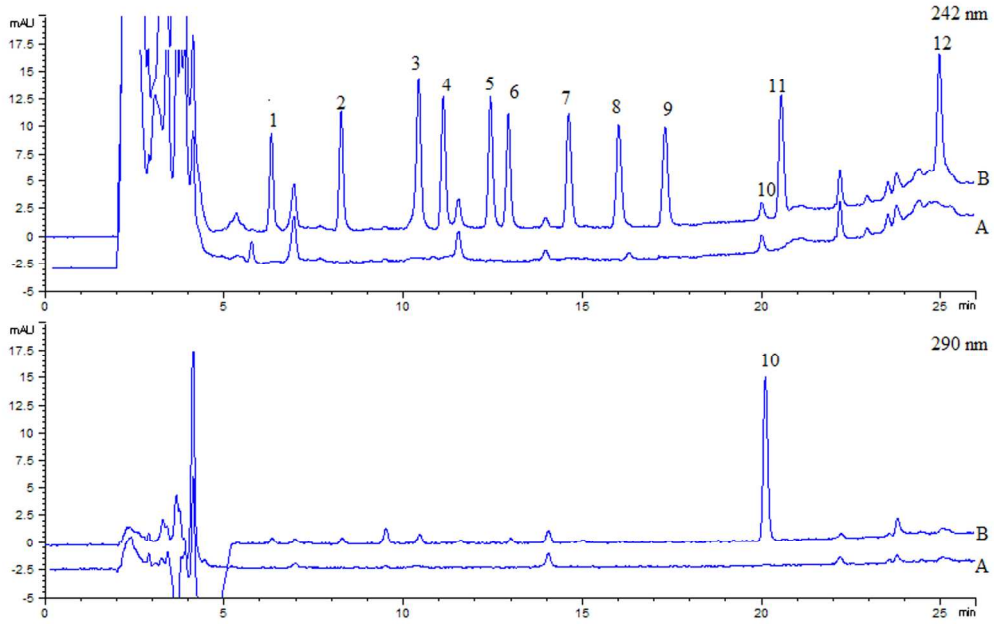
Effect of the amount of MIL-101 (Cr). Extraction, shake for 10 min; pH, 5; the concentration of NaCl, 15%; elution, 1.5 mLof methanol; spiked concentration, 50 μg/ L.
93x54mm (300 x 300 DPI)



Effect of concentration of NaCl. Extraction, shake for 10 min; pH, 5; amount of MIL-101 (Cr), 7 mg; elution, 1.5 mL of methanol; spiked concentration, 50 μg/ L.
94x55mm (300 x 300 DPI)

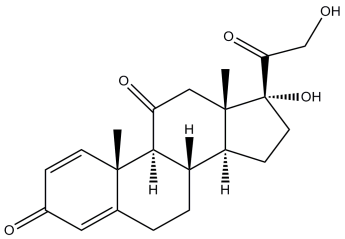
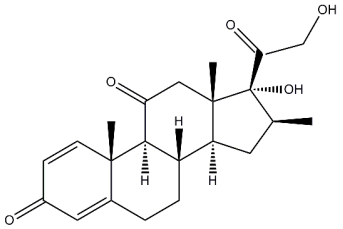
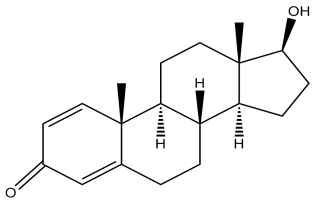
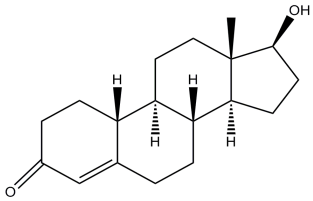
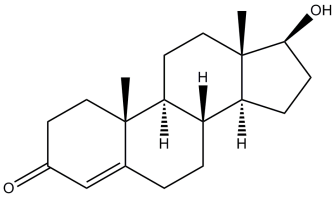
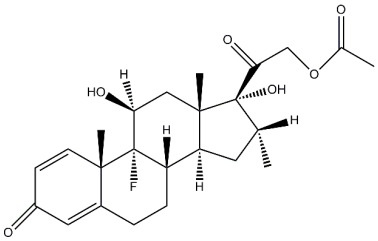


