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Determination of 18 β-glycyrrhetinic acid and its phytosome in cosmetics by derivative UV spectrophotometry and liquid chromatography (HPLC)

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Synopsis

First-order and second-order derivative spectrophotometric methods are proposed for the determination of 18 β-glycyrrhetinic acid (GT) and its phytosome (GTP) in cosmetic formulations (creams and hydrophilic gels). The methods involve preliminary liquid–liquid extraction procedures to eliminate the interferences from the formulation excipients. The extraction steps and the results obtained were verified by a reversed-phase liquid-chromatographic (HPLC) method. The spectrophotometric method proved to be suitable for a reliable quality control of cosmetic formulations containing ≥0.3% of GT, while the HPLC method was found to be of general application.

INTRODUCTION

18 β-Glycyrrhetinic acid (GT) is the aglycone of glycyrrhizinic acid (GZ), the most important component of licorice extracts. The compound is widely used in cosmetics (1,2) and pharmaceuticals (3) for its antiinflammatory properties, due to its inhibitory action on arachidonic acid metabolism (4).

Because of its lipophilic character, GT can be used generally in O/W and W/O emulsions and in oil-based cosmetics such as lipogels, ointments, and anhydrous pastes. Recently the 18 glycyrrhetinic acid phytosome (GTP) has been introduced for cosmetic use (1). It is described as a complex between purified soya phospholipids and GT, dispersible in water, and used also in water-based formulations such as hydrophilic gels.

Analytical methods for the analysis of GT include colorimetric (5) and several HPLC (6–9) procedures. In order to provide alternative selective methods suitable for a practical quality control of cosmetic preparations, derivative UV spectrophotometry was applied.

Derivative spectrophotometry is a well established technique for resolution enhancement; it allows selective discrimination of sharp bands over broad bands in UV spectra, offering an effective approach to the suppression of broad-background matrix absorption (10–12). Thus, in the present study, first and second derivative UV spectroscopy was applied to the determination of GT and GTP in commercially available cosmetic for-

mulations (creams and hydrophilic gels), and the results were compared with those obtained by a reference HPLC method.

EXPERIMENTAL

MATERIALS

18 β-Glycyrrhetinic acid (GT) was obtained from Aldrich Chemie (Switzerland) and glycyrrhetinic acid phytosome (GTP) from Indena (Gruppo Inverni della Beffa, Italy). Testosterone acetate (internal standard) was from Sigma (St. Louis, MO). Methyl, ethyl, propyl, and butyl p-hydroxybenzoates, butylhydroxyanisol (BHA), butylhydroxytoluol (BHT), and sodium dehydroacetate were supplied by Formenti (Italy). Imidazolidinyl urea (IMU) was from Medolla (Milan, Italy). Acetonitrile was obtained from Promochem (F.R.G.), and water was deionized and double-distilled.

Aqueous 0.02 M phosphate buffer solutions (pH 3.0) were prepared by mixing potassium dihydrogen phosphate and phosphoric acid solutions in the proportion required to give the desired pH value.

The GT (0.5 mg ml^{-1}) and GTP (1.5 mg ml^{-1}) stock solutions in 1% ammonium hydroxide and methanolic 1% potassium hydroxide were found to be stable for more than one month at room temperature.

The testosterone acetate solution (0.1 mg ml^{-1}) was prepared in the mixture acetonitrile:phosphate buffer (0.02 M; pH = 3.0) 60:40 (v/v), as were the solutions of the single preservants and antioxidants.

APPARATUS

All the spectrophotometric analyses were performed on a Jasco UVIDEC-610 double-beam spectrophotometer, using 1-cm quartz cells with a slit width of 2 nm. The working settings were scan speed 100 nm min^{-1} and chart speed 20 nm cm^{-1} over the range 500–200 nm. For the derivative mode, $\Delta\lambda = 5$ nm was selected, and absorbance scale expansion ×5 (first derivative mode) and ×15 (second derivative mode) were used.

The HPLC apparatus consisted of a Waters 501 solvent delivery system, an on-line ERMA ERC-3312 degasser, a Rheodyne sample injection valve with a 20-μl sample loop, a variable wavelength UV detector (model 481), and a data module 745. The detector wavelength was set at 248 nm, with the integrator attenuation at 16.

A 250 × 4.5-mm I.D. column packed with 5-μm Hypersil C18 was used at ambient temperature, using as mobile phase a binary mixture consisting of acetonitrile:0.02 M potassium phosphate buffer (pH = 3.0) 65:35 (v/v) or 60:40 (v/v) at a flow rate of 1.2 ml min^{-1}.

CALIBRATION GRAPHS

First derivative method. The zero order UV spectra of GT (7.2–36 μg ml^{-1}) and GTP (30–150 μg ml^{-1}) standard solutions in 1% ammonium hydroxide were recorded, using 1% ammonium hydroxide as the blank. The first order spectrum was then derived. The amplitude of the negative peaks at $\lambda = 248$ nm and $\lambda = 246.4$ nm to the zero line, $^1D_{248}$ and $^1D_{246.4}$, were measured for GT standard solutions and GTP

standard solutions, respectively. The amplitude values were then plotted against the corresponding GT and GTP concentrations to obtain the calibration graphs.

Second derivative method. The GT (7.2–36 μg ml^{-1}) and GTP (30–150 μg ml^{-1}) analytical solutions were prepared in 1% methanolic potassium hydroxyde. The zero-order UV spectra of the solutions were recorded against the solvent blank, and the second-order UV spectra were then derived. The amplitude of the positive peak at 275.2 nm ($^2D_{275.2}$) to the zero line was measured for each standard solution and was plotted against the corresponding concentration to obtain the calibration graph.

HPLC method. Standard solutions of GT (6.0–15.0 μg ml^{-1}) in the mobile phase, containing 4.3 μg ml^{-1} of testosterone acetate as internal standard, were injected into the chromatograph. The ratios of analyte area to internal standard area were plotted against the corresponding analyte concentrations to obtain the calibration graph.

