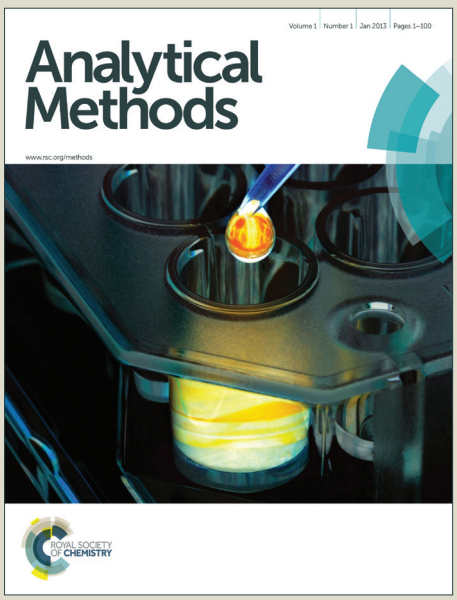
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Simultaneous determination of sixteen fluoroquinolone antibiotics in cosmetics by ultra-performance liquid chromatography/triple quadrupole mass spectrometry with ultrasound-assisted extraction and solid-phase extraction

Xianshuang Meng,[a] Qiang Ma,*[a,b] Qing Zhang,[a] Qing Lv,[a] Yunxia Chen,[a] Hua Bai[a] and Chao Wang[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A rapid, sensitive and reliable analytical method has been developed for the simultaneous determination of 16 fluoroquinolones (FQs) in cosmetic samples by use of ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). The employment of ultrasound-assisted extraction (UAE) and solid-phase extraction (SPE) in sample pretreatment procedures enabled exhaustive extraction of target analytes and effective clean-up of sample matrices prior to instrumental measurement. The UPLC separation of the 16 FQs was performed on an ACQUITY UPLC BEH Shield RP18 column (100 mm × 2.1 mm, 1.7 μm), preceded by a VanGuard pre-column (5 mm × 2.1 mm, 1.7 μm) of the same packing material with gradient elution. The MS/MS acquisition was carried out using positive electrospray ionization (ESI) under the selected reaction monitoring (SRM) mode. The established method was validated in terms of matrix effect, linearity, sensitivity, accuracy, stability, specificity, recovery, and precision, and can be applied for the monitoring of FQ antibiotics residues in cosmetic products to ensure consumer health and protection.

Introduction

Cosmetics are commonly used consumer products that are intended to be placed in contact with human body for the purpose of cleansing, beautifying, promoting attractiveness, or altering appearance. They represent a tremendous global industry involving major markets to be found in the European Union, the United States of America, China, and Japan with approximate retail sales values of 69, 47, 29, and 18 billion Euros, respectively, in 2013, according to the statistics compiled by Cosmetics Europe[1]. As the prime principle, the cosmetic products put on the market must not cause damage to human health under normal or reasonably foreseeable conditions of use. To ensure the highest level of consumer protection, the quality and safety of cosmetics are strictly supervised by the authorities worldwide. In general, the vast majority of cosmetics manufacturers act responsibly, and voluntary addition of prohibited ingredients is very scarce. However, in order to achieve anti-acne, anti-freckle, anti-wrinkle, skin-whitening or astringent efficacy in a short term, illicit substances, *e.g.*, antibiotics[2] and corticosteroids[3,4], might be intentionally added to cosmetic products by a few lawbreakers for economic benefit, hence posing a serious risk to human health.

Fluoroquinolones (FQs) are a large group of broad-spectrum synthetic antibiotics, which have been initially used for the treatment of the infectious diseases of humans and food-producing animals, since they are effective against both Gram-positive and Gram-negative bacteria[5]. Their activity is based on the inhibition of the enzyme DNA gyrase or topoisomerase II, which are responsible for the preservation of the DNA biological activity of the bacteria[6,7]. The cosmetics containing FQ antibiotics may have an immediate effect of relieving the symptom of acne. However, the repeated use over a long period of time, in particular, overexposure to the cosmetics in which the concentration of FQs is unknowing high without any medical supervision, will cause serious side effects. There are indications that the long-term exposure to FQs may result in a series of adverse effects, including central nervous system toxicity, phtotoxicity, cardiotoxicity, genotoxicity, carcinogenicity, *etc*.[8-11] There is also scientific evidence that the use or misuse of FQs may be responsible for the increasing concerns on public health, such as allergic reactions and antibiotic resistance.[12] In view of the health hazard, FQ antibiotics are regulated as prohibited substances in cosmetic products by the European Union,[13] the Association of South East Asia Nations (ASEAN),[14] China,[15] *etc*.

To date, various analytical methods have been developed for the determination of FQs in food matrices, biological specimens, environmental samples, and pharmaceutical formulations,[8,16,17] based on spectrophotometry,[18,19] spectrofluorometry,[20,21] chemiluminescence,[22,23] immunological assay,[24,25] microbiological assay,[26,27] capillary electrophoresis,[28,29] electrochemistry,[30,31] high-performance liquid chromatography with ultraviolet,[32-35] fluorescence,[34,36-38] mass spectrometric,[39] or tandem mass spectrometric[40-43] detection. Nevertheless, there are very limited studies published dealing with the analysis of FQ

antibiotics in cosmetic samples, especially multicomponent analytical methods. Thus, there is an urgent necessity to develop a suitable analytical method for the identification and determination of multiple FQs in cosmetics accurately and sensitively to ensure regulatory compliance.

