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Rapid UHPLC-MS/MS Detection of Prohibited Drugs in Cosmetics Using Pass-Through SPE

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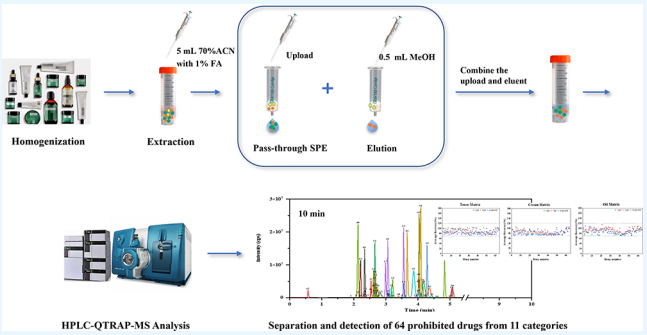
ABSTRACT: Prolonged exposure to drugs prohibited in cosmetics may cause irritation and allergic reactions in humans. In this study, a method using ultrahigh performance liquid chromatography-tandem mass spectrometry was established for the simultaneous determination of 64 prohibited drugs spanning 11 categories, including 39 antibiotics, 8 antiallergics, 7 anesthetics, and 10 hormones. Typical cosmetic samples containing toner, cream, and oil matrices were extracted using a 70% acetonitrile solvent system with 1% formic acid and purified using a pass-through cleanup solid-phase extraction approach with a PRiME hydrophobic−lipophilic balanced column. Analytes covering a wide range of polarities showed excellent recoveries between 70 and 120%, with relative standard deviations of <11%. Excellent sensitivities ranging from 0.1 to 1 μg/kg were achieved with the limits of quantification. This method provides a rapid and comprehensive targeted strategy for the analysis of multiclass prohibited drugs in various cosmetic matrices. Finally, analysis of 20 cosmetic products using the optimized method identified prohibited substances in 6 samples at concentrations spanning 7 orders of magnitude.



1. INTRODUCTION

Cosmetics are commonly used in daily life for personal skin care or to improve appearance and odor; thus, the global cosmetics industry has become a large market. Cosmetic products include a wide variety of toners, creams, essences, and treatment oils; however, social concerns regarding the quality and safety of cosmetic products have risen alongside their popularity. Annex II of the European Regulation 1223/2009 on cosmetic products[1] and China's Safety and Technical Standards for Cosmetics (Version 2022)[2] ban a wide range of compounds from cosmetics, including hormones, antibiotics, and other pharmacologically active substances. However, illegal additives such as antibiotics or hormones are still added to cosmetics to achieve exaggerated functions such as whitening, elimination of freckles, and acne removal, significantly compromising their safety. Long-term exposure to harmful substances in cosmetics may pose a serious risk to human health, with symptoms ranging from relatively mild skin irritation or allergies to a compromised immune system and potential genotoxicity.[3,4] Increasing social concerns have been placed on the safety and quality control of cosmetic products, with the primary concern being the prevention of illegal additives.

Qualitative and quantitative analysis of the residues in cosmetics is currently performed using liquid chromatography (LC),[5,6] gas chromatography (GC),[7] chromatography-tandem mass spectrometry, including LC-MS/MS[8−12] and GC-MS/MS,[13] capillary electrophoresis (CE),[14] and electrochemistry.[15] Mass spectrometry (MS) is considered the "gold standard"[16] owing to its high sensitivity, throughput, and reproducibility; thus, trace multiresidue analysis is commonly performed using ultrahigh performance LC-tandem mass spectrometry (UHPLC-MS/MS).

Cosmetics are complex matrices containing various natural and artificial substances, including emulsifiers, surfactants, fats, waxes, water, preservatives, pigments, and flavors among others. Moreover, different forms of cosmetic matrices, including toners, creams, and oils, may cause disparate matrix effects via interactions between analytes and other constituents or by affecting ionization efficiency. Similarly, illegal additives in cosmetics such as prohibited drugs have various physical and chemical properties, with the oil - water partition coefficients (Log P) investigated in this study ranging from −1.8 to 6.1. The development of an efficient sample preparation method for the simultaneous extraction of such compounds is therefore

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Table 1. Category, CAS Number, Retention Times, and MS Parameters of the 64 Prohibited Drugs[a]

