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Determination of glycolic acid in cosmetic products by solid-phase extraction and reversed-phase ion-pair high-performance liquid chromatography

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Abstract

A procedure has been developed for the assay of glycolic acid in cosmetic products by reversed-phase high-performance liquid chromatography using an ion-pairing method. After dissolution in tetrahydrofuran–water (90:10, v/v), samples were purified by solid-phase extraction using silica-based strong anion-exchange cartridges and analysed directly on an Ultrasphere ODS column with UV detection at 210 nm and methanol–phosphate buffer (2:98, v/v), containing tetrabutylammonium iodide, as the mobile phase. Recovery of glycolic acid from different cosmetic matrices was between 92.4 and 96.2% and the precision of the method was better than 5.4% relative standard deviation. The procedure is rapid, simple, selective and it is suitable for routine analyses of commercial cosmetics. © 1998 Elsevier Science B.V.

Keywords: Cosmetics; Glycolic acid; Organic acids

1. Introduction

In recent years the use of α-hydroxy acids (e.g., glycolic, lactic, malic, tartaric and citric) in cosmetic products has greatly expanded [1–4]. These acids are reported to increase skin hydration [3,4], to induce the removal of the outer layers of the skin [2,5] and to improve some of the visible effects of ageing by reducing lines and wrinkles and stimulating skin cell renewal [1,3,4].

The α-hydroxy acids most commonly used in skin care products are lactic acid and especially glycolic acid [1–3,6]. Different types of glycolic acid-containing cosmetics are marketed [2] including preparations with relatively low concentrations of the acid (generally less than 10%, w/w), formulations employed in beauty salons which generally contain higher glycolic acid levels (up to 30–40%, w/w) and products used by physicians to perform chemical skin peeling containing up to 70% (w/w) glycolic acid. The glycolic acid titre of commercial cosmetics affects the activity of the preparations [2,3] and their potential to cause adverse reactions [1,3] which range from skin irritation to serious skin injuries at high concentrations (e.g., severe burns and swellings), as reported by the US Food and Drug Administration [2]. Hence, the assay of glycolic acid in finished products is particularly important both for quality control purposes and for consumer safety.

The current chromatographic methods for the analysis of short-chain carboxylic acids, including glycolic acid, are based on gas chromatography after

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derivatization [7–9] and high-performance liquid chromatography (HPLC) [10–17]. The latter technique is the method of choice [11,12,17] since the compounds can be analyzed directly, employing four main separation modes, namely ion-exclusion [10–12,14], ion-exchange [12,13], reversed-phase (RP) [12,17] and ion-pair [15,16] chromatography. However, despite the large number of publications which have appeared in the literature on the determination of short-chain organic acids in different matrices [7–17], no chromatographic methods for the assay of glycolic acid in cosmetics have yet been reported.

The present study describes the first chromatographic procedure for the determination of glycolic acid in cosmetic products using ion-pair RP–HPLC. The samples, after rapid and efficient purification on strong anion-exchange (SAX) cartridges, were directly analyzed by HPLC–UV without prior derivatization. The application of the method to the assay of glycolic acid in commercial cosmetics is also demonstrated.

2. Experimental

2.1. Reagents

Glycolic acid, tetramethylammonium iodide and tetrabutylammonium iodide were obtained from Sigma (St. Louis, MO, USA) with purities of at least 98%. Methanol was HPLC grade from Baker (Phillipsburg, NJ, USA). Water was purified by a Modupure Plus system (Continental Water Systems, San Antonio, TX, USA). All other chemicals were of analytical-reagent grade (Carlo Erba, Milan, Italy). Bond-Elut SAX cartridges were supplied by Varian (Harbor City, CA, USA). Commercial cosmetics, from various manufacturers, were purchased from retail stores and from a local pharmacy.

2.2. High-performance liquid chromatography

The HPLC apparatus consisted of a modular chromatographic system (Model 980-PU pump, Model 980-02 solvent programmer and Model 975-UV variable-wavelength UV–Vis detector; Jasco, Tokyo, Japan) linked to an injection valve with a 10-μl sample loop (Model 7125; Rheodyne, Cotati, CA, USA). The detector was set at 210 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 802 RN syringe (10 μl; Hamilton, Bonaduz, Switzerland).

