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Original Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE SIMULTANEOUS ESTIMATION OF HYDROQUINONE, HYDROCORTISONE AND TRETINOIN TERNARY MIXTURE IN TOPICAL FORMULATION

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ABSTRACT

Objective: Development and validation of spectrophotometric and RP-HPLC methods for the simultaneous determination of Hydroquinone (HQ), Hydrocortisone (HC) and Tretinoin (TRT) ternary combination in pharmaceutical preparation.

Methods: The proposed spectrophotometric method was able to determine TRT directly from its absorption spectrum at 362 nm, however, HQ and HC from their first derivative spectra at 284 nm and 252 nm, respectively, without any separation step. The RP-HPLC method was developed using a C_{18} Sunfire© waters column with a mobile phase composed of acetonitrile: phosphate buffer (adjusted to pH 6.1 using ortho-phosphoric acid) in the ratio of 30:70 %, v/v, respectively at a flow rate of 0.8 ml/min. Quantification was based on measuring peak areas at 260 nm.

Results: The spectrophotometric method was able to selectively quantify each of HQ, HC and TRT in the ranges of 10-50 µg/ml, 2-10 µg/ml and 0.5-5 µg/ml, respectively. The RP-HPLC method was able to produce well-resolved peaks after 3.0, 8.2 and 20.2 min, in the ranges of 2-10 µg/ml, 0.1-1 µg/ml and 0.05-2 µg/ml, for HQ, HC and TRT, respectively. The obtained A, D_1 or peak areas values plotted against the concentration of each of the three components showed linear response in the stated ranges. Both methods were validated in terms of linearity, LOD, LOQ, precision, accuracy and selectivity.

Conclusion: Both developed proposed methods were applied for the determination of the active ingredients in the pharmaceutical formulation and the common excipients did not interfere in the analysis. The RP-HPLC method proved to be more sensitive when compared to the applied spectrophotometric method. However, the applied spectrophotometric methods, considered as green analytical chemistry, is a simple, time-saving method that requires minimal use of a hazardous solvent.

Keywords: Hydroquinone, Hydrocortisone, Tretinoin, Ternary mixture, Spectrophotometry and RP-HPLC

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INTRODUCTION

Melasma is a common pigmentation disorder that causes brown or gray patches to appear on the skin, especially those exposed to sunlight. Although melasma is not dangerous to your physical health, it can have serious effects on your emotional well-being. HQ is considered as the first line treatment for melasma which works by lightening the color of the skin patches by reducing the production of melanin [1]. However, HQ is not guaranteed to treat melasma on its own, it is often combined with TRT and HC. This triple combination is an effective combination that helps lightening the color of the melasma patches [1]. Thus, it is of great importance to develop analytical methods able of analyzing and quantifying this triple mixture in its pharmaceutical formulation.

Hydroquinone chemically known as benzene-1, 4-diol or quinol (fig. 1), is an phenol type aromatic organic compound derived from benzene, having the chemical formula C_6H_4 **(OH)** $_2$. Hydroquinone is used in the cosmetic treatment of hyper pigmented skin conditions, as it interferes with the melanin produced by the melanocytes by inhibiting the enzymes that convert tyrosine to dihydroxyphenylalanine [2].

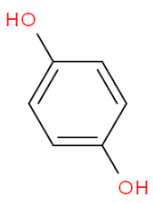

Fig. 1: Hydroquinone

Hydrocortisone chemically known as 11β, 17α, 21-trihydroxypregn-4-ene-3,20-dione (fig. 2), is a naturally occurring pregnane steroid. Hydrocortisone is the main glucocorticoid secreted by the adrenal cortex. It is a corticosteroid hormone receptor agonist with primary glucocorticoid and minor mineralocorticoid effects. As a glucocorticoid receptor agonist, hydrocortisone promotes protein catabolism, gluconeogenesis, capillary wall stability, renal excretion of calcium, and suppresses immune and inflammatory responses [3].

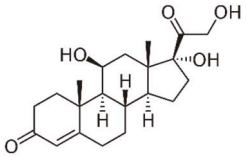

Fig. 2: Hydrocortisone

Tretinoin is a vitamin A derivative. Vitamin A chemically known as (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl cyclohexene-1-yl) nona-2,4,6,8-tetraen-1-ol (fig. 3), is essential for normal growth; and embryonic development. It is used in the treatment of psoriasis, acne vulgaris, and several other skin diseases [4].