The matrices of cosmetic samples are not simple. They usually contain a number of ingredients, such as oily materials (grease, oil, and waxes), surfactants, moisturizers, emulsifiers, pigments, preservatives, *etc*.[44] The complexity of their composition presents a formidable challenge to the analytical task, since the possible presence of interferences that could distort the analytical results is not excludable. To overcome these drawbacks, a two-step sample preparation procedure should be included. One is an extraction step that provides exhaustive recoveries for the analytes of interest, and the other is a clean-up step used to remove the co-extracted compounds. When it comes to chromatographic technology, the introduction of ultra-performance liquid chromatography (UPLC) with sub-2 μm packing particles makes great improvements in chromatographic resolution, analysis speed, and detection sensitivity compared with traditional liquid chromatography.[45,46] A wide variety of stationary phases available offers flexible selectivity for the separation of multiple target analytes. Meanwhile, the utilization of triple quadrupole mass spectrometry can provide better sensitivity, higher selectivity, and more reliable identification of detected analytes using the selected reaction monitoring (SRM) mode.

The objective of this study was to establish a sensitive and reliable analytical methodology for the simultaneous determination of 16 FQ antibiotics in cosmetic products. Both ultrasound-assisted extraction (UAE) and solid-phase extraction (SPE) were employed to enable the efficient extraction and purification in the sample pretreatment procedures. Ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-MS/MS) was applied for the separation and analysis of the multicomponent FQ analytes.

Experimental

Chemicals and reagents

Authentic reference standards of the 16 FQ antibiotics studied in this paper were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Sigma-Aldrich (St. Louis, MO, USA), or National Institute for Food and Drug Control (Beijing, China), respectively. Acetonitrile and methanol of HPLC grade were supplied by Merck (Darmstadt, Germany). Ultrapure water was deionized prior to use by a Millipore Milli-Q water purification system (Bedford, MA, USA). Other chemicals were of analytical grade, and were acquired from Beijing Chemical Co. (Beijing, China). Detailed information about the 16 FQ analytes was given in Table S1.

Preparation of standard solutions

Individual stock standard solutions at the concentration of 500 μg mL^{-1} were prepared by dissolving 25 mg of each fluoroquinolone antibiotic in a 50 mL volumetric flask with appropriate solvents. Norfloxacin (NOR), marbofloxacin (MAR), difloxain (DIF), enrofloxacin (ENR), moxifloxacin (MOX), sarafloxacin (SAR), pefloxacin mesylate (PEF), ciprofloxacin (CIP), enoxacin (ENO), gatifloxacin (GAT), ofloxacin (OFL), danofloxacin mesylate (DAN), and fleroxacin (FLE) were dissolved in 40% aqueous acetonitrile solution containing 1% formic acid; lomefloxacin (LOM), and sparfloxacin (SPA) were dissolved in methanol; pazufloxacin (PAZ) was dissolved in methanol-ammonium hydroxide (9:1). All the stock solutions were further diluted with the initial chromatographic mobile phase composition to a series of concentrations for the construction of standard calibration curves.

Sample collection and pretreatment

37 untreated anti-acne cosmetic samples of different brands and categories, collected in local markets and on the Internet, were subjected to laboratory tests. Aliquots of 0.5 g cosmetic samples were accurately weighed into a 25-mL capped glass colorimetric tube. Acetonitrile - 2% aqueous formic acid (1:2) was added to the 10 mL mark of the tube, and shaken for 30 s on an MS2 vortex mixer (IKA, Staufen, Germany), and then extracted under ultrasonication for 30 min at a frequency of 40 kHz. The extract was decanted into a polypropylene centrifuge tube and centrifuged with a CR 21G high-speed refrigerated centrifuge (Hitachi, Tokyo, Japan) for 10 min at 10,000 rpm. Aliquot of 2 mL clear supernatant was blown to dryness at 40 ºC under a gentle stream of nitrogen employing an N-EVAP nitrogen evaporator (Organomation, West Berlin, MA, USA) and reconstituted with 2 mL of 0.1% aqueous formic acid. The resultant solution was subjected to subsequent solid phase extraction (SPE) clean-up.

The clean-up procedure was performed using a Gilson GX-274 ASPEC automated SPE system (Middleton, WI, USA). An Oasis MCX 3cc/60mg cartridge (Waters, Milford, MA, USA) was pre-conditioned with 3 mL methanol and equilibrated with 3 mL water at a flow rate of 1 mL min^{-1}. The sample extract was then allowed to pass through the cartridge at a flow rate of 1 mL min^{-1}. Firstly, 3 mL of 2% aqueous formic acid solution was used to retain the target fluoroquinolone compounds to the cartridge based on a mixed-mode cation exchange mechanism, followed by 3 mL methanol added to wash out the matrix interference. Finally, 2×1 mL methanol-ammonium hydroxide (4:1) was loaded to elute all the target analytes. The collected eluant was blown to dryness at 40 ºC under a gentle stream of nitrogen, redissolved with 1 mL of the initial chromatographic mobile phase, and filtered through a 0.20-μm Acrodisc GHP membrane (Pall, Port Washington, NY, USA) prior to injection for instrumental analysis.