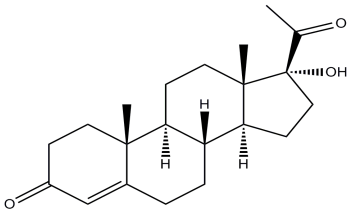
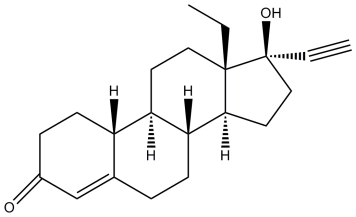
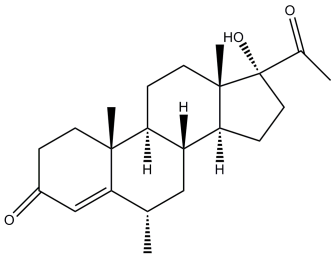
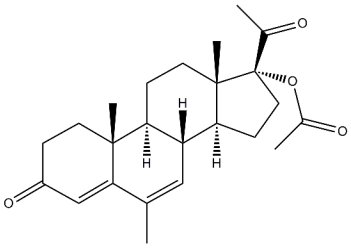
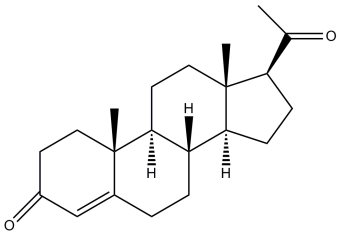
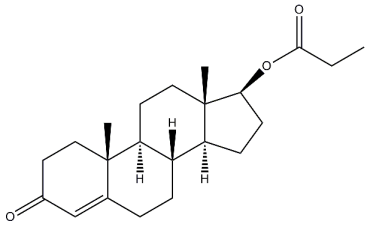
Effect of the volume of elution solvent. Extraction, shake for 10 min; pH, 5; amount of MIL-101(Cr), 7 mg; the concentration of NaCl, 15%; spiked concentration, 50 μg/ L.
97x59mm (300 x 300 DPI)



The chromatograms of (A) blank and (B) spiked sample 1 at 50µg/L of hormones obtained at 242 nm and 290 nm. 1, prednisone; 2, meprednisone; 3, boldenone; 4, nandrolone; 5, testosterone; 6, dexamethasone acetate; 7, 17-hydroxyprogesterone; 8, norgestrel; 9, Medroxyprogesterone; 10, megestrol acetate; 11, progesterone and 12, testosterone 17-propionateand.
215x139mm (300 x 300 DPI)

Table 1 Physical-chemical properties of the hormones

Analyte	CAS number	Molecular weight	pk_a	logKow	Molecular demension (nm×nm)	Summary structure	Structure
Prednisone	53-03-2	358.4	12.36	1.566	0.82×2.03	$C_{21}H_{26}O_5$	
Meprednisone	1247-42-3	372.5	12.38	2.101	0.80×1.60	$C_{22}H_{28}O_5$	
Boldenone	846-48-0	286.4	15.05	3.085	0.61×1.51	$C_{19}H_{26}O_2$	
Nandrolone	434-22-0	274.4	15.06	2.898	0.61×1.45	$C_{18}H_{26}O_2$	
Testosterone	58-22-0	288.4	15.06	3.179	0.61×1.45	$C_{19}H_{28}O_2$	
Dexamethasone acetate	1177-87-3	434.5	12.08	2.654	0.85×2.00	$C_{24}H_{31}FO_6$	