No.	Category	Compound	CAS No.	RT/min	Precursor ion (m/z)	Product ion (m/z)	DP/V	CE/V	ESI mode
1	β-lactams	Penicillin G	69−57−8	3.64	335.1	160, 114	40	13, 45	+
2		Oxacillin	66−79−5	4.13	402.1	160.1, 144	40	16, 35	+
3		Ampicillin	69−53−4	2.34	350.1	106.1, 192	40	22, 20	+
4		Piperacillin	61477−96−1	3.5	518.1	143, 160	80	20, 13	+
5		Amoxicillin	26787−78−0	2.1	366.1	114, 208	40	24, 17	+
6		Tazobactam sodium	89785−84−2	2.1	301	168, 207	60	20, 20	+
7	Cephalosporins (CEP)	Cefazolin	25953−19−9	2.65	454.9	323, 155.8	60	15, 21	+
8		Cephalexin	15686−71−2	2.34	348	157.9, 174	55	13, 21	+
9		Ceftriaxone	73384−59−5	2.36	555	396, 324.1	70	15, 25	+
10		Ceftazidime	72558−82−8	2.2	547	467.8, 166.9	60	16, 34	+
11	Tetracyclines (TET)	Tetracycline	64−75−5	2.68	445.1	410.2, 427.1	80	24, 19	+
12		Doxycycline	24390−14−5	3.2	445	428.1, 154.1	80	24, 35	+
13		Minocycline	13614−98−7	2.4	458.2	423.1, 352	80	33, 40	+
14		Oxytetracycline	2058−46−0	2.54	461.2	426.2, 443	80	25, 17	+
15		Chlortetracycline	64−72−2	3.06	479.1	462, 444	80	24, 28	+
16	Macrolides (MAR)	Clarithromycin	81103−11−9	4.16	748.5	590.5, 158.1	100	24, 33	+
17		Clindamycin	18323−44−9	3	425.3	126.1, 377.1	50	32, 27	+
18	Nitroimidazoles (NIT)	Metronidazole	443−48−1	2.15	172.2	127.9, 82	50	20, 37	+
19		Tinidazole	19387−91−8	2.65	248.2	121.2, 93	80	21, 25	+
20		Nitrofurantoin	67−20−9	2.8	239.2	122, 67	60	28, 28	+
21	Quinolones (QUI)	Enoxacin	74011−58−8	2.52	321	303, 234	80	24, 30	+
22		Fleroxacin	79660−72−3	2.65	370	326.1, 269.2	80	27, 35	+
23		Enrofloxacin	93106−60−6	2.8	360	316.1, 245.1	80	25, 35	+
24		Norfloxacin	70458−96−7	2.6	320.1	276.1, 233.1	80	26, 35	+
25		Pefloxacin	70458−95−6	2.65	334.1	316.1, 290.2	80	27,25	+
26		Ciprofloxacin	93107−08−5	2.7	332.1	288.1, 245.1	80	25, 33	+
27		Ofloxacin	82419−36−1	2.6	362.2	318.1, 261.1	80	26, 38	+
28		Sarafloxacin	91296−87−6	3.04	386	342.3, 299	80	25, 38	+
29		Moxifloxacin	186826−86−8	3.06	402.2	364.2, 260	60	38, 45	+
30		Lomefloxacin	98079−52−8	2.73	352	265, 308.1	80	33, 28	+
31	Sulfonamines (SUL)	Sulfamethoxazole	723−46−6	3.22	254.1	156, 108	65	22, 36	+
32		Sulfadiazine	68−35−9	2.37	251.1	156, 92	40	22, 38	+
33	Other anti-infectives	Fluconazole	86386−73−4	2.8	307	238.2. 220	60	23, 23	+
34		Ketoconazole	65277−42−1	3.92	531.1	489, 255	120	40, 48	+
35		Clotrimazole	23593−75−1	4.3	277	165, 241	95	33, 38	+
36		Econazole	27220−47−9	4.9	381	125, 193	100	34, 23	+
37		Griseofulvin	126−07−8	4.27	353	165, 215	95	26, 26	+
38		Miconazole	22916−47−8	5.1	417	159, 161.1	110	36, 32	+
39		Chloramphenicol	56−75−7	3.33	321	152.1, 256.9	−75	−24, −17	-
40	Antiallergics (ANT)	Loratadine	79794−75−5	4.2	383.1	337.1, 267.1	80	32, 43	+
41		Chlorpheniramine	113−92−8	3.67	275.1	230.2, 167	40	20, 50	+
42		Diphenhydramine	58−73−1	3.87	256.1	167.1, 152	40	15, 51	+
43		Promethazine	60−87−7	3.87	285.1	86, 198	60	19, 28	+
44		Perphenazine	58−39−9	4	404.2	171.1, 143.1	80	29, 38	+
45		Cetirizine	83881−51−0	4.05	389.2	201.1, 166	60	26, 55	+
46		Chlorpromazine	50−53−3	4.37	319.1	86.1, 58.1	60	25, 70	+
47		Cyproheptadine	969−33−5	4	288.1	191.1, 215.1	100	40, 70	+
48	Narcotics (NAR)	Procainamide	51−06−9	2.1	236.1	163, 120	20	23, 41	+
49		Procaine	59−46−1	2.32	237.1	100, 120	35	20, 34	+
50		Chloroprocaine	3858−89−7	2.6	271	154, 100	20	38, 20	+
51		Benzocaine	94−09−7	3.6	166	138, 94	20	17, 25	+
52		Lidocaine	137−58−6	2.65	235.1	86.1, 191.9	60	20, 33	+
53		Tetracaine	94−24−6	3.56	265.1	176, 72	55	23, 54	+
54		Cinchocaine	85−79−0	4.1	344.2	271, 116	40	29, 70	+
55	Hormones (HOR)	Methyltestosterone	58−18−4	4.44	303.2	109, 97	106	30, 28	+
56		Progesterone	57−83−0	5.1	315.1	97, 109	65	27, 33	+
57		Estrone	53−16−7	2.6	271.1	154, 100	45	34, 20	+
58		Hydrocortisone	50−23−7	3.41	363.2	121.1, 105	80	31, 68	+
59		Triamcinolone	76−25−5	4.04	435.2	415.2, 397.2	80	15, 15	+
60		Estradiol	57−91−0	2.6	273.1	100, 156	30	21, 36	+

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Table 1. continued

No.	Category	Compound	CAS No.	RT/min	Precursor ion (m/z)	Product ion (m/z)	DP/V	CE/V	ESI mode
61		Ephedrine	299−42−3	2.2	166.1	148.1, 133.1	40	15, 26	+
62		(−)-Epinephrine	51−43−4	0.56	184.1	166.1, 107	20	15, 30	+
63		Diethylstilbestrol	56−53−1	5	266.9	251.1, 237.1	−100	−34, −36	-
64		Dienestrol	84−17−3	4.68	264.9	249.1, 235.1	−115	−35, −25	-

[a]Quantitative ion.

essential for high-throughput mass analyses. Several simple and facile pretreatment methods with low solvent consumption have been developed in recent years, including vortex extraction[13] and the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method,[17−19] which offer advantages over the conditional solid-phase extraction (SPE) and gel permeation chromatography (GPC).[20,21] While the QuEChERS method achieves rapid qualitative screening the recoveries for the quantitative analysis of some analytes, particularly tetracycline drugs, are unsatisfactory.[20] The hydrophilic−lipophilic balanced (HLB) cartridge showed excellent performance in the detection of multiclass veterinary drug in meat;[23,24] however, the use of HLB in the analysis of cosmetics is limited, with no reported examples of the use of HLB in the analysis of the complex matrices of creams and oils, and only one application in lotion.[25] The PRiME HLB cartridge, with the filling components of reversed-phase N-vinylpyrrolidone and divinylbenzene copolymer, requires no equilibration or washing steps and is therefore easier to use and saves more time than the HLB cartridge. The combination of a one-step vortex and PRiME HLB pass-through cleanup may satisfy the requirements for simple and highly efficient cosmetic sample preparation.