UPLC-MS/MS analysis

The chromatographic separation was conducted on an ACQUITY UPLC system (Waters, Milford, MA, USA) consisted of a binary solvent manager, a thermostatic column compartment and an automatic sampler with a 10 μL injection loop. A Waters ACQUITY UPLC BEH Shield RP18 column (100 mm × 2.1 mm i.d., particle size 1.7 μm), preceded by a Waters VanGuard pre-column (5 mm × 2.1 mm, 1.7 μm) of the same packing material was used for the separation. A binary mobile phase system, an organic part (A) - methanol-acetonitrile (2:3) and an aqueous part (B) - 5 mM ammonium formate solution at pH 3.0, was applied at a flow rate of 0.3 mL min^{-1} with a gradient profile. Initial gradient conditions were set to 12% A and held for 4 min, then increased to 17% A in 1 min. At 9 min, the gradient was programmed to

50% A. Finally, the gradient was returned to the initial conditions at 10 min. The sample and column temperature were maintained at 15 ºC and 40 ºC, respectively. 5 μL of sample was injected for analysis in a partial loop with needle overfilled mode. 200 μL of strong needle wash solvent (50% A) and 600 μL of weak needle wash solvent (12% A) were used for each run.

The MS/MS data were acquired on a Waters Xevo TQ MS tandem quadrupole mass spectrometer (Manchester, UK). The entire column eluent from UPLC was directed to a Z-spray dual-orthogonal electrospray ionization (ESI) interface operated in the positive-ion mode. Typical mass spectrometric parameters were set as follows: Capillary voltage 3.00 kV, extraction voltage 3.00 V, radio frequency (RF) lens voltage 0.10 V, source temperature 150 ºC, nitrogen desolvation gas of 800 L h^{-1} at 500 ºC, cone gas of 50 L h^{-1}. The pressure of the collision chamber, in which argon gas at a flow rate of 0.15 mL min^{-1} was used as the collision gas, was adjusted to 3.2×10^{-3} mbar. The cone voltage and collision energy for each MS/MS transition in the SRM mode were optimized individually and the optimum values are given in Table 1. Data acquisition was performed with multiple channels set based on the retention time of each reference compound. Instrumentation control and data acquisition were operated by MassLynx and TargetLynx software, supplied with the instrument (Waters, Manchester, UK).

Results and discussion

Optimization of UPLC-MS/MS conditions

In an attempt to obtain optimal chromatographic separation and analytical sensitivity for multiple FQ antibiotics, a comprehensive investigation toward critical chromatographic parameters, including stationary phase type, mobile phase composition, column temperature, and flow rate, was conducted in the present study. A mixed standard solution, in which all the 16 FQ analytes were at the concentration of 0.5 μg mL^{-1}, was used for the experiment.

In view of the fact that chemical nature of column packing materials may have a significant effect on chromatographic performance, a wide variety of commercially available UPLC columns with sub-2 μm particles were compared for the separation of the 16 FQs during method development, such as BEH C_{18}, BEH C_8, BEH Phenyl, BEH Shield RP18, HSS C_{18}, HSS C_{18} SB, and CSH C_{18} (all with the dimension of 100 mm × 2.1 mm). These columns vary in selectivity, due to the differences in functionalities, completeness of bonding, presence or absence of endcapping, particle surface area, etc. Besides column stationary phase, mobile phase composition may exert a great influence over selectivity as well. Thus, a combined survey in consideration of both chromatographic columns and mobile phases was carried out. As the most commonly used organic solvents for reversed-phase liquid chromatography, methanol and acetonitrile differ considerably in their selectivity properties. In this work, methanol, acetonitrile, as well as their mixtures in varying proportions (1:4, 2:3, 1:1, 3:2, and 4:1), were paired with water as the mobile phases and undertaken a series of trials. The octanol/water partition coefficients ($\log K_{ow}$) of the target FQs range from 0.72 to 2.93, calculated by ChemDraw Ultra version 7.0 software (CambridgeSoft, Cambridge, MA, USA). To acquire satisfactory chromatographic separation for the FQ compounds in a reasonable period of time, gradient elution programs were applied. For each individual type of column with different types of mobile phases, the corresponding gradient conditions were adjusted thoroughly under the circumstance. In the end, the experimental results demonstrated that the best performance was achieved with the BEH Shield RP18 column as stationary phase and methanol-acetonitrile (2:3) paired with water as mobile phase based on an integrated evaluation in terms of band spacing, peak shape and detection sensitivity. The packing particles of the BEH Shield RP18 column are synthesized on high-pressure bearing ethylene bridged hybrid (BEH) substrate, and contain an embedded carbamate polar group. This bonding chemistry provides unique selectivity and enhanced peak shape for basic compounds, like FQs. A closer look at the chemical structures of FQs may reveal the fact that FQs normally exhibit amphoteric properties because of the presence of two ionisable functional groups, i.e., the carboxylic acid and piperazine moieties (pK_{a1} and pK_{a2} ranging from 5.5 to 6.7, and from 6.8 to 9.4, respectively). In this case, the pH value of the mobile phase has a profound effect on the chromatographic and mass spectrometric behaviour of FQs. For the purpose of exploring the composition of the aqueous part, the optimized organic part (methanol-acetonitrile, 2:3) of the mobile phase was paired with various volatile buffers adjusted to different pH values within appropriate buffer ranges: 5 mM ammonium formate solution (adjusted to pH 3.0 and 4.0 using formic acid, respectively), 5 mM ammonium acetate (adjusted to pH 5.0, and 6.0 with acetic acid, respectively), and 5 mM ammonium bicarbonate (adjusted to pH 8.0, 9.0 and 10.0 with ammonium hydroxide, respectively). The mixture of the 16 FQ analytes were chromatographically separated with different mobile phases and then subjected to the ionization in either positive or negative ESI mode. The best chromatographic performance and analytical sensitivity were found to be obtained in the positive ionization mode when acidifying the mobile phas eluents with 5 mM ammonium formate solution at pH 3.0. In addition to the changes in selectivity brought about by changing the mobile and stationary phases, control of column temperature and flow rate can also be used to improve selectivity and resolution. The influence of column temperature and flow rate was investigated within the ranges from 30 ºC to 55 ºC, and from 0.2 to 0.5 mL min^{-1}, among which 40 ºC and 0.3 mL min^{-1} presented the optimal signal response and peak shape.