ANALYSES OF COMMERCIAL FORMULATIONS

Commercially available creams and hydrophilic gels containing GT and GTP at different concentrations (see Table II) were analyzed.

First derivative method. Quantities of 300 mg of commercial sample 3 (1% GT) and 1 g of gels 4 and 5 (1% GTP) were transferred into a separator containing 50 ml of chloroform and extracted with 3 × 15 ml of aqueous hydrochloric acid solution (pH 2.0). The chloroform solution was then adjusted to the volume of 50 ml in a volumetric flask. An aliquot of 10 ml of this solution was extracted with 1 × 10 ml and 3 × 5 ml of 1% ammonium hydroxide solution. The combined ammoniacal extracts were adjusted to the volume of 25 ml in a volumetric flask. The filtered resulting solutions were then analyzed as described under Calibration Graphs (first derivative method), and the GT and GTP content was calculated by comparison with an appropriate standard solution according to the formula Cs(Du/Ds), in which Cs is the concentration, μg per ml, of GT in the standard solution and Du and Ds are the amplitudes $^1D_{248}$ in the first derivative UV spectrum of the sample solution and standard solution, respectively.

The same sample preparation procedure was applied when the blanks of the cosmetic samples, which contained all the components except GT and GTP, were analyzed.

Second derivative method. This method was applied to the analysis of the samples 4 and 5 (hydrogels, 1% GTP content). A 1.0-g quantity of the product was dissolved in chloroform and washed three times with a solution of hydrochloric acid in water (pH = 2.0). The chloroform extract was adjusted to the volume of 50 ml in a volumetric flask, and an aliquot of 10 ml of the chloroform solution was evaporated to dryness. The residue was dissolved with 1% methanolic potassium hydroxide, diluted to 25 ml in a volumetric flask. The filtered solution was then subjected to the second derivative method, and the amplitude $^2D_{275.2}$ was used to calculate the GTP content in each sample by comparison with the appropriate standard solution, according to the formula Cs(Du/Ds), in which Cs is the concentration, in μg per ml, of GT in the standard solution and Du and Ds are the amplitude $^2D_{275.2}$ in the second derivative UV spectrum of the sample solution and standard solution, respectively. The same sample preparation procedure was applied to the GTP lacking hydrogels.

HPLC method. A suitable amount of sample was dissolved in a mixture of chloroform-methanol 1:1 to obtain a solution containing 13 μg ml^{-1} of GT. An aliquot of 10 ml

of this solution was evaporated to dryness and the residue suspended with the mobile phase containing 4.3 $\mu g\ ml^{-1}$ of testosterone acetate as an internal standard. The volume was adjusted to 10 ml in a volumetric flask. The resulting solution was filtered and then injected into the chromatograph in triplicate. The unknown samples were run concurrently with a standard, and the peak area ratio of GT to internal standard was used to calculate the GT content according to the formula Cs(Ru/Rs) in which Cs is the concentration, in μg per ml, of GT in the standard solution and Ru and Rs are the ratios of the peak responses of the GT peak to the internal standard peak obtained from the sample solution and the standard solution, respectively.

HPLC IDENTIFICATION OF THE COSMETIC FORMULATION COMPONENTS

The sample preparation procedure described under First Derivative Method was applied to all the blanks of the commercial samples, i.e. preparations containing all the ingredients, with the exception of the glycyrrhetinic acid. The aqueous acidic extract was directly injected into the chromatograph. Aliquots of the chloroform solutions before and after the extraction with ammonium hydroxide were evaporated to dryness; the residue was suspended in acetonitrile:phosphate buffer (0.02 M; pH = 3) 60:40 v/v, and the volume adjusted to 10 ml in a volumetric flask. The filtered solutions were then injected into the chromatograph. A 5-ml aliquot of the ammoniacal solutions was acidified with 0.2 ml of 17% phosphoric acid solution in water, diluted to the volume of 10 ml with acetonitrile, and injected into the chromatograph.

The components of the analyzed cosmetics (preservatives and antioxidants) were identified through their retention times, by comparison with the pure standards.

DETERMINATION OF THE GT CONTENT IN GTP

HPLC method. A solution of 28.5 $\mu g\ ml^{-1}$ of GT in the mobile phase, containing 4.3 $\mu g\ ml^{-1}$ of testosterone acetate as internal standard, was injected into the chromatograph in triplicate and run concurrently with a solution containing 120 $\mu g\ ml^{-1}$ of GTP (solvent and concentration of internal standard being the same).

The peak area ratios of GTP to internal standard were then calculated and the amount of GT in GTP determined by comparison with the GT standard solution. The GT content in GTP was found to be 34.9%.

Spectrophotometric second derivative method. The zero order spectra of a solution of GT (c = 30.7 $\mu g\ ml^{-1}$) and GTP (c = 74.9 $\mu g\ ml^{-1}$) in methanolic potassium hydroxide were recorded. The second derivative spectra were then derived, and the GT content in GTP was calculated by comparing the GTP $^2D_{277}$ value to the GT one (GT in GTP = 34.2%).

RESULTS AND DISCUSSION

SPECTROPHOTOMETRY

The zero-order UV spectra of GT and GTP in neutral medium show the same absorption maximum at 248 nm, which is slightly shifted (to 258 nm) at basic pHs. On the other

hand, the spectra (13,14) of the preservatives (p-hydroxybenzoates contained in the cosmetic formulations), which overlap the GT band at neutral pHs, show a remarkable bathochromic shift at alkaline pHs. This results in an improved, although partial, resolution obtained between the GT and the parabens absorption maxima in 1% ammonium hydroxide (Figure 1).

