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Determination of undecylenic and sorbic acids in cosmetic preparations by high performance liquid chromatography with electrochemical detection☆

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Abstract

A highly sensitive and selective method for the determination of sorbic (SA) and undecylenic acid (UA) in cosmetic formulations by a high performance liquid chromatography method with electrochemical detection (ECD) is described. The pre-column derivatizations of SA and UA and the internal standard (cyclohexanoic acid (cHA)) were carried out using 1-(2,5-dihydroxyphenyl)-2-bromoethanone (2,5-DBE) as an electroactive labeling reagent previously synthesized in our lab. The resulting electroactive esters were separated by isocratic elution of a 5 μm Hypersil CN column with acetonitrile–acetate buffer eluent. The compounds were detected by a porous graphite electrode set at an oxidation potential of +0.45 V. The analytical method developed in this study is suitable for quality control assays of complex cosmetic formulations containing sorbic and/or UA.

Keywords: Sorbic acid; Undecylenic acid; Cyclohexanoic acid; HPLC; Electrochemical detection; Derivatization; 1-(2,5-Dihyroxyphenyl)-2-bromoethanone; Cosmetic

1. Introduction

Undecylenic acid (UA), a monounsaturated fatty acid, is the active ingredient in a number of over-the-counter (OTC) antifungal spray powders, that also exhibits in vitro antibacterial and antiviral activity [1–4]. For these reasons UA has been proposed as topical microbicides to help prevent the spread of sexually transmitted diseases [5]. Due to its bactericidal and fungicidal and antiviral properties UA and its derivatives (salts, esters, amides and other derived surfactants) are used in cosmetic formulations such as foot preparations, including athlete's foot gel, deodorant sticks, and antidandruff shampoos. Several perfumery bases can be prepared from UA and its derivatives [6,7].

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Few assay methods have been described for the analysis of UA in pharmaceutical, cosmetic and biological material. Colorimetric [8], titrimetric [9], high performance liquid chromatography (HPLC) methods with fluorescent detection, using 4-bromomethyl-7-methoxycoumarin, has been used to improve the sensitivity and selectivity in biological samples. The fluorescent derivative was detected by photodiode-array and fluorescence detectors [10].

Sorbic acid (*trans*,*trans*-2,4-hexadienoic acid) (SA) is an antimycotic agent used as preservative in pharmaceutical, cosmetic and food products. During the last decades SA and its potassium salt have been accepted as 'generally recognized as safe' substances [11] and have become the leading preservatives for food as well as for pharmaceutical and cosmetic preparations [12]. Several assay methods have been described for the analysis of SA in pharmaceutical and biological material, including thin layer chromatography (TLC) techniques [13,14] and titrimetric method [15]. Colorimetry and spectrophotometry [16,17] have been described, as well as polarography [18] and GLC methods [19,20]. In addition, in the last 5 years methods using capillary zone electrophoresis (CZE) procedures [21], cyclodextrin-modified CZE and micellar electrokinetic capillary chromatography (MECC) have also been reported [22,23]. Several liquid chromatographic methods coupled with UV detection [24–31] for the simultaneous determination of benzoic and SA are reported. Both compounds were simultaneously monitored at 235 nm in food samples with a detection limit of 25 μg ml^{-1} [31].

The HPLC technique is the method of choice due to its precision and simplicity. Direct detection of UA and SA is troublesome due to the lack of chromophoric and fluorescent groups, and therefore, short UV wavelengths must be used. This results in a deficiency of sensitivity and in an increase of the interference from matrix components.

HPLC coupled with electrochemical detection (ECD) represents a very sensitive technique for the determination of many important substances. Moreover, enhanced selectivity is obtained by HPLC–ECD because of the limited number of substances that undergo redox reactions under certain conditions [32]. In a previous paper we synthesized 1-(2,5-dihydroxyphenyl)-2-bromoethanone (2,5-DBE), a new electro-chemical probe, useful for the electrogenic labeling of a carboxylic acid in HPLC analysis with ECD [33,34].

In the present paper we describe a highly sensitive and selective HPLC–ECD method with isocratic elution for the simultaneous determination of SA and UA after pre-column derivatization with 2,5-DBE. For this purpose we established and optimized a novel reaction of derivatization of SA, UA and cyclohexanoic acid (cHA) (used as internal standard), to form electroactive esters quantifiable by HPLC coupled with ECD. Moreover, the applicability of this procedure to the assay of SA and UA in different cosmetic formulations was investigated as well as several factors that might influence the final results of the method.