The principal mass spectrometric parameters were tuned by use of an IntelliStart automated system optimization program provided with the instrument (Waters, Manchester, UK) via direct flow injection at 10 μL min^{-1}. The most abundant ions for each individual FQ antibiotics were all observed as the protonated ions $[M+H]^+$ in full scan mode. These pseudo-molecular ions were selected as the precursor ions for further collision-induced dissociation to identify the resulting product ions. For the detection by UPLC-MS/MS, qualitative identification was based on the retention times and two diagnostic product ions for each FQs. A comparison with QC samples was made with acceptable tolerance of ±2.5% for retention time and of ±20-30% for relative ionic abundance according to the European Union Commission Decision 2002/657/EC.[47] Two transitions in the SRM acquisition mode were selected for quantitative analysis, but only one was

used for quantitation. Table 1 gives the optimum MS/MS analysis parameters and proposed identities of the mass fragments for the 16 FQ compounds. The SRM chromatogram of the 16 FQs was shown in Figure 1.

Optimization of sample pretreatment conditions

Ultrasound-assisted extraction (UAE), as an inexpensive, simple, and efficient extraction technique, is commonly used in the pretreatment of cosmetic samples.[48] The enhancement in extraction obtained by use of UAE is mainly attributed to the effect of acoustic cavitations produced in the solvent by the passage of an ultrasound wave. Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample, increasing the contact surface area between the solid and liquid phase. To simplify the experimental procedures while maintaining exhaustive extraction, UAE was used for the extraction of FQs from cosmetics in this study. In many published literatures, acetonitrile in an acidic medium was widely adopted as the extraction solvent for the determination of FQs in food and environmental samples.[49-53] Considering the ionization efficiency and the compatibility with mass spectrometry, acetonitrile forcified with formic acid solution, was employed. Mixtures of acetonitrile and 2% aqueous formic acid solution in various proportions (1:1, 1:2, 1:3, 1:4, 2:1, and 3:2) were compared to investigate the extraction yield. Figure S1 presents the extraction yields of six selected FQs (NOR, CIP, PEF, DAN, ENR, and MAR), among which 2% aqueous formic acid-acetonitrile (2:1) led to the highest extraction efficiency above 87.9%, and was chosen as the best solvent in the following experiments. Liquid-to-solid ratio (volume/mass) is another critical factor to be optimized for UAE method. Aliquots of 0.5 g of cosmetics was fixed, and volumes of 5 to 30 mL of extraction solvent were evaluated. Figure S2 demonstrates the influence of the liquid-to-solid ratio on the extraction yield of six selected FQs (NOR, CIP, PEF, DAN, ENR, and MAR). The results indicated that an increase of extraction efficiency of FQs could be observed with the increase of the solvent volume from 5 to 10 mL, and maintained almost constant (above 92%) from 10 to 30 mL. Hence, 10 mL was selected as the volume of extraction solvent. Extraction duration was also investigated from 10 to 60 min. As shown in Figure S3, the response of NOR increased from 10 to 20 min, and remained nearly unchanged from 20 to 60 min. As to MAR, PEF and ENR, the signal intensity reached the highest level at 30 min and decreased from 30 to 60 min. While the intensities of CIP and DAN were nearly constant before 30 min and decreased at 40 min. Thus, 30 min was selected as the extraction duration.