The application of the derivative UV analyses to the basic analytical solutions allowed a selective determination of GT in the cosmetic vehicles. In fact, the first derivative spectrum of standard and sample ammoniacal GT solutions, compared to the one obtained from the respective blank, show a specific negative peak at 248 nm for GT, wavelengths at which the blank is zero (Figure 2). The amplitude of this negative peak, $^1D_{248}$, was found to be linearly correlated to the concentration of GT in the cosmetic formulation (Table I).

A similar procedure was followed for the analysis of hydrogels formulations, but in this case the parabens interference was found to be more intense, owing to a greater ratio of excipient to GT in this type of formulation (Figure 3). Using the first derivative method, the negative amplitude at 246.4 nm can be used for the selective determination of GT in the presence of the excipients (Figure 4). Accordingly, linear calibration graphs were obtained when the amplitude $^1D_{246.4}$ to the zero line was plotted against the GT concentrations. However, for the determination of GTP in the hydrogels it was found

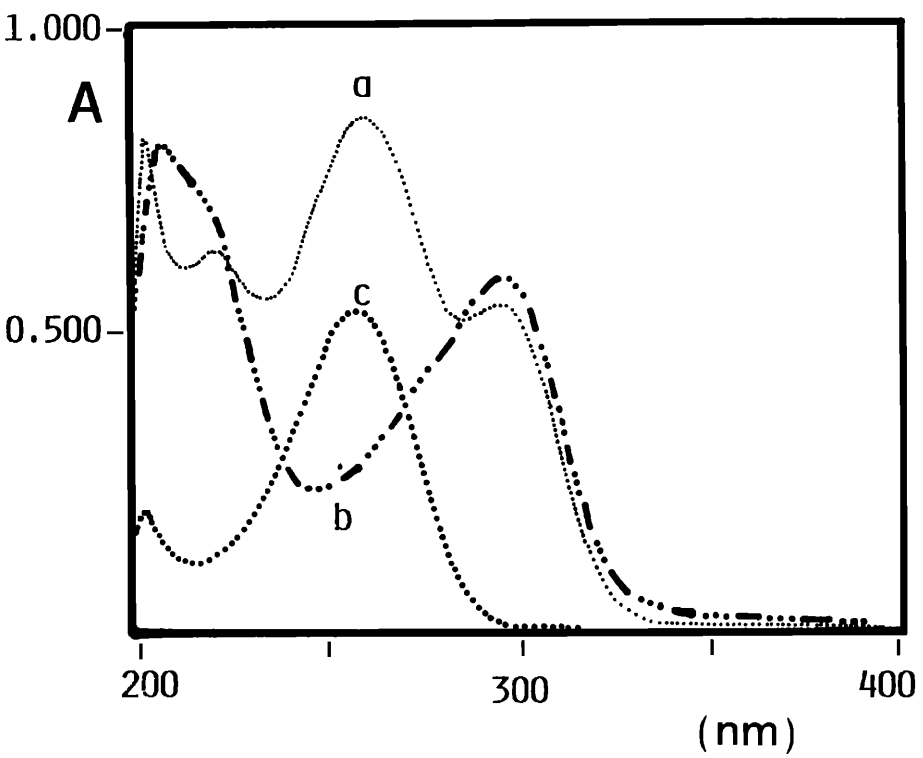

Figure 1. Absorption zero-order UV spectra of: (a) ammoniacal extract from a cream sample containing 1% GT; (b) ammoniacal extract of the same cream without GT; (c) glycyrrhetinic acid in 1% ammonium hydroxide (c = 21.1 μg ml^{-1}).