In this study, we established a method for the simultaneous determination of 64 prohibited drugs spanning 11 categories, including 39 antibiotics, 8 antiallergics, 7 anesthetics, and 10 hormones, using UHPLC-MS/MS coupled with pass-through cleanup SPE in three types of cosmetic matrices. This method offers simple, rapid, efficient analysis with low solvent consumption, was successfully applied to the analysis of several cosmetic samples from markets, and has shown great potential for cosmetic risk evaluation.

2. MATERIALS AND METHODS

2.1. Prohibited Drugs Selection. The requirements for the approval of prescription drugs in China are much stricter than those for functional cosmetics such as antiacne, whitening and anti-itch products. Despite being prohibited by the Chinese Safety and Technical Standards for Cosmetics, various antibiotics, hormones, antihistamines, and other drugs are still illegally added to functional products by manufacturers to increase the effect of the product. Developing targeted screening and quantitative analytical methods to identify prohibited drugs illegally added to cosmetics is therefore of vital importance. Antibiotic drugs comprise a wide variety of compounds that are classified according to their structure, including β-lactams, cephalosporins, tetracyclines, macrolides, nitroimidazoles, quinolones, sulfonamides, lincomamides, aminoglycosides and chloramphenicols. Such compounds can be illegally added to functional cosmetics. For example, antiacne toner or essential oils may be supplemented with antibiotics and hormones; whitening creams and essence products may contain nitroimidazoles; and anti-itch gels or creams can contain narcotics or antiallergics. The compounds targeted in this study comprise 64 prohibited drugs spanning 11 categories, including 39 antibiotics representing 8 categories, 8 antiallergics, 7 anesthetics, and 10 hormones.

2.2. Materials and Reagents. Standards of the prohibited drugs were purchased from Alta Scientific (Tianjin, China). The categories and CAS numbers of these drugs are shown in Table 1. Individual stock solutions of the 64 prohibited drugs in methanol (100 μg/mL) were prepared. Working standard mixtures (1 μg/mL) were diluted with a 1:1 v/v mixture of methanol and water. Beta-lactams, cephalosporins, and tetracyclines were diluted before use because of their instability. All solutions were stored in brown glass vials at −20 °C.

Water, methanol, acetonitrile (LC−MS grade), and formic acid were purchased from Fisher Scientific (Waltham, MA, USA). A Kinetex F5 chromatography column (100 mm × 2.1 mm, 2.6 μm) was acquired from Phenomenex (California, USA). Oasis PRiME HLB (3 cc, 60 mg) cartridges were purchased from Waters (Milford, MA, USA).

2.3. Samples Collection. Samples of cosmetic products, including toner, cream and treatment oil were purchased from local supermarkets (Beijing, China) and online stores (China). All samples were stored at room temperature and shaken manually before use.

2.4. Sample Preparation. A mixed working standard solution was added to homogenized cosmetic samples (1.00 ± 0.01 g) in 50 mL centrifuge tubes. After standing for 5 min, 70% acetonitrile solvent (5 mL) containing 1% formic acid was added, vortexed for 5 min at 2500 rpm on a multichannel vortex (Tuohe Ltd., Shanghai, China), and ultrasonicated (LumTech Ltd., Beijing, China) for 5 min. The extraction solution was centrifuged at 10 000 rpm for 5 min at room temperature, and the supernatant was subjected to SPE cleanup. The supernatant (1 mL) was loaded onto a PRiME HLB cartridge on a multichannel SPE instrument (J2 Scientific, USA) and passed through at a flow rate of 1 drop/s; the cartridge was further eluted with methanol (0.5 mL). All effluents were collected and thoroughly mixed. The solution was filtered through a 0.22 μm filter membrane prior to analysis by UHPLC-MS/MS.

2.5. UHPLC-MS/MS Analysis. UHPLC-MS/MS was performed using a UHPLC system (LC-30AD, Shimadzu, Japan) equipped with a Kinetex F5 column (100 mm × 2.1 mm, 2.6 μm, Phenomenex, USA) and coupled with a triple quadrupole mass spectrometer (SCIEX QTRAP 6500, AB SCIEX, Singapore) equipped with a Turbo V ion source. The 64 compounds listed in Table 1 were separated using a mobile phase consisting of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B). A gradient elution was applied as follows: 0−0.5 min, 3% B; 0.5−0.6 min, 3%−15% B; 0.6−4.5 min, 15%−75% B; 4.5−4.6 min, 75%−95% B; 4.6−5.5 min, 95% B; 5.5−6.0 min, 95%−3% B; 6.0−10.0 min,