UPLC-MS/MS analysis can offer high sensitivity and selectivity, which however may suffer from the interference of matrix effect. In this work, an SPE protocol was incorporated prior to instrumental analysis for sample clean-up and enrichment. Considering the amphoteric properties of FQs, an Oasis MCX mixed-mode cation-exchange cartridge (3cc/60mg), which is based on a dual mechanism of both strong cation-exchange and reversed-phase retention, was used as the clean-up sorbent to remove endogenous interference originated from cosmetic sample matrices. Aliquots of 0.5 g blank cosmetic sample was spiked with the 16 FQs (20 mg/kg) and extracted following the procedure aforementioned. 2 mL of extract was loaded onto the cartridge, and 2% aqueous formic acid solution was used as the first washing agent to lock the target FQ compounds in the sorbent by cation-exchange action. Experimental results demonstrated that 3 mL was adequate to retain all the target compounds and also effective in washing the majority of hydrophilic matrices. The clean-up protocol was further optimized mainly in terms of the second washing agent and the elution solvent. Methanol was selected as the second washing agent to remove the neutral and acidic co-extractive substances in the cosmetic extracts that may contribute to matrix effect. With the increasing volume of methanol from 1 to 5 mL applied, the impurities were gradually eluted out. When methanol of less than 3 mL was loaded, the entire target FQs were well retained on the absorbent. However, there are FQs observed to be washed out the cartridge when even more methanol was used. For the purpose of eluting all the target compounds with the least volume of elution solvent, mixtures of methanol fortified with ammonium hydroxide solution in different proportions (95:5, 90:10, 80:20, 70:30, and 60:40) were tested. By taking into account of both the elution effect and the convenience for the following nitrogen blowing procedure, methanol versus ammonium hydroxide (80:20) was adopted as the elution agent. A further investigation revealed that 2 mL of the elution agent was enough for the experiment in which 99% of the 16 FQs could be eluted out of the cartridge.

Method validation

The established method was validated in terms of matrix effect, linearity, sensitivity, accuracy, stability, specificity, recovery, and precision.

For the construction of calibration curves and the determination of LODs and LOQs for all the FQ compounds, standard mixed solution and spiked samples were prepared, respectively. The peak areas of the quantitative transition for each analyte versus the concentration were linear in the range studied. LODs and LOQs values were determined as the concentration of analyte giving signal response 3 and 10 times to the noise background separately. All data obtained from the calibration curve are summarized in Table 2. Specificity of the method was evaluated by the absence of any potential interference at the chromatographic elution time of the investigated compounds in the same UPLC-MS/MS run. For checking the specificity, six lotion and six cream samples free of the target chemicals were treated and analyzed with the proposed method. There were no interfering peaks around the retention times of all the studied analytes. To study the recovery of the method, the average recoveries with relative standard deviations (RSDs) for each component were determined at three spiked levels corresponding to LOQ, 1.5×LOQ and 2×LOQ [8]. The testing results are recorded in Table 2. The recoveries ranged from 80.7% to 113.2% with RSDs between 1.8% and 8.6%. The stability of the 16 FQs was tested by placing blank samples spiked at 10 μg kg^{-1} under two types of conditions: (a) refrigerated at 4ºC for one week and examined every day; (b) kept in dark place at ambient temperature for one month and examined every week. The stability (expressed as RSDs), ranged from 3.7% to 9.5% under the (a) condition and varied from 7.7% to 11.7% under the (b) conditon, indicating good stability for the studied FQs. The experimental results are summarized in Table 3. Matrix effect,

mainly caused by the chromatographic coelution of the endogenous substances originating from sample matrix, may interfere with the ionization process, and the accuracy of analysis results. Since matrix effect may significantly enhance or suppress the ionization efficiency and thereby increase or reduce the sensitivity of the analytes of interest, it should be evaluated prior to quantitation. In this study, the slope comparison method [25-27] was utilized to assess the matrix effect. The sample extracts, which were spiked with appropriate amounts of standards as done for the recovery measurement, were used to construct calibration curves. Afterwards, the slopes of the calibration curves from the standard addition experiments were compared with those obtained from the standards in neat solvents at the same concentration levels. The ratio of 1 denotes that matrix does not exert an influence toward the ionization, otherwise indicating ionization enhancement (>1) or suppression (<1) [25]. As are shown in Table 3, the slope ratios range between 0.86 and 1.11 for the 16 FQs in lotion and cream matrices, indicating that the interfering substances in cosmetic samples were effectively eliminated through the SPE clean-up protocol.

Method application

The developed method was applied for the determination of FQs in 37 commercial anti-acne cosmetics of different brands, origins, and matrices, that were obtained in local markets or on the Internet. Each sample was analyzed in duplicate. The results demonstrated that the 16 FQs were not found in any samples. To further validate the accuracy of the method, two commercial pharmaceuticals of similar matrices to cosmetics (one norfloxacin cream and one ofloxacin cream) were tested using the developed method. Both ingredients were identified, with the analyte content determined in compliance with the label.