Separations were performed on a 5-μm Ultrasphere ODS column (150×4.6 mm I.D.; Beckman, San Ramon, CA, USA) fitted with a guard column (LiChrospher RP-18, 5-μm particles, 4×4 mm I.D.; Merck, Darmstadt, Germany) and eluted with 0.025 *M* sodium phosphate buffer (pH 7.2)–methanol (98:2, v/v) containing 0.002 *M* tetrabutylammonium iodide. The mobile phase was filtered through GV-type filters (0.22-μm; Millipore, Bedford, MA, USA) and deaerated on-line by a Model ERC-3311 automatic solvent degasser (Erma, Tokyo, Japan). The column temperature was maintained at 32°C using a Model 7990 Space Column Heater (Jones Chromatography, Hengoed, UK). Chromatography was performed under isocratic conditions, at a flow-rate of 0.6 ml/min. Other columns used in this study included a Spherisorb SAX (5-μm particles, 250×4.6 mm I.D.; Phase Separations, Deeside, UK), an Apex ODS (5-μm particles, 250×4.6 mm I.D.; Jones Chromatography, Hengoed, UK) and an Hypersil ODS (5-μm particles, 200×2.1 mm I.D.; Shandon, Runcorn, UK). The injection of sodium nitrate, with methanol–water as eluent, was used to determine the hold-up time. The identity of the glycolic acid peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method.

2.3. Sample preparation

The cosmetic product (0.2–1.0 g, according to the glycolic acid content) was accurately weighed into a 50-ml volumetric flask and dissolved in tetrahydrofuran–water (90:10, v/v) under sonication (5 min). After dilution to volume, a portion (1 ml, <0.03 mmol of glycolic acid) of the resulting sample was applied (at a flow-rate of ca. 1 ml/min) to a Bond-Elut SAX cartridge (sorbent mass, 500 mg) which was pre-conditioned with 4 ml of methanol, 4 ml of the HPLC mobile phase, 2 ml of water and 2 ml of tetrahydrofuran–water (90:10, v/v). The extraction

column was then washed with 2 ml of methanol and, after light drying by vacuum, eluted with 4.5 ml of the HPLC mobile phase. The latter fraction was made up to volume (5 ml) and a portion (5 μl) of this solution was directly analyzed by HPLC.

2.4. Assay validation

A cream (oil-in-water emulsion) and a gel test sample were prepared in the laboratory by adding glycolic acid at levels of 2 and 10% (w/w) to the formulation components (cream excipients were shea butter, sesam oil, sweet almond oil, methyl silicone, polyoxyethylene sorbitan monostearate, polyoxyethylene stearyl ether, sodium lauryl sulphate, butylated hydroxyanisole, *p*-hydroxybenzoic acid ethyl ester, glycerin, imidazolidinyl urea, triethanolamine, yarrow extract, water; gel excipients were hydroxyethylcellulose, triethanolamine, glycerin, imidazolidinyl urea, water). The percentage recovery was calculated by comparing the peak areas of glycolic acid extracted from the test samples with those obtained by direct injections of an equivalent concentration of the analyte dissolved in the mobile phase.

Calibration curves of peak areas versus concentration were generated with placebo extracts spiked with known amounts of glycolic acid in the concentration range 15–500 μg/ml.

The chromatographic precision was evaluated by repeated analyses ($n=6$) of the same sample solution obtained from a cream containing 2% (w/w) glycolic acid. The precision of the method was calculated by Bond-Elut SAX extraction and HPLC assay of independent samples ($n=6$) from the same cream formulation.

3. Results and discussion

3.1. Chromatography

The objectives of this study were to develop a HPLC method for the rapid and accurate determination of glycolic acid in complex cosmetic matrices (e.g., creams, gels, liquid detergents).

Preliminary experiments were carried out on a silica-based strong anion-exchange column with UV detection at 210 nm and phosphate buffer (pH 3.5) as eluent. Marked overlapping of glycolic and lactic acid was observed, which is a major drawback since these two α-hydroxy acids are frequently used concurrently in cosmetic products [1–3]. Reversed-phase packings have been reported by several researchers [12,15] to produce enhanced separation efficiency for low-molecular-mass aliphatic acids compared to ion chromatographic techniques. Consequently, an octadecyl-silica (C_{18}) stationary phase was selected for subsequent analyses using UV detection (210 nm). This chromatographic system provides additional advantages for application in quality control laboratories since it requires a conventional HPLC column and equipment. Separation of short-chain organic acids has been achieved on C_{18} packings using low pH (2.2–2.5) aqueous eluents [12,17], although partial overlapping of some component peaks occurred [17]. Under these conditions, resolution of glycolic and lactic acid was obtained. However, glycolic acid was weakly retained on the different RP sorbents examined in this investigation (see the Section 2), eluting close to the void volume region (k, 0.27–0.30) where reduced resolution power and increased interference from unretained matrix components hamper accurate determinations.