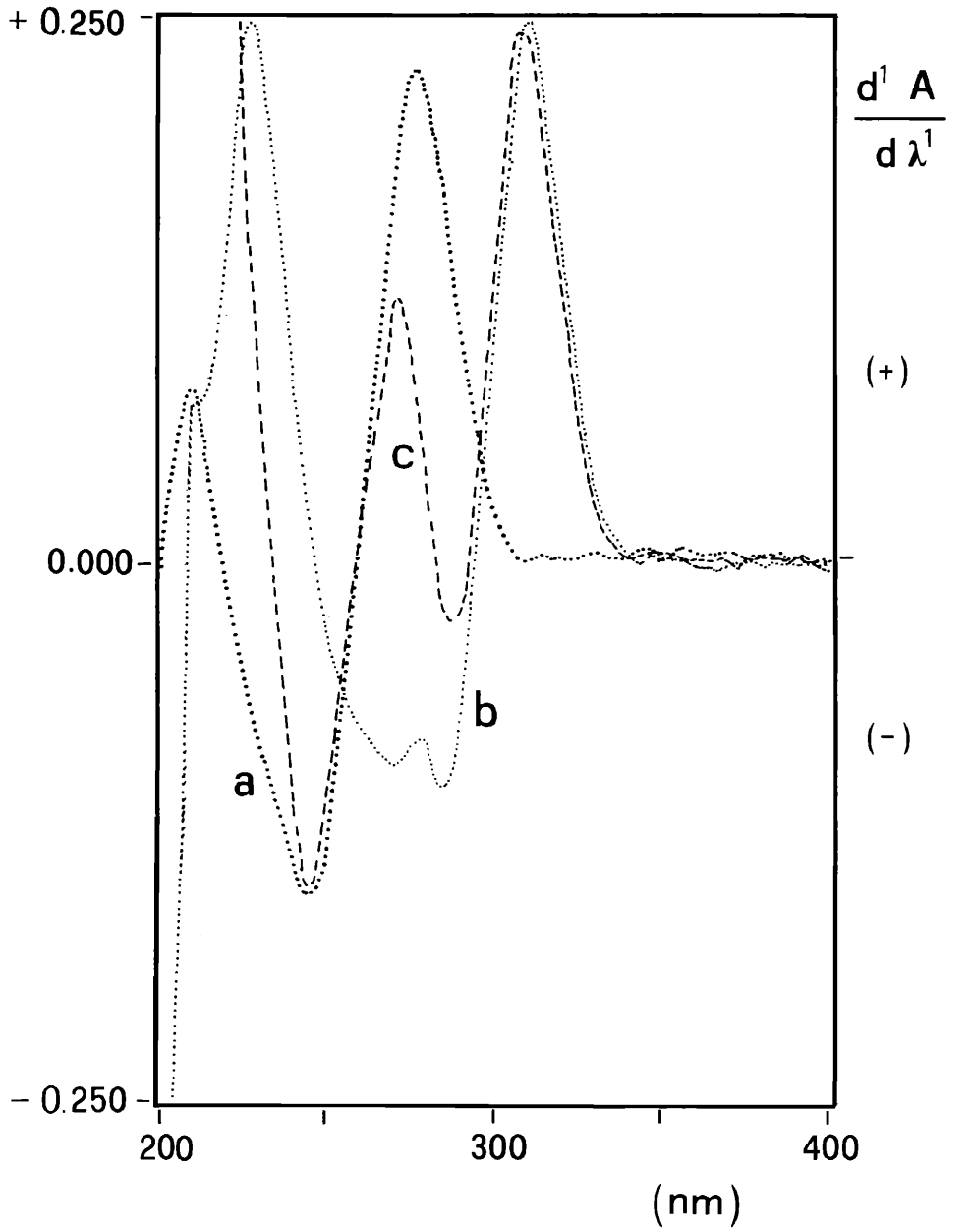

Figure 2. First-order derivative UV spectra of: (a) glycyrrhetinic acid in 1% ammonium hydroxide (c = 21.1 μg ml^{-1}); (b) ammoniacal extract from a cream sample without GT; (c) ammoniacal analytical solution from a cream sample containing 1% GT.

more convenient to use a second derivative method, measuring the amplitude of the positive peak at 275.2 nm, where the blank spectrum is zero (Figure 5). A linear calibration graph was obtained by plotting $^2D_{275.2}$ against the corresponding concentration (Table I).

Table I
Data for the Calibration Curves (n = 6) for the Determination of Glycyrrhetinic Acid (GT) and Its Phytosome (GTP) by Derivative UV Spectrophotometry and HPLC

	Methods	Slope	Intercept	Correlation coefficient	Working range μg ml^{-1}
GT	$^1D_{248}$	0.00710	−0.00270	0.99991	7.2–36
	$^2D_{275.2}$	0.00428	−0.00417	0.99996	7.2–36
	HPLC	0.06407	0.00725	0.99873	6.0–15
GTP	$^1D_{246.4}$	0.00169	−0.00330	0.99945	30–150
	$^2D_{275.2}$	0.00150	−0.00320	0.99940	30–150

The comparison of GT and GTP second derivative spectra [ratio of the positive peak (277 nm) to the negative peak (250 nm)] showed a higher ratio value for GTP, due to a lower negative peak, the positive peak amplitude, $^2D_{277}$, being unchanged. The peak at 277 nm was therefore used to calculate the real GT content in GTP. The assay result, confirmed by the HPLC method described above, allows expressing the GTP content in the analyzed cosmetics in terms of GT percentage.

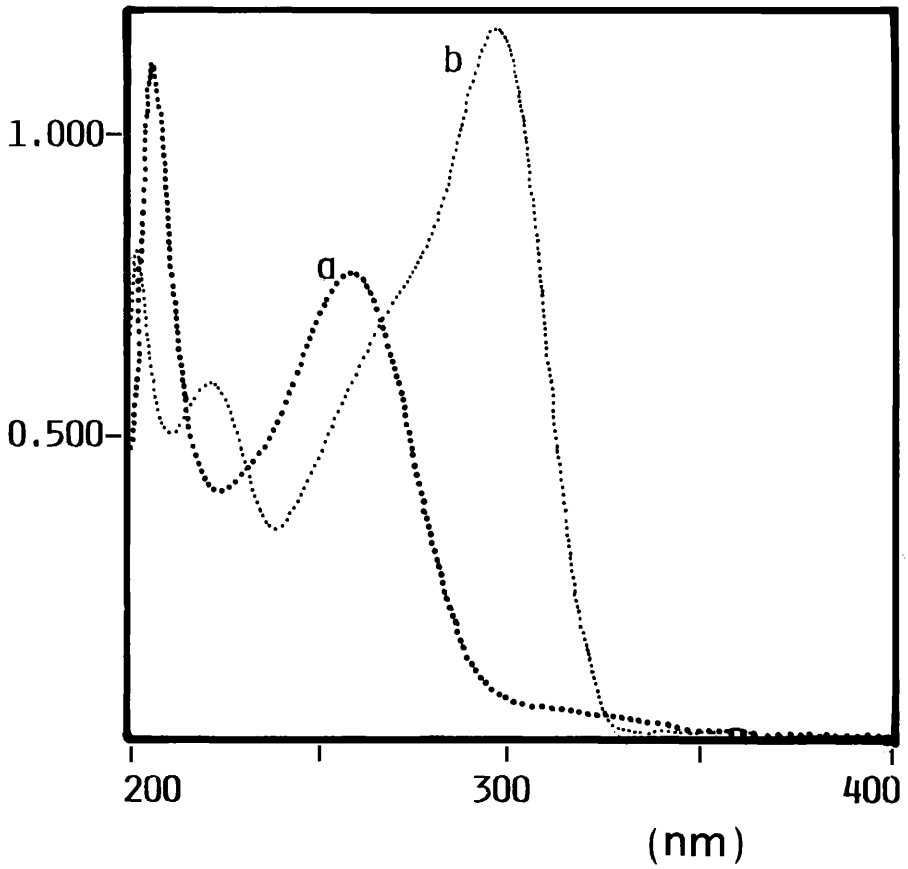

Figure 3. Zero order UV spectra of: (a) glycyrrhetinic acid phytosome in 1% ammonium hydroxide (c = 86.6 μg ml^{-1}); (b) ammoniacal extract from a hydrogel formulation containing 1% GTP.