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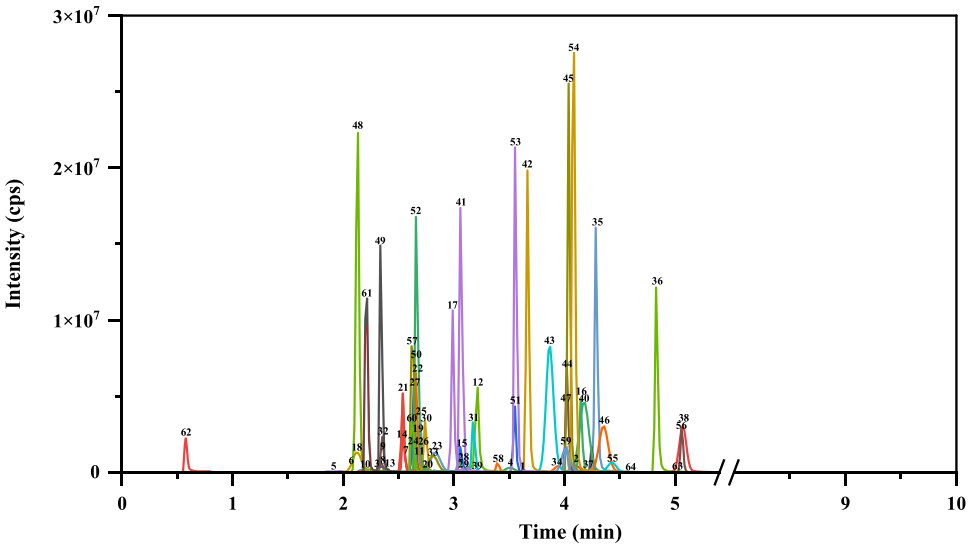

Figure 1. Optimized UHPLC-QTRAP-MS extracted ion chromatograms of 64 compounds (10 ng/mL). (Drug numbers refer to Table 1).

3% B, at a flow rate of 0.4 mL/min. The column oven and the autosampler were maintained at 40 and 10 °C, respectively. The injection volume was 5 μL.

Tandem MS was performed using both positive and negative electrospray ionization (ESI) with scheduled multiple reaction monitoring (sMRM). The optimized parameters included a source temperature of 350 °C, curtain gas pressure of 25 psi, CAD gas pressure of 12 psi, GAS 1 pressure of 55 psi, GAS 2 pressure of 50 psi, positive ion spray voltage of 5500 V, and negative voltage of − 4500 V. The scheduled MRM parameters, including the retention time, declustering voltage−potential (DP), and collision energy (CE) are shown in Table 1. The total ion chromatograms of the 64 compounds are shown in Figure 1. Data was acquired and processed using the SCIEX OS software (version 3.3.1.43).

2.6. Method Validation. The developed method was validated by considering the specificity, linearity, limits of quantification (LOQs); matrix effects (MEs), recovery, and repeatability (intra- and interday precision).

The specificity was assessed by analyzing blank samples to identify any potential interference from the matrix. Additionally, the ion ratios of each prohibited drug in the various matrices were measured to evaluate consistency. According to the revised EU criteria (2021/808/EC),[1] the ion ratio is within the ± 40% tolerance of different relative intensity. Matrix-matched calibration curves were constructed at concentrations of 0.1, 0.5, 1.0, 5.0, 10, 25, 50, 100 ng/mL to enable quantification of the target compounds in the cosmetics samples. LOQs were defined as the minimum detectable amount of analyte in spiked samples with a signal-to-noise ratio (S/N) of 10. The MEs of the three matrices were evaluated using the absolute response values of spiked cosmetic samples (A) and standards in the pure solvent (water/methanol 1:1, v/v) (B) at the same concentrations (1, 5, and 20 μg/kg), calculated by eq 1:

$$\mathrm{ME}(\%) = [(\mathrm{A} - \mathrm{B})/\mathrm{B}] \times 100 \quad (1)$$

The recovery and repeatability of the optimized method were evaluated using blank matrix samples spiked with three concentrations (1, 5, and 20 μg/kg). The intraday precision was determined by taking six replicate measurement on the same day, and interday precision was measured by taking measurements on three consecutive days (n = 18). The precision was expressed as the relative standard deviation (RSD).

3. RESULTS AND DISCUSSION

3.1. UHPLC-MS/MS Analysis. Various mobile phase compositions were compared to achieve strong ionization and rapid separation of these drugs. Compared with methanol, acetonitrile provided more effective elution. The addition of formic acid (0.1%) to both the A and B mobile phases enhanced the ionization efficiency and provided satisfactory peak shapes for all 64 compounds through a 10 min elution. To obtain the optimal conditions for the separation of multiclass drugs, we compared two C18 columns commonly employed for this purpose: ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 μm) and Kinetex F5 (100 mm × 2.1 mm, 2.6 μm). The pentafluorophenyl propyl stationary phase used in the Kinetex F5 column provides high separation of polar compounds such as prohibited drugs. The Kinetex F5 column exhibited better peak shapes than the ACQUITY HSS T3 column, along with high resolution and suitable particle size.

The scheduled MRM parameters were optimized by direct infusion into the mass spectrometer at a flow rate of 7 μL/min. The most abundant fragment ion and the next most abundant ion were selected as the quantitative and qualitative product ions, respectively. The sMRM retention time tolerance (± s) was 100, and the target compounds were acquired at various time periods to reduce overlap and obtain good peak shapes. The mass parameters of the ESI source, including ion spray voltage, source temperature, curtain gas, collision gas, GAS 1, GAS 2, DP, and CE, were finely adjusted as shown in Table 1 to ensure optimum signal intensity. The response of all drugs showed good linearity ($r^2 \geq 0.99$, Table S3). All 64 prohibited drugs from 11 different categories were separated with high sensitivity and selectivity under the optimized UHPLC−MS/MS conditions.