2. Materials and methods

2.1. Apparatus

The HPLC apparatus consisted of two Model 510 pumps, a Model 712 WISP auto-injector, and an electrochemical detector (Model 5100A Coulchem; ESA, Bedford, MA, USA) which consisted of a control module and an analytical cell (Model 5010) containing two on-line porous graphite coulometric electrodes. The analysis was performed in the oxidative mode. The ED sensitivity range and response time were set at 100 nA and 10 s, respectively. Data from the detector were collected and elaborated by a computer using MAXIMA 820 software (Waters Assoc., Milfond, MA, USA). The mobile phase was filtered through a GS-type filter (0.22 μm, Millipore, Bedford, MA, USA) and degassed on-line with a model ERC-3311 solvent degasser (Erma, Tokyo, Japan).

Mass spectra were recorded on ZAB-2SE mass spectometer at 70 eV (VG Analytical). IR spectra were recorded on a Perkin–Elmer 1720 spectrophotometer as KBr disks. NMR spectra were performed on a Varian Inova 200 MHz spectrometer using $CDCl_3$ with tetramethylsilane as

internal standard. Elemental analysis for C, H, N was obtained on a Carlo Erba 1106 Analyzer (Milano, Italy) and agreed with theoretical values within ±0.4%. UV absorption spectra were recorded on a Uvikon 860 spectrometer (Kontron, Zurich, Switzerland) in a MeOH solution. Analytical TLC was performed on Merck 60 F_{254} silica gel plates. Column chromatography was performed by the flash procedure.

2.2. Chemicals

2,5-Dihydroxyacetophenone (2,5-DAP), phenyltrimethylammonium bromide tribromide was obtained from Fluka (Buch, Switzerland). SA, UA and cHA were purchased from Sigma (St. Louis, MO, USA). HPLC-grade methanol, acetonitrile and water were obtained from Carlo Erba (Milano, Italy), other chemicals used were of reagent grade or better.

2.3. Cosmetic formulations

The cosmetic formulations (bath foam and cream) were prepared from Professor Santo Scalia in his laboratory.

2.4. Synthesis of electroactive derivatization reagent and standards

2.4.1. 1-(2,5-Dihyroxyphenyl)-2-bromoethanone (2,5-DBE)

The derivatization reagent 2,5-DBE was synthesized as previously reported [33,34]. Briefly, phenyltrimethylammonium bromide tribromide ($PTMABr_3$ (6.6 mmol) was slowly added to a solution of 2,5-DAP (6.6 mmol) in 20 ml of dry THF. The mixture was stirred overnight at room temperature (21 °C) and checked by TLC with eluent cyclohexane:ethylacetate (7:3, v/v). The precipitate that formed was removed by vacuum filtration and purified by flash chromatography (cyclohexane:ethylacetate 7:3 v/v) giving 960 mg of 2,5-DBE (yield 63%).

UV: 255 nm, ε (M^{-1} cm^{-1}) 10 356. IR (KBr) cm^{-1} 3335 (OH), 1620 (C=O). ^{1}H-NMR ($CDCl_3$) δ 11.4 (s, 1H OH); 11.1 (1**H**, O**H**); 7.3–6.7 (m, 3**H**, Ar**H**); 4.4 (s, 2H C$\mathbf{H}_2$). MS (*m*/*z*); 232 ($M^+ + 2$), 230, 150, 136, 108.

2.4.2. 1-(2,5-Dihydroxyphenyl)-2-ethanone-2-sorbate (2,5-DE-SE)

2,5-DBE (0.347 mmol) was added to a solution of SA (1.04 mmol) in 5 ml of dry THF containing 110 μl of triethylamine (1.08 mmol) and heated at 70 °C for 4 h. The mixture was diluted with 20 ml of H_2O and was extracted three times with diethyl ether. The organic layer was washed with saturated $NaHCO_3$, then with H_2O, dried (Na_2SO_4), evaporated and purified by flash chromatography (chloroform:ethyl acetate, 8:2, v/v).

IR (Nujol, cm^{-1}) 3423 (**OH**), 1714 (CO), 1638 (Ph–**CO**); MS (*m*/*z*): 262 (M^+), 221, 151, 137, 109, 95, 54, 41. ^{1}H-NMR ($CDCl_3$, δ) 11.13 (s, 1H, OH), 7.39–7.27 (m, 1H; CH), 6.99–6.26 (m, 3**H** Ph), 6.19–6.14 (m, 1H CH), 5.90–5.82 (d,m 1H, CH), 5.29 (s, 2H C$\mathbf{H}_2$), 1.82–1.80 (d, 3H, CH_3). UV: λ 261 nm, ε 21 839 M^{-1} cm^{-1}; λ 359 nm, 2895 M^{-1} cm^{-1}.