17-hydroxyprogesterone	68-96-2	330.5	13.03	3.040	0.67×1.76	$C_{21}H_{30}O_3$	
Norgestrel	6533-00-2	312.5	13.09	3.368	1.14×1.50	$C_{21}H_{28}O_2$	
Medroxyprogesterone	520-85-4	344.5	13.03	3.576	0.68×1.77	$C_{22}H_{32}O_3$	
Megestrol acetate	595-33-5	384.5	n.a.	3.748	0.99×1.78	$C_{24}H_{32}O_4$	
Progesterone	57-83-0	314.5	n.a.	3.827	0.62×1.75	$C_{21}H_{30}O_2$	
Testosterone 17-propionate	57-85-2	344.5	n.a.	4.654	0.68×1.73	$C_{22}H_{32}O_3$	

logKow: octanol-water partitioning coefficients. Molecular weights, logKow and pk_a are obtained from SciFinder scholar database.

Table 2 Analytical performance

Analyte	Regression equation	Correlation coefficient	Liner range (μg/L)	LOD (μg/L)	LOQ(μg/L)
Prednisone	A=1.359*c*-0.129	0.9999	2.87-183.8	0.84	2.81
Meprednisone	A=1.264*c* +1.797	0.9997	4.05-259.0	0.91	3.04
Boldenone	A=1.920*c*+1.948	0.9998	1.56-200.0	0.44	1.46
Nandrolone	A=1.792*c* +0.873	0.9999	1.56-200.0	0.45	1.49
Testosterone	A=1.792*c*+1.909	0.9999	1.56-200.0	0.44	1.48
Dexamethasone acetate	A=1.430*c*+1.572	0.9997	1.60-204.5	0.46	1.54
17-hydroxyprogesterone	A=1.768*c*+0.458	0.9999	1.62-207.2	0.43	1.44
Norgestrel	A=1.714*c*+1.111	0.9998	1.56-200.0	0.41	1.38
Medroxyprogesterone	A=1.779*c*+0.230	0.9999	1.63-208.3	0.47	1.56
Megestrol acetate	A=2.360*c*+2.619	0.9999	1.56-200.0	0.36	1.20
Progesterone	A=1.781*c*+0.566	0.9999	1.56-200.0	0.46	1.54
Testosterone 17-propionate	A=1.811*c*+0.518	0.9997	1.46-187.2	0.45	1.50

Table 3 Precision and recovery

Analytes	Added (μg/L)	Intra-day (n=5)		Inter-day (n=5)	
		Recovery (%)	RSD(%)	Recovery (%)	RSD(%)
Prednisone	5	97.8	4.3	94.3	3.2
	50	97.7	5.3	101.4	3.2
	100	99.8	5.9	100.5	5.8
Meprednisone	5	92.9	1.9	92.7	2.2
	50	99.1	2.3	98.6	3.3
	100	97.3	4.8	96.4	3.3
Boldenone	5	96.3	1.3	96.9	2.2
	50	95.2	2.0	96.3	4.8
	100	95.8	3.5	96.7	4.4
Nandrolone	5	99.7	6.1	96.4	3.8
	50	96.7	1.2	97.5	4.0
	100	96.6	3.3	99.0	3.4
Testosterone	5	94.1	3.8	94.1	4.8
	50	94.9	0.9	99.2	2.7
	100	99.1	3.2	98.5	3.8
Dexamethasone acetate	5	99.5	2.9	96.1	4.6
	50	98.9	1.5	98.0	3.1
	100	100.5	4.6	101.9	2.8
17-hydroxyprogesterone	5	97.2	2.2	94.3	4.1
	50	97.4	1.2	99.3	2.6
	100	94.1	2.7	99.9	5.6
Norgestrel	5	98.0	0.9	96.4	2.0
	50	98.0	2.5	100.2	5.7
	100	94.2	4.2	97.8	4.7
Medroxyprogesterone	5	102.0	2.3	97.7	5.1
	50	94.7	1.3	94.8	3.9
	100	98.0	3.2	98.0	5.4
Megestrol acetate	5	97.7	2.8	95.3	4.3
	50	96.1	2.1	94.9	4.1
	100	97.1	2.5	96.5	3.7