Conclusions

In the present study, a sophisticated UPLC-MS/MS method was developed for the sensitive detection of 16 FQs in cosmetic products. The incorporation of UAE and SPE techniques in the sample preparation allows for exhaustive extraction of the target analytes and effective clean-up of the sample matrices prior to instrumental analysis. The described method has been validated with good specificity, excellent sensitivity, and satisfactory accuracy. The overall LOQs for each individual FQs were all better than 5 μg kg^{-1}, and the total run time was less than 10 min.

Acknowledgements

This work was financially supported by the Youth Talent Program of Chinese Academy of Inspection and Quarantine, the Quality Inspection Public Welfare Scientific Research Foundation of China (2012104013-4), and the National Key Technology Research and Development Program of China (2013BAK04B03).

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[a] *Chinese Academy of Inspection and Quarantine, No. A3, Gaobeidian North Road, Chaoyang District, Beijing 100123, China. Fax: +86 85772625; Tel: +86 85773279; E-mail: maqiang@caiq.gov.cn*
[b] *Weldon School of Biomedical Engineering, Purdue University, 206 South Martin Jischke Drive, West Lafayette, IN 47907, USA.*

† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

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Tables

Table 1 MS/MS parameters for the 16 FQs investigated in this study

FQs	Precursor ions (*m/z*)	Product ions (*m/z*)	CV [b] (V)	CE [c] (eV)	Relative ion ratio	Acceptable tolerance (%)
MAR	363.3 $[M+H]^+$	345.2 $[M+H-H_2O]^+$, 320.2[a] $[M+H-C_2H_5N]^+$	30	15, 20	0.89	±20
FLE	370.3 $[M+H]^+$	326.3[a] $[M+H-CO_2]^+$, 269.2 $[M+H-CO_2-C_3H_7N]^+$	30	18, 26	0.91	±20
ENO	321.3 $[M+H]^+$	303.2[a] $[M+H-H_2O]^+$, 232.2 $[M+H-H_2O-C_2H_4-C_2H_5N]^+$	30	20, 22	0.31	±25
PAZ	319.2 $[M+H]^+$	301.3[a] $[M+H-H_2O]^+$, 276.2 $[M+H-CO_2]^+$	40	18, 26	0.70	±20
OFL	362.3 $[M+H]^+$	318.3 $[M+H-CO_2]^+$, 261.2[a] $[M+H-CO_2-C_3H_7N]^+$	30	20, 27	0.85	±20
PEF	334.3 $[M+H]^+$	316.3[a] $[M+H-H_2O]^+$, 290.3 $[M+H-CO_2]^+$	30	19, 20	0.65	±20
NOR	320.3 $[M+H]^+$	302.2[a] $[M+H-H_2O]^+$, 276.2 $[M+H-CO_2]^+$	30	17, 20	0.66	±20
CIP	332.2 $[M+H]^+$	314.2[a] $[M+H-H_2O]^+$, 231.2 $[M+H-CO_2-C_3H_7N]^+$	30	18, 20	0.50	±20
DAN	358.3 $[M+H]^+$	340.3[a] $[M+H-H_2O]^+$, 255.3 $[M+H-H_2O-C_2H_4-C_3H_7N]^+$	35	18, 20	0.13	±30
LOM	352.3 $[M+H]^+$	308.3 $[M+H-CO_2]^+$, 265.2[a] $[M+H-CO_2-C_2H_5N]^+$	35	17, 25	0.59	±20
ENR	360.3 $[M+H]^+$	342.3 $[M+H-H_2O]^+$, 316.3[a] $[M+H-CO_2]^+$	30	19, 20	0.97	±20
DIF	400.3 $[M+H]^+$	382.3 $[M+H-H_2O]^+$, 356.3[a] $[M+H-CO_2]^+$	32	20, 22	0.89	±20
SAR	386.3 $[M+H]^+$	368.3[a] $[M+H-H_2O]^+$, 342.3 $[M+H-CO2]^+$	32	18, 22	0.71	±20
GAT	376.3 $[M+H]^+$	358.3 $[M+H-H_2O]^+$, 332.3[a] $[M+H-CO_2]^+$	30	18, 20	0.86	±20
SPA	393.3 $[M+H]^+$	349.3[a] $[M+H-CO_2]^+$, 292.3 $[M+H-CO_2-C_3H_7N]^+$	40	20, 25	0.97	±20
MOX	402.3 $[M+H]^+$	384.3[a] $[M+H-H_2O]$, 358.3 $[M+H-CO_2]^+$	33	19, 22	0.44	±25

[a] Transitions used for quantification and the other is employed for the completion of the identification.

[b] CV: Cone voltage.

[c] CE: Collision energy.