2.4.3. 1-(2,5-Dihydroxyphenyl)-2-ethanone-2-undecilenate (2,5-DE-VE)

2,5-DBE (0.340 mmol) was added to a solution of UA (1.02 mmol) in 5 ml of dry THF containing 110 μl of triethylamine (1.06 mmol), and heated at 70 °C for 4 h. The mixture was diluted with 20 ml of H_2O and was extracted three times with diethyl ether. The organic layer was washed with saturated $NaHCO_3$, then with H_2O, dried (Na_2SO_4), evaporated and purified by flash chromatography (hexane:ethyl acetate, 1:1, v/v).

IR (Nujol, cm^{-1}) 3323 (**OH**) g 1721 (CO), 1657 (Ph–CO); MS (*m*/*z*) 334 (M^+), 151, 137, 91, 81, 55, 41. ^{1}H-NMR ($CDCl_3$, δ) 11.62 (s, 1H, OH), 7.05–6.90 (m, 3H Ph), 5.82 (m, 1H, CH), 5.29 (s, 2H, CH_2), 4.95 (t, 1H, CH), 2.54–2.47 (t, 1H, CH), 1.75–1.68 (m, 2H C$\mathbf{H}_2$), 1.32 (s, 2H, CH_2). UV: λ 254 nm, ε 5711 M^{-1} cm^{-1}; λ 359 nm, 3226 M^{-1} cm^{-1}.

2.4.4. 1-(2,5-Dihydroxyphenyl)-2-ethanone-2-cyclo-hexanoate (2,5-DE-cHE)

2,5-DBE (0.340 mmol) was added to a solution of cHA (1.02 mmol) in 5 ml of dry THF containing 110 μl of triethylamine (1.06 mmol), and

heated at 70 °C for 4 h. The mixture was diluted with 20 ml of H_2O and was extracted three times with diethyl ether. The organic layer was washed with saturated $NaHCO_3$, then with H_2O, dried (Na_2SO_4), evaporated and purified by flash chromatography (cyclohexane:ethyl acetate:CH_3CN 8:2:0.1 v/v).

IR (Nujol, cm^{-1}) 3421 (**OH**) g 1710 (**CO**), 1655 (Ph–CO); MS (*m/z*) 278 (M^+), 167, 137, 111, 109, 83, 56, 41. ^{1}H-NMR ($CDCl_3$, δ) 11.23 (s, 1H, OH), 7.03–6.87 (m, 3H, Ph), 5.27 (s, 2**H**, CH_2), 1.68–1.36 (m, 2H, C**H**$_2$). UV: λ 254 nm, ε 6626 M^{-1} cm^{-1}, λ 360 nm, 3513 M^{-1} cm^{-1}.

2.5. Standard solutions

Standard solutions in the concentration range 50–200 ng ml^{-1} were prepared by accurately diluting, with acetonitrile, known amounts of a stock solution containing 200 μg ml^{-1} of the electroactive esters 2,5-DE-SE, 2,5-DE-UE and 2,5-DE-cHE (internal standard).

2.6. Analysis of cosmetic formulations

Ten grams of individual cosmetic formulation were transferred to a 50 ml volumetric flask along a suitable amount of internal standard (cHA) and treated with 33 ml of HCl 0.1 N and 10 ml of THF. The mixture was homogenized in an ultrasonic bath for 10 min at 35 °C. Successively the mixture was extracted three times with methylene chloride. The organic layer was dried (Na_2SO_4) and evaporated under nitrogen. The residue was dissolved in 10 ml THF anhydrous and utilized for the derivatization procedure. In order to check that no interfering peaks were present in the cosmetic matrix, a blank mixture containing the cosmetic excipients (Table 1) was prepared and subjected to the derivatization procedure.

2.7. Derivatization procedure

The derivatization was achieved by adding an appropriate amount of a stock solution of 2,5-DBE (600 μg ml^{-1}) and triethylamine (390 μg ml^{-1}) in THF anhydrous to 1 ml of the THF solutions containing SA, UA and IS (cosmetic samples, standard or blank). The molar ratio between 2,5-DBE and SA, UA and IS was fixed at about 10:1. The reaction mixture was heated at 70 °C for 45 min. After cooling the reaction mixture was filtered on 45 μm. Teflon membrane and 50 μl was diluted to 50 ml with the eluent and analyzed by HPLC.