Progesterone	5	96.4	1.3	96.9	3.3
	50	94.6	1.8	95.6	4.2
	100	98.1	4.2	98.2	4.9
Testosterone 17-propionate	5	96.6	3.2	97.1	3.3
	50	96.1	2.1	96.6	3.1
	100	98.5	2.5	100.1	3.5

Table 4 Analytical results for fresh spiked samples

Sample	Added (μg/L)	Prednisone		Meprednisone		Boldenone		Nandrolone		Testosterone		Dexamethasone acetate		17-hydroxyprogesterone		Norgestrel		Medroxyprogesterone		Megestrol acetate		Progesterone		Testosterone 17-propionate	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	10	91.4	2.4	94.0	0.8	94.3	4.3	91.8	3.3	90.4	3.7	91.4	0.7	91.2	1.7	89.5	1.7	93.9	1.7	91.1	2.7	96.5	4.0	96.1	0.6
	100	93.2	6.1	92.4	1.0	92.2	3.0	91.9	1.2	91.2	0.5	92.7	0.8	90.1	2.9	94.1	2.9	92.6	0.7	89.8	2.0	91.2	2.1	90.0	3.5
2	10	92.1	1.6	96.8	1.5	102.4	6.7	92.3	3.8	98.6	3.7	98.4	1.5	103.3	2.1	98.8	2.1	97.3	0.3	97.4	4.3	100.3	5.1	101.4	1.7
	100	96.0	2.3	93.3	2.1	100.7	3.7	94.4	2.1	99.8	2.0	98.1	1.6	100.9	1.8	98.5	1.8	98.6	1.3	100.6	1.2	102.8	2.0	103.2	1.1
3	10	93.2	1.2	96.5	1.1	98.2	5.0	96.0	1.8	98.6	2.4	93.2	3.2	99.5	2.1	95.2	2.1	96	1.1	94.3	6.0	91.5	2.3	95.3	0.3
	100	93.0	2.5	95.2	0.8	96.1	0.8	95.8	2.1	100.6	0.5	96.5	2.3	99.5	1.0	98.6	1.0	95.3	0.3	96	1.0	99.7	0.2	92.6	1.4
4	10	93.9	1.2	98.6	1.8	100.4	1.9	101.3	2.0	98.9	4.1	99.2	1.8	102.9	1.8	102.3	1.8	101.5	2.1	98.5	3.9	101.4	2.0	98.5	0.6
	100	96.8	1.8	99.6	3.4	99.6	2.9	98.4	0.1	99.5	1.9	97.4	0.6	99.9	5.3	102.7	5.3	100.3	3.9	98.2	4.1	103.4	0.9	99.8	0.5

Table 5 Comparison of the present method with other methods

Method	Extraction time(min)	Clean-up steps	Extraction solvent(mL)	Recovery (%)	RSDs (%)	Detection	LOD	LOQ	Ref.
D-μ-SPE	10	0	0.0	92.1-102.0	⩽6.1	HPLC-DAD	0.36-0.91	1.20-3.04	this work
HILME	2	0	0.07	93.2-114.2	⩽5.2	UPLC-UV	0.03-0.24	0.10-0.79	6
PMME	5	0	0.0	83-119	⩽7.7	HPLC-UV	2.2-4.6	7.7-15.3	17
MSEBLLME	40	0	-	91.3-106.2	0.2-5.5	HPLC-DAD	0.91-1.01	3.04-5.97	18
LLE-SPE	10	1	10.0	88-98	⩽4.0	HPLC-DAD	100-600	-	12
LLE	10	0	100	91.1-97.1	1.2-13.8	HPLC-DAD	400-700	1300-2100	7
LLE	10	0	100	91.5-97.5	0.6-14.8	HPLC-MS	14-18	46-52	7