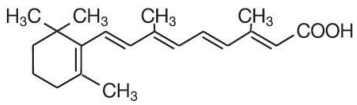

Fig. 3: Tretinoin

Several analytical methods have been reported for the determination of Hydroquinone in pure and pharmaceutical dosage forms using spectrophotometry [5–8], colorimetric reaction [9], FTIR [9] and capillary electrophoresis [10]. In the presence of treinoin, Hydroquinone has been determined by stability-indicating chromatographic methods [9, 11] and spectrophotometric chemometric method [12]. Hydroquinone has also been assayed in presence of tretinoin and mometasone furoate by RP-HPLC [13, 14]. Tretinoin has been determined by spectrophotometric colorimetry [15], Hydroquinone, retinoic acid and beta-methasone has been determined by HPLC [16, 17].

In this work, a new RP-HPLC method and spectrophotometric method have been developed for the simultaneous assay of HQ, HC and TRT ternary combination in synthetic mixtures and pharmaceutical preparation. The suggested spectrophotometric method was able to resolve the overlapped spectra by simple manipulation step; the absorbance values corresponding to HQ, HC and TRT could be easily obtained with easy preparation steps and minimal time consumption. The developed RP-HPLC was also able to resolve the overlapped peaks with reasonable retention time. Both methods were validated according to ICH guidelines [18] and the results obtained were compared using t-test and F-test [19].

MATERIALS AND METHODS

Apparatus

The spectrophotometric measurements were carried out on a Jasco V-530 double beam UV-Vis Spectrophotometer connected to a computer loaded with Jasco UVPC software and HP Deskjet 5652 printer. The absorption spectra were recorded using 1 cm quartz cells.

HPLC system (waters 1525) is composed of a binary HPLC pump, connected to a water 2487 dual wavelength absorbance detector. The liquid chromatographic system is equipped with waters 717 plus autosampler. Liquid separations are performed on RPC Sunfire © C18 analytical column (250 x 4.6 mm x 5µm) at ambient temperature. The mobile phase used was degassed and filtered by passing through 0.5-µm pore size membrane filter at a pumping speed of 30L/min (Glassco diaphragm Vacuuum pump). The samples were also filtered using PTFE 0.2 µm Minisart SRP 15 (Sartorius Stedim) disposable filters.

Chemicals

HQ, HC, and TRT supplied by Pharmadex-Lebanon were used as working standards. Acetonitrile (SIGMA-ALDRICH CHROMASOLV® for HPLC>99.9%), KH_2PO_4 (Merck KGaA), KOH ((SIGMA-ALDRICH) were used as solvents for the mobile phase preparation. The pharmaceutical preparation was supplied by Pharmadex-Lebanon.

Preparation of standard solutions

Standard stock solutions

HQ standard stock solution (4 mg/ml) was prepared by accurately transferring 100 mg into 25-ml volumetric flask.

HC standard stock solution (1 mg/ml) was prepared by accurately transferring 25 mg into 25-ml volumetric flask.

TRT standard stock solution (0.05 mg/ml) was prepared by accurately transferring 5 mg into 100-ml volumetric flask.

The powder in each flask was dissolved and diluted to volume with acetonitrile.

Working standard solutions

Accurate volume (1-ml) of the prepared standard stock solutions were separately diluted with acetonitrile in 3 separate 10-mL volumetric flasks to obtain working standard solutions having the concentrations of 0.4 mg/ml HQ, 0.1 mg/ml HC and 0.005 mg/ml TRT.

From the above working standard solutions, further dilutions are done by transferring 1-ml from each working standard solution into three separate 10-ml volumetric flasks to obtain diluted working standard solutions having the concentrations of 40 µg/ml HQ, 10 µg/ml HC and 0.5 µg/ml TRT.

Calibration graph for the spectrophotometric method

Into a series of 10-mL measured flasks, volumes from the above diluted working standard solutions of HQ, HC and TRT were transferred and diluted with acetonitrile to give the final concentration range stated in the table 2. The absorbance values of each solution were recorded at 1-nm interval in the wavelength range of 200-400 nm using acetonitrile as blank.

Calibration curves were constructed relating, the absorbance value for TRT at 362 and the D_1 values of HQ and HC at 300 and 252 nm, respectively, to the corresponding concentrations in the ranges stated in table 2. The regression equations, that relate the A or D_1 values at the stated λ_{max} to the corresponding concentration of each drug in its calibration graph, were computed (table 2).

Preparation of HQ, HC and TRT synthetic mixtures for the spectrophotometric method

Accurate volumes of the diluted working standard solutions of HQ, HC and TRT were transferred into five separate 10-mL calibrated flasks and diluted to the mark with acetonitrile to give synthetic mixtures containing HQ, HC, and TRT in the ratios stated in table 3, 4 and 5. The absorbance values of the mixtures were recorded at 362 nm and the D_1 values of the mixtures were recorded at 300 nm and 252 nm, using acetonitrile as blank.