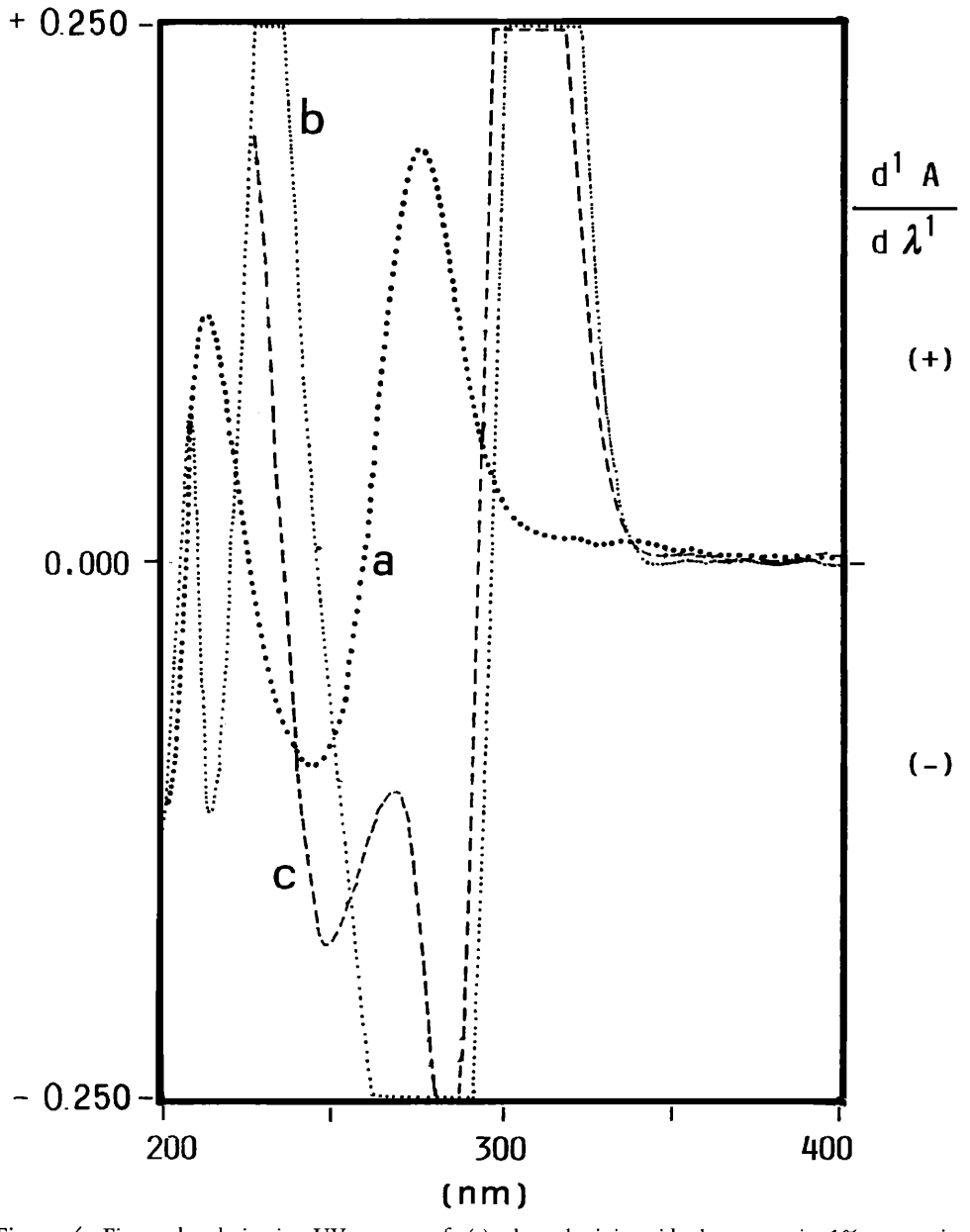

Figure 4. First-order derivative UV spectra of: (a) glycyrrhetinic acid phytosome in 1% ammonium hydroxide (c = 86.6 μg ml^{-1}); (b) ammoniacal extract from a hydrogel sample without GTP; (c) ammoniacal analytical solution from a hydrogel sample containing 1% GTP.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The chromatographic analyses were performed on a Hypersil 50DS column at ambient temperature, using as mobile phase a binary mixture consisting of acetonitrile:potassium phosphate buffer (0.02 M; pH = 3), 65:35 (v/v), at a flow rate of 1.2 ml min^{-1} with