In order to enhance the retention of short-chain carboxylic acids in RP–HPLC, hydrophobic ion-pair reagents, such as alkylammonium salts and protonated amines have been used as mobile phase additives by some authors [15,16]. However, conditions were not optimized specifically for the chromatography of the hydrophilic α-hydroxy acids and, in particular, for the resolution of glycolic acid from the other α-hydroxy acids (lactic, malic, citric, tartaric) used in the formulation of cosmetics [1–4]. In the present study, methanol–phosphate buffer eluents containing tetramethyl- or tetrabutylammonium salts were examined in conjunction with a C_{18} column. The glycolic acid elution time was not affected by the addition of tetramethylammonium iodide (2 m*M*) to the eluent (2%, v/v, methanol in 0.025 *M* phosphate buffer, pH 7.2) while appreciable retention of the acid occurred with tetrabutylammonium as the counter-ion in the mobile phase (see Fig. 1). At ion pairing concentrations of 1 and 2 m*M*, the retention factor of glycolic acid rose to 0.92 and

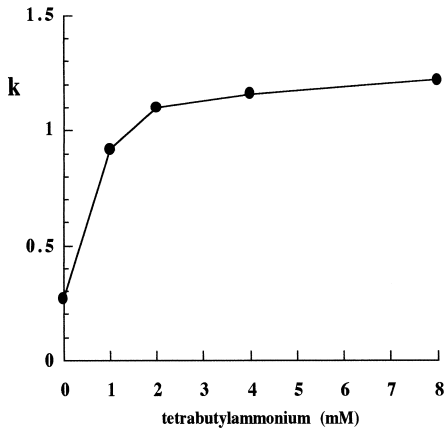

Fig. 1. Dependence of the retention factor of glycolic acid on the concentration of tetrabutylammonium. The mobile phase also contained 0.025 *M* sodium phosphate buffer (pH 7.2)–methanol (98:2, v/v). Column, Ultrasphere ODS; flow-rate, 0.6 ml/min; UV detection, 210 nm.

1.10, respectively. Little enhancement of the analyte retention (Fig. 1) was achieved by further increasing the tetrabutylammonium salt concentration (up to 8 m*M*), which was consequently set at 2 m*M*. Under the chromatographic conditions outlined above, satisfactory retention and peak symmetry were attained for glycolic acid, with baseline separation of the critical peak pair formed by glycolic and lactic acids (see Fig. 2). The other α-hydroxy carboxylic acids commonly present in cosmetics [2–4] were also

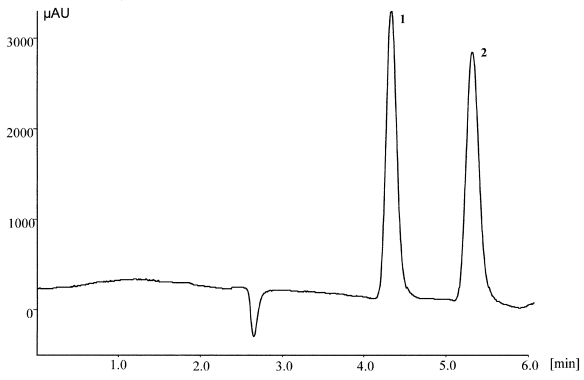

Fig. 2. Typical HPLC separation of a standard mixture of glycolic (51 μg/ml) and lactic (50 μg/ml) acid. The mobile phase was 0.025 *M* sodium phosphate buffer (pH 7.2)–methanol (98:2, v/v) containing 0.002 *M* tetrabutylammonium iodide. The other operating conditions were as in Fig. 1. Peaks: 1=glycolic acid, 2=lactic acid.

completely resolved from glycolic acid (malic acid *k*, 3.5; tartaric acid *k*, 3.6; citric acid *k*, 11.9), thus confirming the selectivity of the method. When the injected solution deviated in composition from the mobile phase, a negative system band developed which eluted after glycolic acid. Since peak deformation has been reported to occur [18] even if there is good resolution between analyte and system peaks, standard and samples were dissolved in the mobile phase to eliminate the influence of the system peak.