2.8. Optimization of the derivatization reaction

To investigate optimum conditions for derivatization, solutions containing SA (6.0 μg ml^{-1}), UA (8.0 μg ml^{-1}) and cHA (7.50 μg ml^{-1}) were incubated at 50, 70 and 90 °C and at appropriate time intervals samples were taken and analyzed immediately by HPLC.

2.9. Chromatographic conditions

Derivatized samples of 5 μl were injected into a 5 μm Hypersil CN column (150 × 4.6 mm; Alltech, Deerfield, IL, USA). Separations were performed with a mobile phase of acetonitrile–sodium acetate buffer (pH 6.0; 0.1 M) (60:40 v/v) at room temperature (21 °C) with a flow rate of 1.0 ml min^{-1} and internal standardization was used.

2.10. Optimization of electrochemical detection

In order to optimize the detection of the electroactive esters several parameters were examined such as oxidation potential, hydrodynamic

Table 1
Excipients composition of the cosmetic formulations

Cream	Bathfoam
Methyl silicone capryl glucoside, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, cetearyl alcohol, octyldodecanol, *p*-hydroxybenzoic acid ethyl ester, glycerin, citric acid, EDTA	Cocamidopropyl betaine, magnesium laureth sulphate, citric acid, EDTA, glycerine

voltammograms, pH and the ionic strength of the eluent.

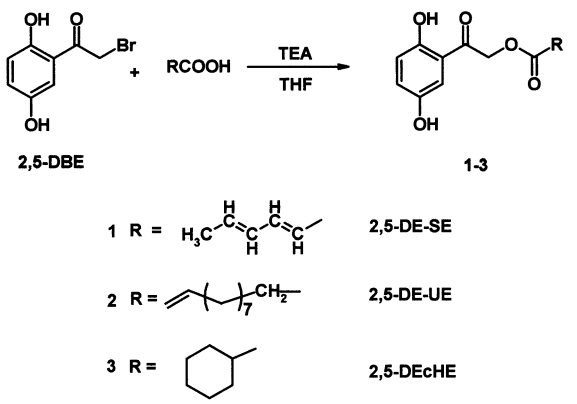
3. Results and discussion

3.1. Optimization of derivatization procedure

Scheme 1 represents the SA, UA and cHA reaction of esterification with 2,5-DBE to give the electroactive esters 2,5-DE-SE, 2,5-DE-UE and 2,5-DE-cHE by nucleophilic substitution. The reaction conditions were optimized with respect to high electroactive derivative yield, short reaction time and clean chromatograms. At 50 °C the reaction was incomplete after 100 min whereas at 90 °C additional HPLC peaks were observed suggesting the reagent or the ester derivatives were undergoing some decomposition. The time course of the derivatization for SA, UA and cHA at 70 °C is shown in Fig. 1. For sensitivity analysis at low concentrations of SA, UA and cHA the optimal heating time for the derivatization reaction was concluded to be 45 min for both acids. The electroactive esters remain stable in the reaction mixture up to 12 h after the optimum.

3.2. Chromatography

Chromatographic separations were carried out under reverse phase conditions on a 5 μm Hypersil



Scheme 1. Derivatization reaction of SA, UA and cHA with 2,5-DBE to give the electroactive esters 2,5-DE-SE (1) 2,5-DE-UE (2) and 2,5-DE-cHE (3).

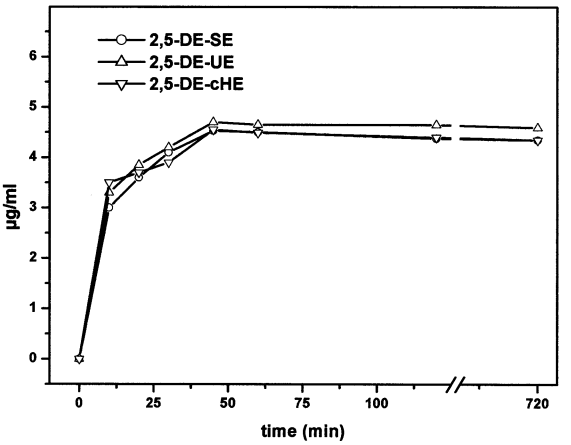


Fig. 1. Time course of the derivatized esters 2,5-DE-SE, 2,5-DE-UE and 2,5-DE-cHE in the optimization of derivatization study.