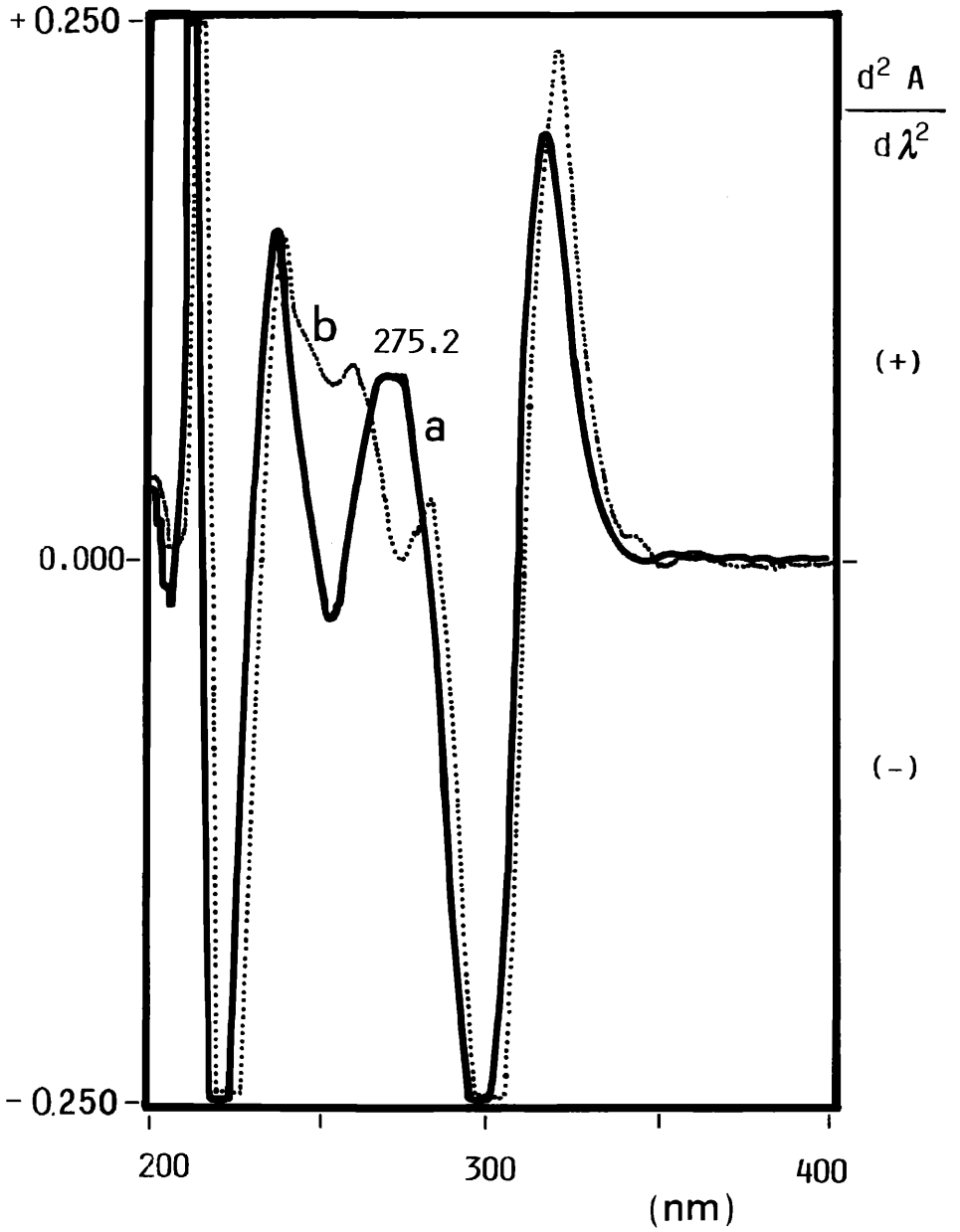

Figure 5. Second-order derivative UV spectra of: (a) methanolic analytical solution from a hydrogel sample containing 1% GTP (solid line); (b) methanolic solution from the same hydrogel preparation without GTP.

UV detection at 248 nm. Under these conditions, GT and the internal standard (testosterone acetate) were appropriately separated from the other components of the cream (Figure 6). A linear calibration graph was obtained by plotting the ratio of GT area to internal standard area against the corresponding GT concentrations (Table I). All the samples were analyzed by the HPLC method described, and the results, summarized in

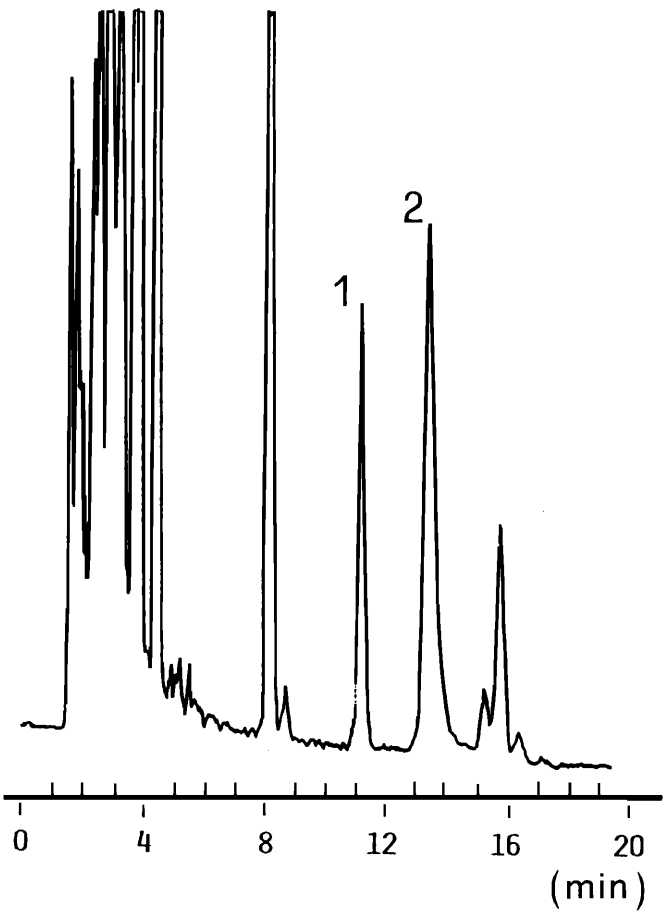

Figure 6. Representative HPLC chromatogram from a cream sample containing 0.06% glycyrrhetinic acid; 1 = internal standard (testosterone acetate); 2 = glycyrrhetinic acid. Column: 5 μm Hypersil C18, 250 × 4.5 mm I.D. Mobile phase: acetonitrile:0.02 M potassium phosphate buffer (pH = 3.0) 65:35 (v/v) at a flow rate of 1.2 ml min^{-1}. Detection: UV at 248 nm.

Table II, proved to be in good agreement with the derivative spectrophotometric methods. The same chromatographic conditions were used to determine the GT content in GTP; the result obtained (34.9%) was in agreement with that from the spectrophotometric assay (34.2%).