Table 2 Method validation results for the 16 FQs

FQs	Retention time (min)	Linearity				LODs ($\mu g\ kg^{-1}$)	LOQs ($\mu g\ kg^{-1}$)	Recovery (%, *n*=3)	RSDs (%)
		Slope	Intercept	R^2	Range ($\mu g\ L^{-1}$)			1×, 1.5×, 2×LOQs	
MAR	2.08	16.65	50.644	0.9946	10-1000	0.6	1.7	90.2, 88.6, 97.7	4.6, 2.9, 2.8
FLE	2.34	262.808	-658.407	0.9980	10-200	1.4	4.1	102.7, 83.7, 92.4	3.3, 3.6, 3.9
ENO	2.35	288.853	-790.835	0.9940	10-200	0.5	1.4	86.7, 80.7, 112.5	4.7, 5.9, 3.4
PAZ	2.52	104.111	-543.797	0.9968	10-200	0.6	1.8	92.1, 88.3, 96.2	2.9, 6.9, 3.0
OFL	2.64	3091.48	-4011.8	0.9991	10-1000	1.6	5.0	104.4, 97.8, 86.1	3.8, 7.6, 2.4
PEF	2.82	1023.08	-1433.99	0.9961	10-200	0.8	2.3	83.9, 90.8, 92.5	2.9, 4.7, 2.2
NOR	2.91	47.8779	-57.6714	0.9983	10-1000	0.8	2.3	98.4, 96.3, 97.5	2.6, 6.2, 3.5
CIP	3.19	235.443	-90.8929	0.9987	10-200	0.4	1.3	103.5, 100.0, 99.1	4.0, 8.6, 3.2
DAN	3.56	536.864	1240.67	0.9993	10-200	0.7	2.0	98.2, 97.8, 102.7	3.8, 7.6, 1.8
LOM	3.58	1769.04	-308.654	0.9930	10-200	0.7	2.1	87.5, 95.1, 90.4	3.2, 7.1, 2.6
ENR	3.95	1961.64	-134.47	0.9996	10-1000	0.8	2.8	96.2, 93.3, 104.2	2.9, 3.4, 2.4
DIF	5.45	1717.08	11335.7	0.9918	10-1000	0.8	2.7	89.3, 96.2, 97.7	3.0, 6.6, 2.1
SAR	5.64	1193.73	802.916	0.9995	10-1000	0.5	1.5	83.7, 88.1, 92.8	2.7, 4.3, 2.7
GAT	5.87	347.687	-246.444	0.9988	10-200	0.8	2.8	87.6, 92.4, 105.1	4.2, 3.0, 3.6
SPA	6.49	1824.86	7920.53	0.9961	10-200	0.7	2.2	91.4, 102.5, 113.2	3.9, 5.4, 2.9
MOX	6.79	883.49	-1620.43	0.9927	10-200	0.5	1.7	91.7, 85.4, 96.8	3.0, 7.2, 4.3

Table 3 Intra- and inter day precision, stability (n=6, expressed as %RSD) and matrix effect of the 16 FQs

FQs	Intra-day/inter-day precision (low spiked level, %)[a]		Intra-day/inter-day precision (medium spiked level, %)[b]		Intra-day/inter-day precision (high spiked level, %)[c]		Stability (RSDs, %)	Matrix effect[f]	
	Lotion	Cream	Lotion	Cream	Lotion	Cream		Lotion	Cream
MAR	6.4/9.1	7.2/9.4	3.9/ 5.0	5.8/10.2	6.8/7.9	4.0/6.9	6.1[d]/8.9[e]	0.87	1.02
FLE	4.9/8.8	6.9/10.3	3.4/8.1	2.4/4.6	5.4/8.7	4.5/6.9	9.5/9.1	0.86	1.08
ENO	6.5/10.1	8.2/8.4	4.0/7.7	8.1/10.4	4.8/5.4	3.4/8.7	8.8/11.7	0.89	0.87
PAZ	5.6/7.3	7.6/9.8	3.4/6.9	5.7/9.4	1.9/4.6	4.2/6.4	5.9/9.7	1.05	1.07
OFL	2.1/4.4	5.5/7.9	3.4/7.7	6.1/8.7	3.9/3.2	4.4/6.6	3.7/8.6	0.88	0.97
PEF	4.2/7.0	7.2/9.7	4.1/8.3	3.6/7.4	6.8/9.1	6.6/8.9	6.4/7.7	0.90	0.88
NOR	6.7/9.4	4.9/6.9	4.7/6.7	7.2/9.9	3.2/9.3	2.8/5.4	8.3/9.6	0.93	1.00
CIP	3.9/7.2	8.1/9.6	4.0/7.4	5.6/9.6	2.7/8.7	4.5/8.7	6.3/10.5	0.99	0.99
DAN	5.1/8.2	9.7/7.1	5.3/7.8	5.1/9.3	4.7/5.5	5.3/5.1	6.2/10.4	1.04	1.03
LOM	1.9/5.2	6.9/8.7	4.5/5.3	5.5/8.1	3.2/2.4	5.0/7.4	5.5/9.2	1.03	0.95
ENR	4.2/4.9	6.4/9.6	3.7/9.2	3.7/6.7	3.9/7.8	2.9/3.6	4.5/9.9	0.93	0.95
DIF	3.3/3.9	7.3/9.0	4.4/6.9	7.3/6.4	4.8/6.6	2.8/5.1	6.5/9.3	1.04	1.12
SAR	4.2/6.7	6.1/6.0	4.0/6.4	6.6/8.7	7.7/6.9	6.2/9.7	6.9/10.2	1.06	1.03
GAT	3.7/6.8	6.8/8.4	4.5/7.0	2.9/3.8	5.7/8.4	3.7/3.9	4.6/8.1	1.06	1.04
SPA	2.3/2.6	5.1/6.9	3.8/4.6	6.6/8.9	2.2/6.8	3.9/5.9	3.8/8.4	1.01	1.00
MOX	3.2/9.7	4.9/8.8	4.5/8.0	6.7/9.0	4.4/5.0	3.1/7.2	7.8/8.0	1.06	1.11

[a, b, c] Low, medium and high spiked level were set as 5, 10 and 20 μg kg^{-1}, respectively.