3.2. Solid-phase extraction

To enable the direct determination of glycolic acid by HPLC–UV without derivatization, non-selective short-wavelength (205–210 nm) UV detection is required [12,14,15] which results in increased interference from matrix components. However, the selectivity of the HPLC–UV assay of glycolic acid can be enhanced in the sample preparation step by efficient clean-up procedures. Purification methods based on solid-phase extraction (SPE) techniques were examined. Although C_{18} silica cartridges have been employed for the extraction of short-chain carboxylic acids from water samples [19], disposable columns pre-packed with a silica-based strong anion exchanger were selected for this investigation since the solvents (i.e. methanol, tetrahydrofuran, chloroform) used for the effective dissolution of cosmetic cream matrices [20–22] inhibit adsorption of glycolic acid on hydrophobic C_{18} sorbents. Moreover, the anion exchanger provides more selective extractions, compared to the C_{18} phase, as a result of higher-energy ionic interactions [23]. Published reports [8,17,24] have demonstrated that glycolic acid is extracted from aqueous samples using disposable columns packed with silica bonded to quaternary ammonium groups (SAX). In the present investigation, the SAX cartridges were found to efficiently retain glycolic acid in tetrahydrofuran–water (90:10, v/v), which was chosen as the solvent for sample preparation to increase the solubilization of heterophasic formulations such as creams [20–22], thus preventing possible analyte losses due to occlusion in insoluble matter. Moreover, the HPLC mobile phase was included in the cartridge conditioning step since it produces improved peak symmetry and baseline output compared with conditioning solvents

containing water, acetate buffer (20 m*M*, pH 4.5) or phosphate buffer (20 m*M*, pH 7.2). After washing the extraction column with methanol, to remove non-ionic endogenous components, quantitative desorption (recovery rates >95.5%) of glycolic acid was obtained by elution of the cartridge with 4.5 ml of the HPLC mobile phase. The direct injection of the SPE eluent is thus fully compatible with the RP ion-pair conditions.

3.3. Accuracy and precision

The accuracy of the method was determined by recovery experiments. A cosmetic cream and gel placebos were spiked with glycolic acid at 2 and 10% (w/w) and subjected to the assay procedure. In Table 1 the recoveries and relative standard deviations (R.S.D.) after repeated analyses are given. The average recoveries of glycolic acid from the cream and gel matrices were satisfactory with values ranging from 92.4 to 96.2%. Moreover, the recovery was found to be consistent between different batches of SAX cartridges.

In order to evaluate the precision of the method, a sample of cream containing 2% (w/w) glycolic acid was assayed (see Section 2). The precision, determined on six replicate assays, was represented by R.S.D. values of 1.9% and 3.8% for the chromatographic and the methods' precision, respectively.

3.4. Selectivity

Figs. 3 and 4 show for the cream and gel preparations the comparative HPLC traces of placebos (Fig. 3A, Fig. 4A) and spiked placebos (Fig. 3B, Fig. 4B). The chromatograms clearly indicate that there is no interference from the formulation excipients. In contrast, direct injection of the placebos onto the HPLC, after dissolution of the product in tetrahydrofuran–water and filtration, produced spurious peaks in the glycolic acid retention window and late-eluting substances that interfered with successive analyses.

Table 1
Recovery of glycolic acid added to experimental formulations

Formulation	Amount added (%, w/w)	Recovery %[a]
Cream	2	92.4 (2.8)
	10	93.7 (2.2)
Gel	2	94.3 (2.1)
	10	96.2 (3.4)

[a] Each value is the mean (R.S.D.) of six determinations.