Table III illustrates the composition of the commercial cosmetic preparations analyzed. Using a modified mobile phase composition (60% acetonitrile) at a flow rate of 1 ml min^{-1}, good resolution among the different components of the cream was achieved. Under these chromatographic conditions, the HPLC method was able to identify all the substances isolated in the different stages of the extraction procedure for the sample preparation (Figures 7 and 8). The identification was based on retention times, compared with those of the individual components dissolved in the same solvent. The described clean-up procedure, compared with a previously described method (9), was found to be more convenient, providing cleaner analytical solutions, longer column life, and more symmetrical peaks.

Table II
Assay Results for Glycyrrhetinic Acid Determination in Commercial Cosmetic Formulations by Derivative UV Spectrophotometric and HPLC Methods[a]

		First derivative		Second derivative		HPLC	
Sample	Formulations[c]	Found (%)	RSD (%)	Found (%)	RSD (%)	Found (%)	RSD (%)
1	Emulsion O/W (0.06% GT)	—	—	—	—	100.8	0.57
2	Emulsion O/W (0.06% GT)	—	—	—	—	101.2	0.44
3	Emulsion O/W (1% GT)	98.3[b]	3.29	ND	ND	99.8	0.76
4	Hydrogel (1% GTP)	101.8	1.66	99.3	0.93	100.4	0.84
5	Hydrogel (1% GTP)	100.8	1.91	99.5	1.32	99.6	0.53

[a] Average of five determinations, expressed as percentage of the claimed content.
[b] On account of the mean recovery value of 90% for this type of formulation.
[c] Other active principles: 2. olive oil, gaiazulene; 5. rosemary extract, panthenol, birch leaf extract, polisorbate biosulfur.
ND = Not done.

ANALYSES OF COMMERCIAL FORMULATIONS

The sample preparation was optimized to eliminate as much as possible the interfering components (Table III), whose absorption bands fall in the same spectral area as GT and GTP. The analyses of samples 3, 4, and 5 by the first derivative method required a preliminary elimination of the interfering imidazolidinylurea. The sample was dissolved in chloroform and washed by an aqueous hydrochloric acid solution to eliminate imidazolidinylurea. A subsequent extraction of the chloroformic solution with 1% ammonium hydroxyde solution allowed GT and the parabens to be obtained selectively in basic medium. The basic extraction allows removal of dehydroacetic acid, butyl p-hydroxybenzoate, BHT, and BHA, which are kept dissolved in the chloroformic phase. The performance of the extraction steps was checked by HPLC analysis. The resulting alkaline solution was then subjected to the first derivative spectrophotometric analysis.

Table III
Excipient Components of the Analyzed Cosmetic Formulations

	Analyzed samples				
Preservatives, antioxidants	1	2	3	4	5
Methyl paraben	X	X	X	X	X
Ethyl paraben	X	X	X		
Propyl paraben	X	X	X	X	X
Butyl paraben	X	X	X		
Sodium dehydroacetate	X	X	X		
IMU	X		X	X	X
BHT			X		
BHA	X	X			

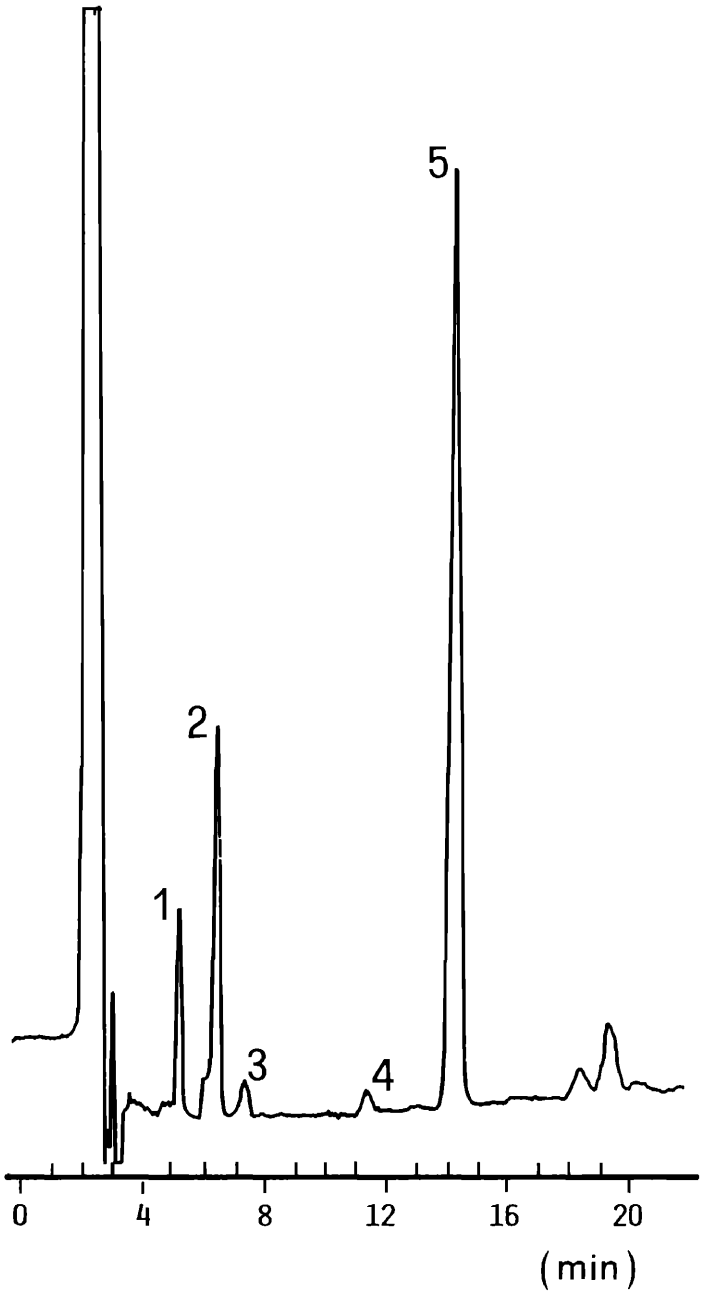

Figure 7. Chromatographic (HPLC) separation of the cream components left in the chloroformic solution after extraction with 1% ammonium hydroxide: 1 = methyl p-hydroxybenzoate; 2 = dehydroacetic acid; 3 = ethyl p-hydroxybenzoate; 4 = propyl p-hydroxybenzoate; 5 = butyl p-hydroxybenzoate. Column: 5 μm Hypersil C18, 250 × 4.5 mm I.D. Mobile phase: acetonitrile:0.02 M potassium phosphate buffer (pH = 3) 60:40 (v/v) at a flow rate of 1.0 ml min^{-1}. Detection: UV at 248 nm.