3.2. Optimization of Sample Pretreatment. Ensuring the efficient extraction of target analytes and subsequent cleanup to remove matrix interference are the primary challenges associated with cosmetic sample pretreatment,[9] particularly in the case of multiclass drugs with various physical properties. The SPE method, which uses Oasis Prime HLB

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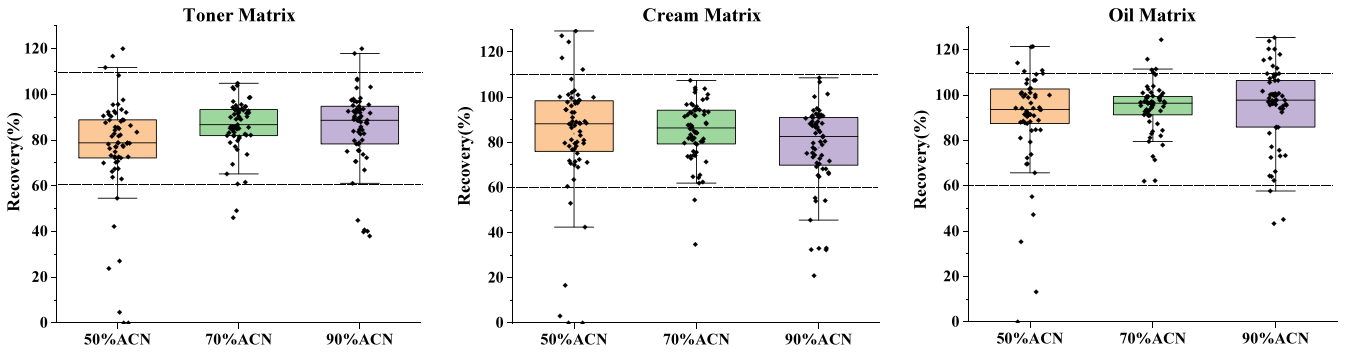

Figure 2. Recovery distribution of 64 drugs from toner, cream, and oil cosmetics using extraction solvents with various proportion of acetonitrile (50%, 70% and 90%).

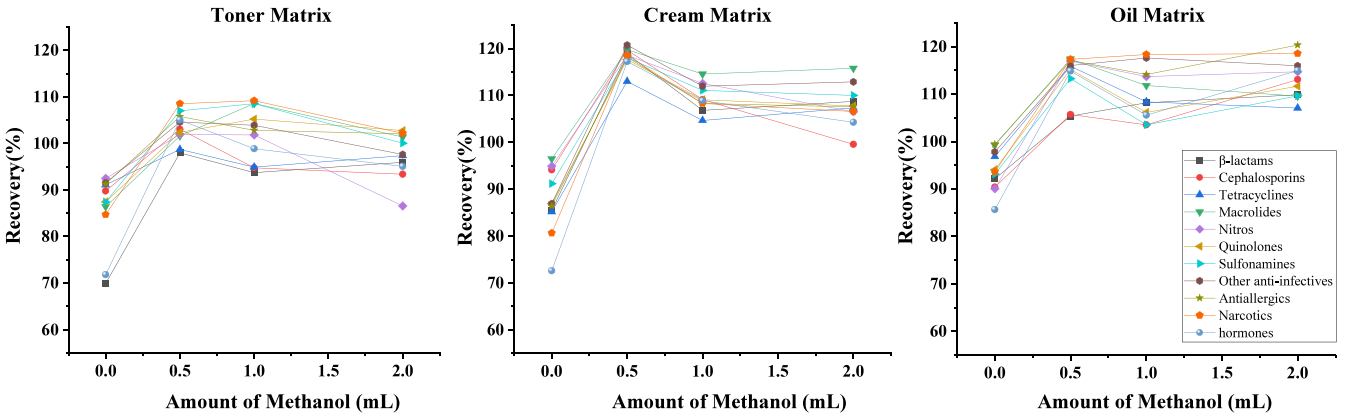

Figure 3. Effect of the amount of methanol (0, 0.5, 1, and 2 mL) on the average recovery of 11 categories of drugs from three cosmetic matrices.

cartridges comprising reversed-phase N-vinylpyrrolidone and a divinylbenzene copolymer, omits the activation, equilibration, and washing steps and therefore provides efficient sample pretreatment with high sensitivity. Oasis Prime HLB cartridges effectively remove fats and pigments from complex cosmetic matrices. To achieve optimal extraction efficiency, critical parameters, including the composition of the extraction solvent and eluent volume, were investigated. The pass-through cleanup SPE procedure developed in this study requires only extraction and purification steps.

3.2.1. Effect of Different Extraction Solvents. The extraction solvent determines the solubility of the analytes in the samples and prevents the coextraction of other matrix interferents. Acetonitrile, methanol, water, and mixtures thereof are frequently used as extraction solvents.[26,27] The extraction efficiency of acetonitrile is superior to that of methanol, which reduces coextraction of the matrix and induces protein precipitation, particularly in the case of quinolones in cosmetics.[28] Extraction with aqueous buffers can limit the coextraction of nonpolar matrix interferents, which is important in the analysis of highly polar compounds such as tetracyclines and sulfonamides.[22]

In the present study, homogenized toner, cream and treatment oil matrix samples were fortified with the 64 prohibited drugs at a concentration of 100 μg/kg. Subsequent analyses were performed in triplicate. The target compounds were divided into 11 categories with a wide range of polarities. Various acetonitrile/water mixtures (50:50, 70:30, and 90:10, v/v) were tested with the addition of 1% formic acid during the extraction step. The extraction solvents should be formulated immediately before use owing to the degradation of formic acid over time. Many of the target compounds contain thioglycoside and tertiary amine structures that are highly soluble in polar solvents and acidic aqueous solutions. Beta-lactams and cephalosporins antibiotics are unstable and can undergo hydrolysis or molecular rearrangement under neutral or physiological conditions; thus, poor recoveries (<20%) of these drugs were obtained in the absence of formic acid. The spiked samples were then cleaned with an Oasis Prime HLB cartridge. The recoveries of the 64 drugs using the different extraction solvents are presented in Figure 2. The optimal recovery of the maximum number of drugs was achieved using 70% acetonitrile. Other proportions achieved very low recoveries (<40%) of several hormones, or too high a recovery (>120%) in the case of some β-lactams.