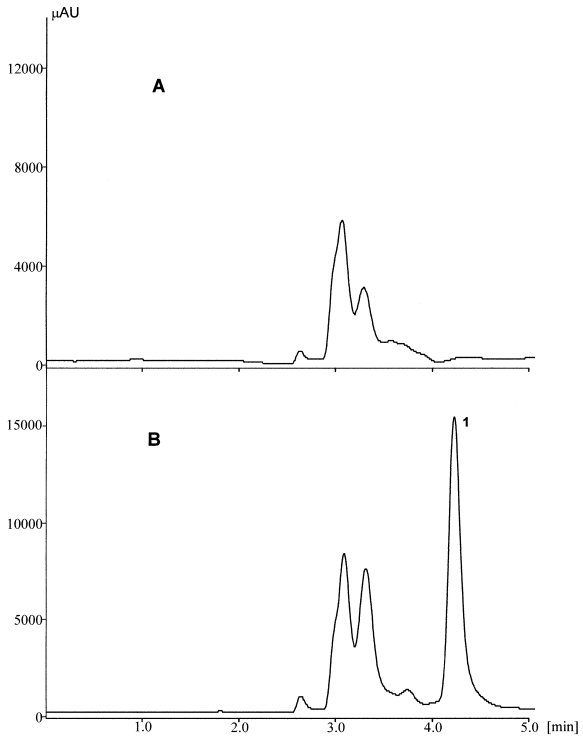

Fig. 3. HPLC chromatograms of (A) a cream placebo extract and (B) the same product spiked with 2% (w/w) of glycolic acid. Operating conditions and peak identification as in Fig. 2.

3.5. Linearity and quantification limit

Calibration curves ($n=6$) were linear in the range 15–500 μg/ml (slope, 497.6±12.4), with correlation coefficients greater than 0.998. The intercepts with the *y*-axis were not significantly different from zero ($p>0.05$).

The minimum quantifiable amount (i.e., 15 μg/ml which corresponds to a concentration in the cosmetic of 0.4%, w/w) was one-half the minimum level of glycolic acid normally used in the formulation of cosmetics [1,3].

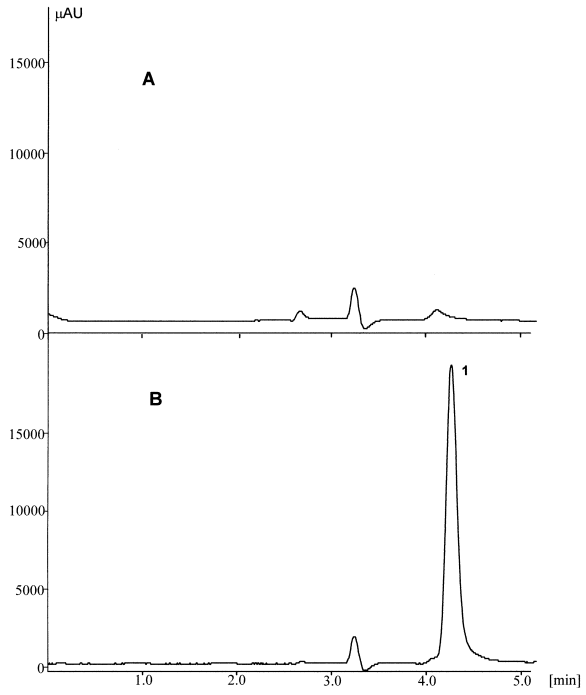

Fig. 4. HPLC traces of (A) a gel placebo extract and (B) the same product spiked with 2% (w/w) of glycolic acid. Operating conditions and peak identification as in Fig. 2.

3.6. Application

Four different cosmetic preparations, all commercially available and containing markedly different concentrations of glycolic acid, were assayed using the procedure developed in this study (Table 2). The results obtained confirm the precision of the method and show compliance with the label claim. Moreover, the data presented demonstrate that the effectiveness of the SPE technique, described here, is not affected by the type of cosmetic matrix, the method being applicable also to formulations, such as shampoos, containing high levels of anionic surfactants (e.g., alkyl and alkyl ether sulphates) [25] with great affinity to the anion-exchange sorbent.

Table 2
Assay results for the HPLC analysis of glycolic acid in commercial cosmetic products

Sample	Label claim (%, w/w)	Found[a] (%, w/w)	% of label	R.S.D.
Shampoo	0.8	0.72	90.0	5.4
Day cream	8.0	7.63	95.4	1.5
Gel A	10.0	9.61	96.1	3.1
Gel B	50.0	43.30	86.6	3.0

[a] Each value is the mean of five determinations.

In conclusion, the first HPLC method has been developed for the selective and rapid determination of glycolic acid in cosmetics. After purification on SAX cartridges, the samples were directly assayed by HPLC–UV, the entire procedure taking less than 25 min to perform. Moreover, the use of multisample apparatus designed for SPE cartridges enables several isolations to be carried out simultaneously. Because of minimal sample preparation, good accuracy and precision, the method is suitable for quality control analyses of finished cosmetic products.

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