The second derivative method was applied to samples 4 and 5, which contained just methyl and propyl p-hydroxybenzoate, imidazolidinylurea, and no antioxidants. The sample preparation was therefore simpler: After the acid extraction to eliminate IMU,

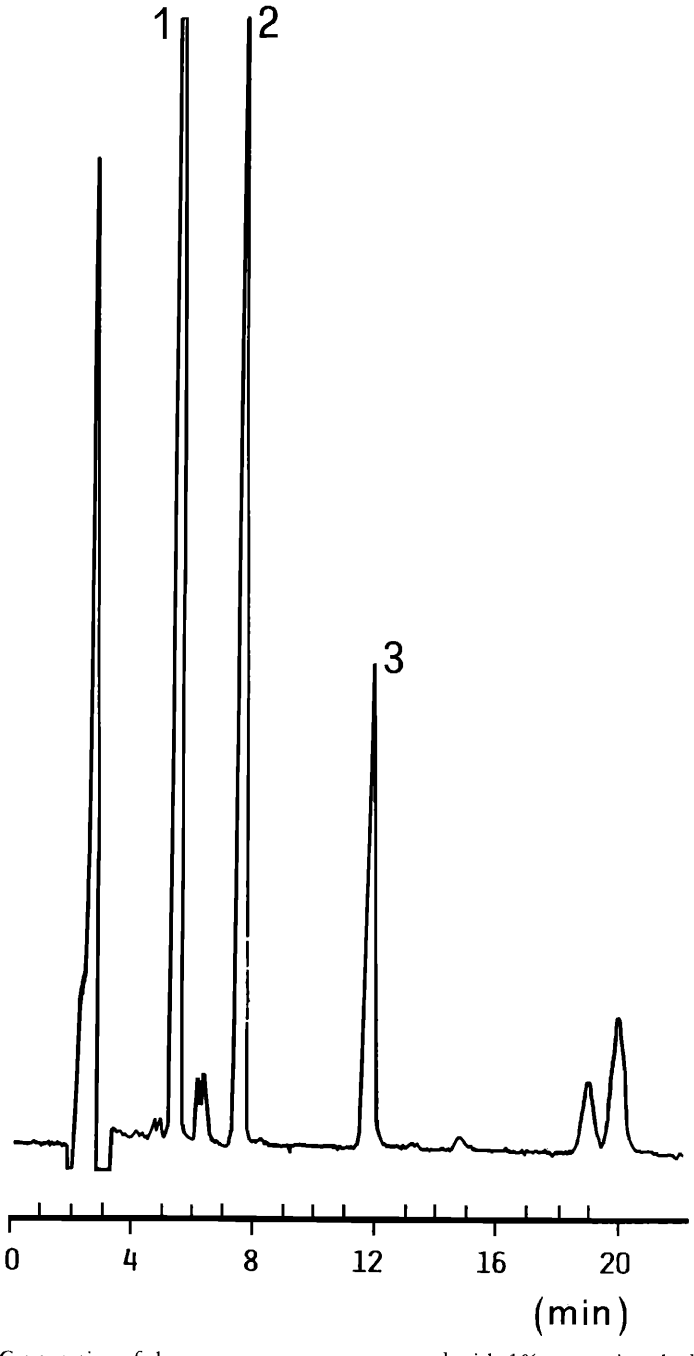

Figure 8. HPLC separation of the cream components extracted with 1% ammonium hydroxide solution. Chromatographic conditions as in Figure 7. 1 = methyl paraben; 2 = ethyl paraben; 3 = propyl paraben.

the chloroform solution was evaporated to dryness. The residue, containing just GT and the p-hydroxybenzoates, was dissolved in methanolic potassium hydroxide, and the resulting solution was used for the second derivative determination.

Experiments carried out with hydrogels spiked with a known amount of GTP provided essentially quantitative recoveries by both the spectrophotometric and HPLC procedures. On the other hand, when cream 3 was analyzed, the recovery obtained by the spectrophotometric procedure was about 90%, owing to a minor percentage of the analyte remaining in the chloroformic solution. This recovery value, however, can be considered adequate to the purpose.

Concerning samples 4 and 5, both the first and second derivative methods were applied, but the second derivative method proved to be more suitable because the blank presents a zero over a wider range of wavelengths.

To obtain information about the determination limit for GT in the examined formulations by the described spectrophotometric method, laboratory-made cosmetic preparations containing GT over the concentration range 0.015–1.5% were analyzed. For the analysis of the formulations examined in this work, the results show the spectrophotometric method to be suitable with content of GT $\geq$0.3%; for lower concentrations, the interference from the excipients made the method inaccurate. However, it is likely that for different formulations with lower excipient-to-GT ratios, a higher sensitivity for GT can be achieved.

CONCLUSION

Derivative UV spectrophotometry and liquid chromatography (HPLC) proved to be effective techniques suitable for the determination of GT and GTP in cosmetic formulations. The described derivative UV method showed good reproducibility and accuracy for GT contents $\geq$0.3% in the examined formulations. This technique can be considered a useful alternative approach when HPLC instrumentation is not available, its application field being dependent on the formula of the GT preparation. HPLC was confirmed to be a versatile analytical tool useful as a reference method and able to selectively determine small amounts of GT in complex cosmetic matrices.

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