The optimal recoveries of 60−110% from the spiked samples were obtained using an acetonitrile/water mixture (70/30, v/v), which was then further optimized.

3.2.2. Optimization of the Elution Volume. The recoveries of penicillin G, dienestrol and (−)-epinephrine were less than 60%; thus, the elution procedure was further optimized to improve extraction efficiency. Methanol is a common elution solvent that limits the coextraction of mineral oil and long-chain hydrocarbons,[8,21] particularly from cream and oil matrices. Extraction solvents containing various quantities of methanol (0, 0.5, 1, and 2 mL) were examined to optimize recoveries from the three spiked cosmetic matrices.

The use of methanol significantly increased the absolute recoveries of the three drugs, which were significantly below 60%, and the average recoveries of all 11 categories of drugs to satisfactory levels. Elution with 0.5 mL methanol greatly improved the average recoveries of β-lactams, hormones and

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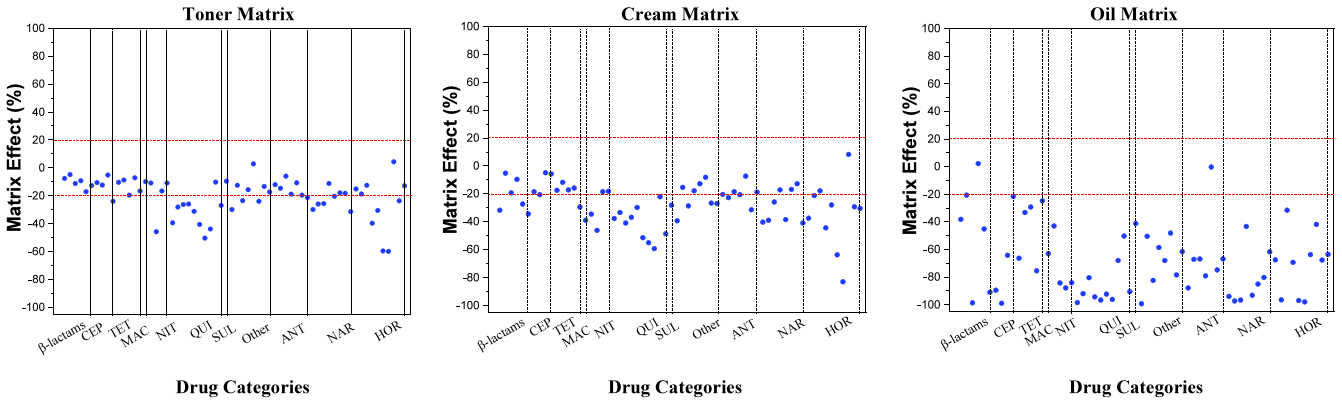

Figure 4. Matrix effects in UHPLC-MS/MS analysis of 11 categories of drugs in three cosmetic matrices.