CN column using a binary eluent, acetonitrile–sodium acetate 0.1 M (60:40 v:v), at a flow rate of 1 ml min^{-1} with an injected volume of 5 μl. The analysis was complete within 11 min and the retention times were 3.93 min for 2,5-DE-SE, 4.60 min for 2,5-DE-cHE (internal standard) and 9.97 min for 2,5-DE-UE. This chromatographic system gave a complete and rapid baseline resolution of electroactive esters, and therefore, was adopted in the following cosmetic formulation analysis-studies.

3.3. Optimization of detection

Several parameters were examined in order to optimize the ECD of the electroactive compound synthesized in this study. Under the chromatographic conditions mentioned above, the electroactive ester derivatives responded to the ED oxidation potential higher than +0.2 V. Enhanced responses for all electroactive compounds were obtained as the working electrode potential was increased from +0.2 to +0.6 V. With additional applied potential no further increase in the peak height occurred and a rise in the background current was observed.

Electroactive properties of the compounds 2,5-DBE, 2,5-DE-SE, 2,5-DE-UE and 2,5-DE-cHE (internal standard) were also examined by their hydrodynamic voltammograms (Fig. 2). Inspec-

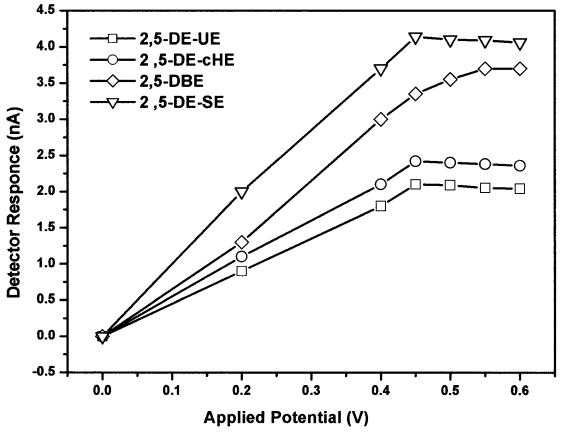


Fig. 2. Hydrodynamic voltammograms of the electroactive compounds 2,5-DBE, 2,5-DE-SE, 2,5-DE-UE and 2,5-DE-cHE.

tion of data reported in Fig. 2 shows that increasing the applied potential from 0.2 to 0.6 V the detector response was enhanced for all the electroactive compounds. The best potential was +0.45 V because for higher potentials the detector response decreased progressively for all electroactive derivatives whereas an increase was observed only for the derivatization reagent 2,5-DBE.

The ECD performance was markedly influenced by the ionic strength of the mobile phase. With increasing concentrations of the sodium acetate buffer from 0.05 to 0.10 M an increase in the electrochemical detector response of 6% was observed. No significant improvement in the detector response was achieved by further increasing the buffer molarity that was consequently fixed at 0.1 M and the pH at 6.00.

3.4. Linearity and detection limit

The linearity of response was examined for the electroactive esters in the range 50–200 ng ml^{-1}. The correlation coefficients of the linear regression of the standard curves were consistently greater than 0.99. The detection limit was determined by analyzing progressively lower concentrations of the electroactive esters and were found to be 0.95, 0.72 and 0.78 pmol ml^{-1} for 2,5-DE-SE, 2,5-DE-UE and 2,5-DE-cHE, respectively, for a signal/noise ratio of 3:1 ($n = 3$) with an injected volume of 5 μl.

3.5. Accuracy and precision

The accuracy of the assay was determined by repetitive analysis of blank cosmetic preparations spiked with 50, 100 and 200 ng ml^{-1} of SA and UA standards and then subjected to derivatization. The accuracy of the assay was determined by comparing the measured concentration to its true value. The repeatability of the method was evaluated by replicate analysis of the above mentioned blank formulation spiked with a known amount of SA and UA standards and was expressed as R.S.D. (Table 2). The inter-day precision data are reported in Table 3.

3.6. Analysis of cosmetic formulations

UA and SA have a poor detectability in the ultraviolet range, and therefore, the HPLC analysis with UV detection, is difficult. In general, when compounds have a very low ultraviolet absorbance, one would attempt to derivatize them for detection enhancement and/or to evaporate the extract for their enrichment. In this case extreme care must be taken to avoid losses due to volatilization when concentrating the extract. HPLC in conjunction with a pre-column chemical derivatization, using the electroactive-labeling reagent 2,5-DBE, constitutes an effective approach to overcome the problem. The HPLC–ECD method developed in this study was applied to the assay of SA and UA in cosmetic dosage forms.