[d] Blank samples spiked at 10 μg kg^{-1}, refrigerated at 4 °C for one week.

[e] Blank samples spiked at 10 μg kg^{-1}, kept in dark place at ambient temperature for one month.

[f] Calculated by the slope comparison method.

Figure

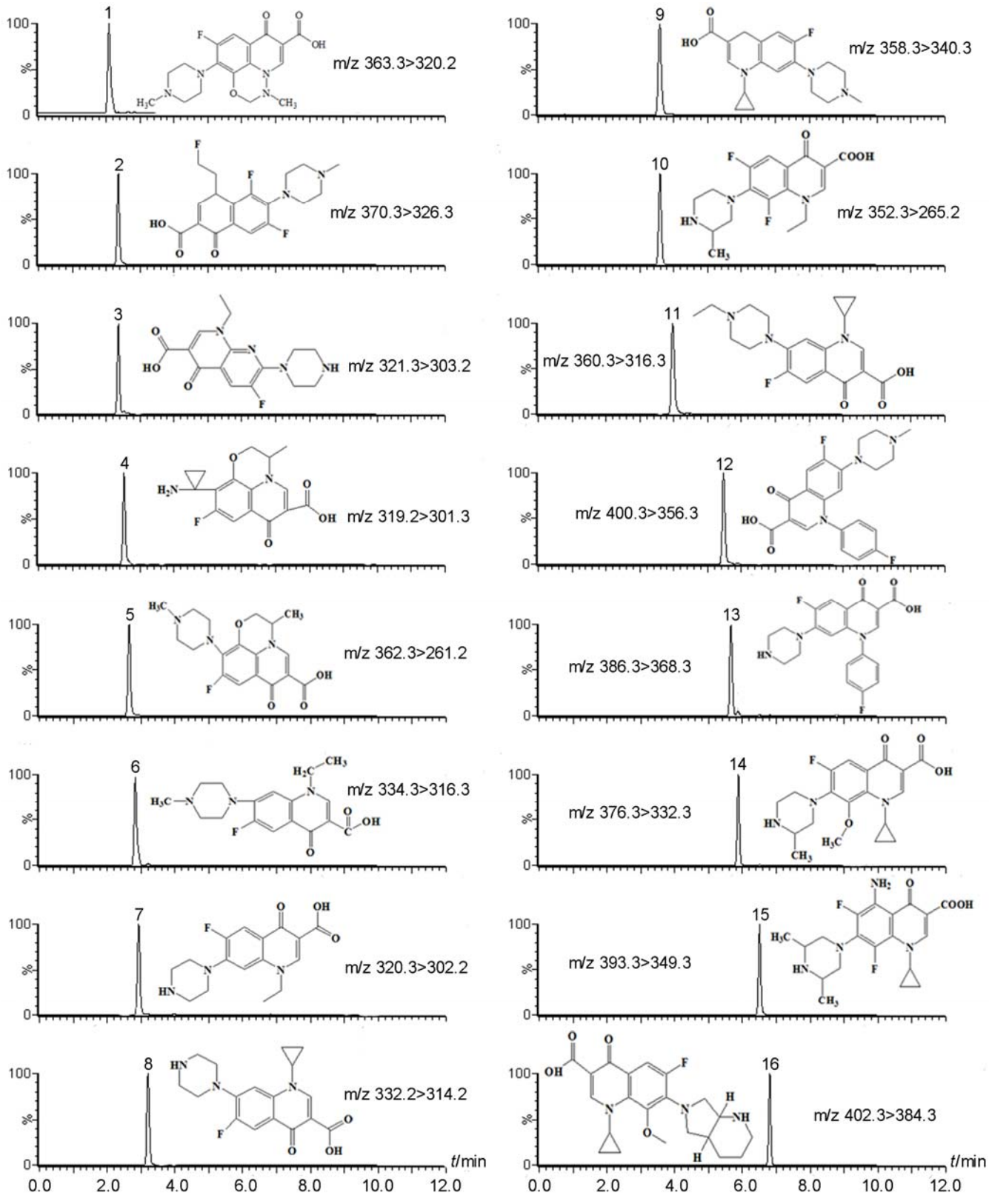

Fig. 1. (black and white)
UPLC-MS/MS chromatograms of a standard mixture of the 16 FQs at the concentrations of 0.5 μg mL^{-1}.