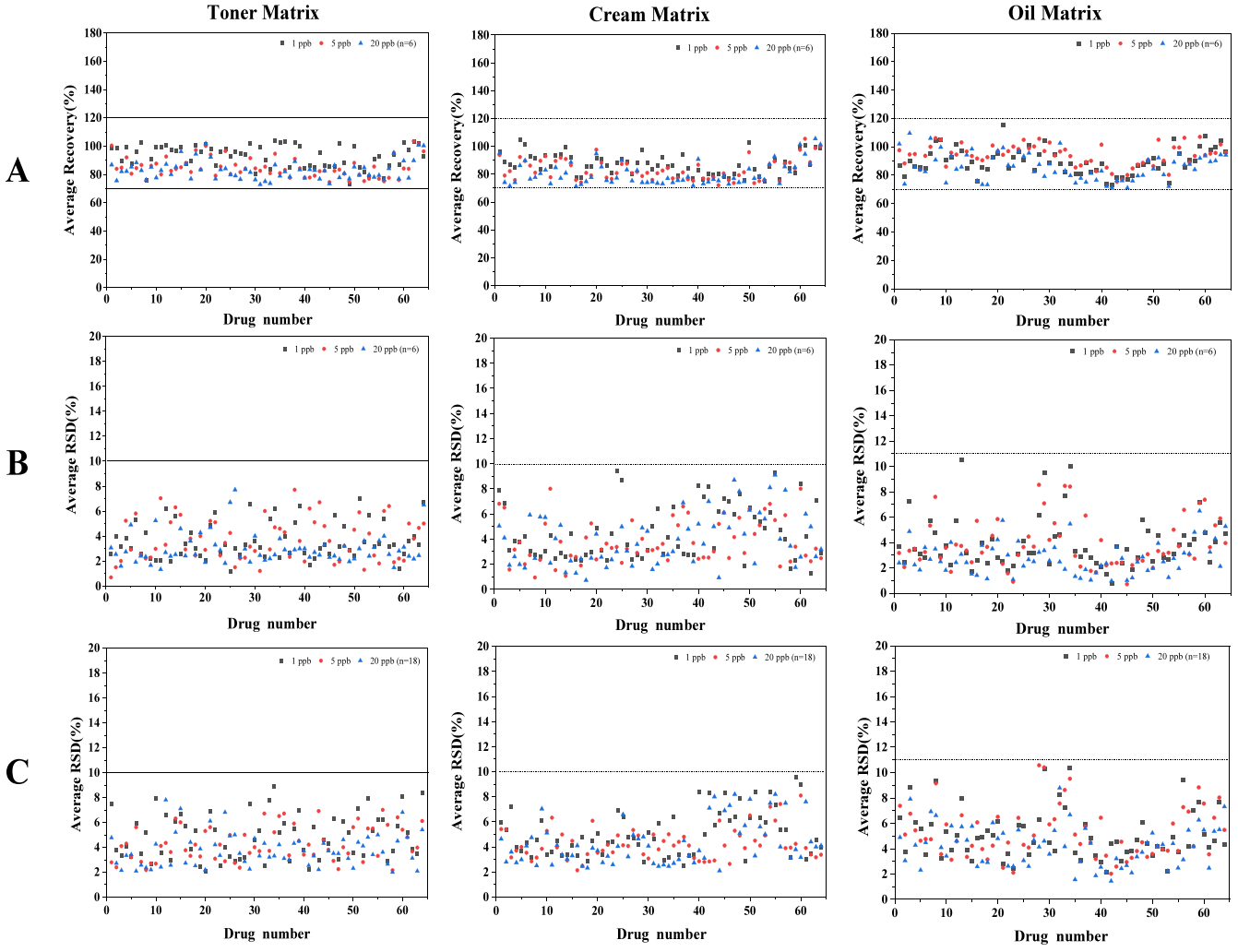

Figure 5. Recoveries, intraday precision and interday precision at the low, medium, high spiked concentrations in three cosmetic matrices. (A) The average recovery of 64 prohibited drugs in toner, cream and oil matrix (B) The intraday precision of average RSD (n = 6) of 64 prohibited drugs in toner, cream and oil matrix (C) The interday precision of average RSD (n = 18) of 64 prohibited drugs in toner, cream and oil matrix. The number of each prohibited drug corresponds to Table 1.

other drugs (Figure 3). In particular, the absolute recovery of dienestrol from the toner matrix increased from 19.2% to 98.2% with the use of 0.5 mL methanol, but fell to 65.3% and further to 61.9% as the quantity of methanol increased to 1 and 2 mL, respectively, while the absolute recovery of penicillin G increased from 46.1% to 86.8%, decreased to 75%, and subsequently increased to 89.5% under the same conditions. In the cream matrix, the absolute recovery of dienestrol increased from 34.8% to 114% in the presence of 0.5 mL methanol and decreased to 101.7% and 79.7% as the methanol content increased to 1 and 2 mL, respectively. In the oil matrix, the absolute recovery of (−)-epinephrine increased from 22.3% to

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Table 2. Comparison of the Proposed Method with Previously Published Methods

Method	Matrix	Cleanup sorbent	Sample preparation time (min)	Recovery (%)	LOQs (μg/kg)	Matrix effect (%)
pass-through SPE in this work	Toner, cream, oil	PRiME HLB	20	70−120	0.1−1	−99−8
pass-through SPE [24]	Toner, lotion	PRiME HLB	20	17−120	3880−45440	−100−100
QuEChERS [8]	cream	dSPE MR-Lipid	37	85−121	0.1−2.7	61−114
Ultrasonic and vortex extraction [12]	Toner, cream, oil	0.22 μm filter	25	82−115	210−1150	−42−615

113.5%, fell to 110.4%, and subsequently increased to 114.4% with the addition of 0.5, 1, and 2 mL of methanol, respectively. The recoveries of all drugs from the three matrices are listed in Table S4. These data indicated that the addition of methanol significantly affects the elution of low-polarity drugs, including dienestrol (Log P 5.9) and diethylstilbestrol (Log P 5.07), as well as unstable β-lactams. Additionally, elution with 0.5 mL of methanol afforded higher recoveries than elution with 1 or 2 mL, likely because higher amounts coeluted more impurities that generated stronger matrix interference. Hence, subsequent experiments included methanol (0.5 mL) during cleanup elution.

The effluent of the two steps was thoroughly mixed and directly analyzed using rapid UHPLC-MS/MS without lyophilization and redissolution. This method avoids the hours-long drying process and is therefore less time-consuming than all previously reported methods without sacrificing sensitivity. Satisfactory recoveries (70 to 120%) of 64 prohibited drugs spanning 11 categories were obtained.

3.3. Method Validation. The method developed in this study was validated using toner, cream, and treatment oil as representative matrices. The validation parameters included the linearity, LOQ, matrix effects, accuracy, and precision. The linearity and LOQ results are summarized in Table S3.

3.3.1. Selectivity and Linearity. Blank matrix samples and ion ratios were analyzed to evaluate the selectivity of the method. The mass spectra of all three blank matrix samples showed no signal at the retention times of the target drugs, demonstrating the absence of a false-positive signal from matrix interference. The accuracy of the quantitative MRM methods was qualitatively confirmed using the two most abundant product ions. The ion ratio of the 64 prohibited drugs in different cosmetic matrices is within the ± 40% tolerance of relative deviation from standard solutions (Table S2), demonstrating the high qualitative accuracy of the method in both positive and negative ion modes.

The linearity was evaluated using blank calibration standards matrix-matched to each analyte. The calibration curves of all analytes showed correlation coefficients (R^2)≥0.99, ranging from 0.9902 to 0.9999, indicating excellent linearity of all 11 categories of drugs within each linear range.

3.3.2. Matrix Effect. The ionization of the analytes in tandem mass spectrometry is frequently subjected to interference from the complex cosmetics matrix, which can increase or inhibit the signals, affecting the ionization efficiency and accuracy of the quantitative results owing to the highly susceptible ESI source.[29] In this study, MEs of three cosmetic matrices were evaluated (Figure 4). The vast majority of analytes in the toner, cream, and oil matrices exhibited matrix suppression effects. The oil matrix showed the strongest MEs (|ME|>50%) relative to the other two matrices, whereas the toner matrix typically exhibited the weakest MEs. The multiclass analysis of drug residues in cosmetics should therefore investigate all types of matrices. In particular, cream and oil matrices should not be ignored.

In the present study, matrix-matched standard curves were adopted to minimize matrix interference, improve accuracy, and obtain satisfactory recoveries of the 64 prohibited drugs.

(CEP: cephalosporins, TET: tetracyclines, MAC: macrolides, NIT: nitroimidazoles, QUI: Quinolones, SUL: sulfonamides, Other: other anti-infectives, ANT: Antiallergics, NAR: Narcotics, HOR: hormones. Each blue point corresponds to one drug. The drug categories are listed in Table 1.)