Two different cosmetic preparation (cream and bathfoam) containing SA and UA were subjected to the derivatization and HPLC analysis. The high sensitivity achieved by ECD permitted an accurate quantification of the preservatives present in the cosmetic preparations. Fig. 3 shows a representative chromatogram of a cosmetic cream formulation. The results are presented in Table 4 and were found to be in good agreement with the label claim and demonstrated the precision of the method. No interfering peaks were observed in the blank formulations subjected to derivatization and HPLC analysis.

Table 2
Intra-day accuracy and precision in the analysis of SA and UA

True concentration (ng ml^{-1})	Accuracy mean ± S.D.[a]		R.S.D.[b](%)	
	SA	UA	SA	UA
50	98.7 ± 3.33	98.6 ± 3.21	3.37	3.26
100	99.6 ± 2.85	99.45 ± 2.76	2.86	2.78
200	100.9 ± 1.43	100.5 ± 1.42	1.42	1.41

[a] $n = 6$.
[b] $n = 6$.

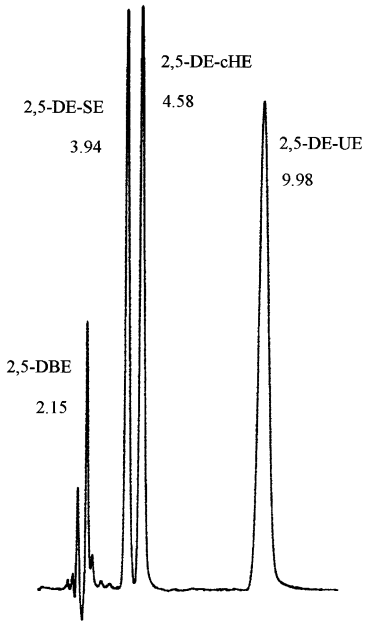


Fig. 3. Representative HPLC–ECD chromatogram of cosmetic cream formulation. 2,5-DBE (1.58 min), 2,5-DE-SE (3.94 min), 2,5-DE-cHE (4.58 min), 2,5-DE-UE (9.9 min).

4. Conclusion

Although HPLC is a versatile technique for the analysis of compounds in complex matrices, such as biological, pharmaceutical or cosmetic, the poor ultraviolet absorbance of SA and UA makes the direct HPLC separation and quantification difficult due to the interfering substances. The preferred method for a HPLC analysis of SA and UA in complex matrices is the direct conversion into a detectable compound without complicated steps to isolate it.

The derivatization of SA and UA acid with the electroactive labeling reagent 2,5-DBE yields stable and highly sensitive electroactive esters which are easily quantifiable by the HPLC–ECD technique. The applied potential of +0.45 V permits the selective oxidation of the electroactive esters without interference from the excipients present in the cosmetic matrix because of the limited number of substances that can undergo redox reactions under these conditions.

This HPLC–ECD method offers a means of enhancing the selectivity and sensitivity of conventional HPLC–UV analysis of the poorly absorbing

Table 3
Inter-day accuracy and precision in the analysis of SA and UA

True concentration (ng ml^{-1})	Accuracy mean ± S.D.[a]		R.S.D.[b](%)	
	SA	UA	SA	UA
50	98.40 ± 3.91	98.80 ± 3.53	3.97	3.57
100	98.31 ± 3.56	99.65 ± 3.21	3.62	3.22
200	101.30 ± 1.59	100.75 ± 1.63	1.57	1.62

[a] $n = 6$.
[b] $n = 6$.

Table 4
HPLC–ECD assay results for SA and VA in cosmetic formulations

Cosmetic preparation	Label claim (%)		Found ± R.S.D. ($n = 6$) (%)	
	SA	UA	SA	UA
Cream	0.1345	0.0800	96.20 ± 3.85	96.30 ± 4.52
Bathfoam	0.2000	0.1600	101.35 ± 2.98	99.82 ± 3.75

UA. In conclusion, the high selectivity, good accuracy, repeatability and sensitivity of the HPLC–ECD technique developed in this study makes it suitable for quality control assays of complex cosmetic formulations containing SA and UA. In addition the methods proposed may be suitable with some modification to quantification of SA and UA in biological samples.

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