3.3.3. Accuracy and Precision. The accuracy and precision of the method were evaluated by measuring the recoveries and RSDs. The recoveries were assessed using standards of the 64 prohibited drugs spiked with low, medium and high concentrations (1, 5, and 20 μg/kg, respectively) in three typical matrices. The lowest concentration was evaluated near the LOQs, which were typically 1 μg/kg. All drugs were quantitatively detected above the LOQ in the toner, cream, and oil matrix samples. Satisfactory recoveries of all drugs between 70% and 120% were achieved in the toner, cream, and oil matrix samples (Figure 5. A).

The RSD was determined by analyzing samples spiked with three different concentrations (1, 5, and 20 μg/kg). The intra- and interday precision was determined by taking six replicate measurements in 1 day and by taking measurements over three consecutive days, respectively (Figure 5. B, C). Excellent repeatability (RSD < 11%) was observed in all three matrices (Tables S4−6), demonstrating the effectiveness of the methodology in the analysis of the prohibited multiclass drugs in typical cosmetic samples.

3.4. Comparison with Other Methods. Table 2 compares the method developed in this study with established UHPLC-MS/MS-based methods, including SPE and QuEChERS. Previously reported SPE methods include pass-through SPE,[24] QuEChERS employs a cleanup sorbent consisting of dSPE MR-Lipid,[8] while ultrasonic and vortex extraction rely on purification via silica gel chromatography and filtration with a 0.22 μm filter.[12] The method developed in this study achieved satisfactory recoveries and sensitivities in the analysis of cream and oil matrices. Additionally, the pass-through SPE-UHPLC-MS/MS method was faster than QuEChERS and ultrasonic and vortex extraction methods, while exhibiting weaker Mes and optimum LOQs. Considering all the factors, the pass-through SPE-UHPLC-MS/MS method presented herein offers highly efficient, simultaneous determination of multiclass prohibited drugs in various cosmetic matrices while consuming fewer resources than previously established methods.

3.5. Application to Cosmetics Products. The established method was applied to the analysis of 20 commercially available cosmetics, including toners, essences, shampoos, hair conditioners, moisturizing creams, baby repair creams (Fule cream), acne creams, essence oils, hair oils, and masks purchased from local supermarkets (Beijing, China) and online stores (China). Prohibited substance were identified

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in six samples (Table 3) representing severe misuse of antibiotics.

Table 3. Analysis of the Prohibited Drugs in the Cosmetics Products

Sample Name	Sample type	Compound name	Concentration (μg/kg)
Baby repair cream-1	Cream	Miconazole	17.1
		Diphenhydramine	13.5
Baby repair cream-2	Cream	Diphenhydramine	0.1
VE-cream	Cream	Econazole	9.2
		Miconazole	3.4
Acne-cream	Cream	Metronidazole	7.8×10^6
		Miconazole	7.4×10^3
		Ofloxacin	112.4
		Diphenhydramine	5.2
Hair essence-oil	Oil	Miconazole	0.5
Nose Pack-mask	Toner	Miconazole	0.3

Surprisingly, several products reportedly extracted from pure herbs contained prohibited drugs, with one acne cream containing a very high concentration of metronidazole (7.8×10^6 μg/kg). Although cream products are used only on the skin, metronidazole at such high concentrations can easily penetrate the skin, enter the human body and weaken the immune system. Metronidazole is a class 2B carcinogen with potential genotoxicity according to the International Agency for Research on Cancer. The extracted ion chromatograms of the acne cream are shown in Figure S1. Miconazole, a highly effective, safe, and broad-spectrum antifungal drug, was the most frequently detected drug among all the screened prohibited compounds, and is likely added owing to its effectiveness against almost all pathogenic fungi.

Additionally, the optimized method detected extremely low concentrations of diphenhydramine (0.1 μg/kg) and miconazole (3.4 μg/kg) in creams. Because the addition of drugs in cosmetics is prohibited, such drugs are typically added in low concentrations: thus, detecting the illegal addition of prohibited drugs requires methods with LOQs of the order of parts per billion (ppb).

4. CONCLUSION

A rapid pass-through cleanup SPE approach coupled with UHPLC-MS/MS was established for the simultaneous determination of 64 prohibited drugs from 11 categories in three typical cosmetic matrices. This method offers high sensitivity and selectivity, high linearity, optimal recoveries, and high precision. Furthermore, the method was successfully applied to the analysis of 20 commercially available cosmetic products, identifying prohibited substances in six of the 20 samples. The established method broadens the simultaneous detection range and shortens the analysis time of drugs in a single experiment relative to those of conventional methods. In addition, the low solvent consumption of the method substantially reduces analysis time and cost. This rapid, simple, and effective analytical method is suitable for the routine analysis of prohibited drugs in various cosmetic matrices. Although several governments have banned the addition of drugs to cosmetics, standards must be continuously developed and updated. This analytical method is expected to facilitate surveillance of the illegal addition of high and low concentration of drugs to different types of cosmetic products by effectively and simultaneously screening and quantifying various hazardous and banned substances in cosmetics. In future research, this method could be extended to encompass a broader range of batches and a larger number of cosmetic products. Furthermore, this rapid and robust pass-through SPE sample preparation method demonstrates potential for the extraction of other categories of contaminants and prohibited substances in various cosmetics matrices, thereby better ensuring consumer health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c01319.

Detailed information on each standard of the 64 prohibited drugs; ion ratios of prohibited drugs in three typical cosmetic matrices; linearity and LOQs of 64 prohibited drugs in three typical cosmetic matrices (toner, cream, and oil); recovery, intraday precision, and interday precision of the developed method at three spiked concentrations in toner, cream, and oil matrix; MRM chromatograms for the acne-cream sample (PDF)

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Notes

The authors declare no competing financial interest.

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