Calibration graphs for RP-HPLC method

Into a series of 10-mL measured flasks, volumes from the above diluted working standard solutions of HQ, HC and TRT were transferred and diluted with acetonitrile to give the final concentration range stated in table 2. The above solutions were filtered using 0.2 µm disposable filters. 20µL portions of the prepared solutions of HQ, HC and TRT were separately injected in triplicates and chromatographed under the chromatographic conditions mentioned above. The peak area values were plotted against the corresponding concentrations to obtain the calibration graph for each drug. The concentrations of HQ, HC and TRT from the corresponding calibration graphs were computed.

Synthetic mixtures for RP-HPLC method

Accurate volumes from the diluted working standard solutions of HQ, HC and TRT were transferred into five separate 10-mL calibrated flasks and diluted to the mark with acetonitrile to give synthetic mixtures containing HQ, HC and TRT in the ratios stated in tables 3, 4 and 5. The solutions were filtered using 0.2 µm disposable filters. Volumes of 20 µl portions of the mixture solutions were injected in triplicates and chromatographed under the chromatographic conditions mentioned above. The peak areas for each drug were measured and the corresponding concentrations in the mixtures were derived referring to its own calibration graph.

Pharmaceutical preparation

Accurately one gram of the pharmaceutical ointment containing 40 mg/ml HQ, 10 mg/ml HC and 0.5 mg/ml TRT was weighed in a 50-mL beaker. 50-mL acetonitrile was added and manual extraction with the help of a thin metallic glass rod was performed. The extract was transferred into a 100-mL volumetric flask; successive proportions of the solvent were added to the ointment to ensure full extraction from the base. The obtained extract was transferred to the flask and diluted to the mark with acetonitrile to prepare the pharmaceutical stock solution. From this prepared solution and for the application of the spectrophotometric method, 1-mL was transferred into a 10-mL volumetric flask and diluted to the volume with the same solvent to obtain the ratio of 40 µg/ml HQ, 10 µg/ml HC and 0.5 µg/ml TRT. The absorbance value of the mixture was recorded at 362 nm and the D_1 value of the mixture was recorded at 284 nm and 252 nm, using acetonitrile as blank. From the pharmaceutical stock solution and for the application of the RP-HPLC method, 1-mL was transferred into a 100-mL volumetric flask and diluted to the volume with the same solvent to obtain the ratio of 4 µg/ml HQ, 1 µg/ml HC and 0.05 µg/ml TRT.

RESULTS AND DISCUSSION

The extensive overlapping between the absorption spectra of HQ, HC and TRT (fig. 4), is sufficiently clear, rendering the resolution of HQ and HC by direct spectrophotometry difficult. However, as shown in fig. 4, TRT could be directly determined from the absorbance spectra at 362 nm where there is no interference from HC and HQ. The use of the first derivative approach was able to resolve and determine each of HQ and HC in their ternary mixture (fig. 5). The D_1 spectrum of HQ exhibit a zero crossing at 252 nm, which allows the selective determination of HC. Whereas, measurement of the D_1 value of the mixture at 284 nm allows the determination of HQ.

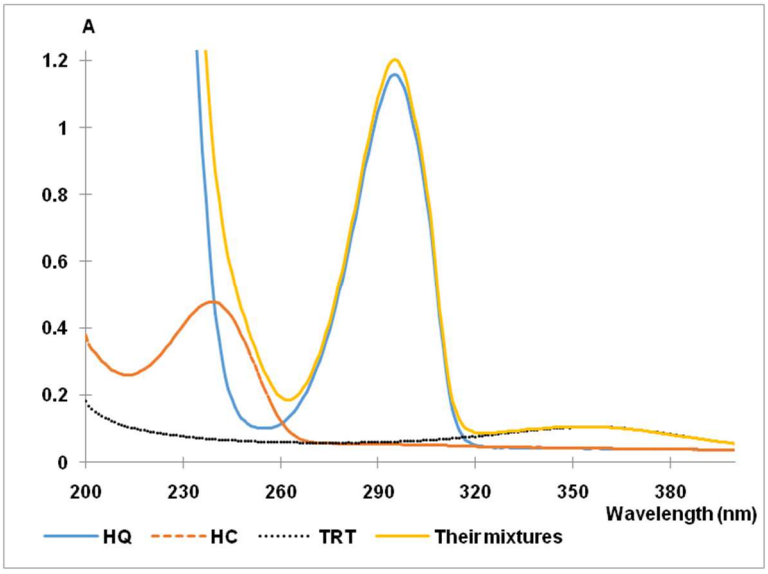

Fig. 4: Absorption curves of 40 µg. ml^{-1} HQ in methanol, 10 µg. ml^{-1} of HC in methanol, 0.5 µg. ml^{-1} TRT in methanol and their mixture

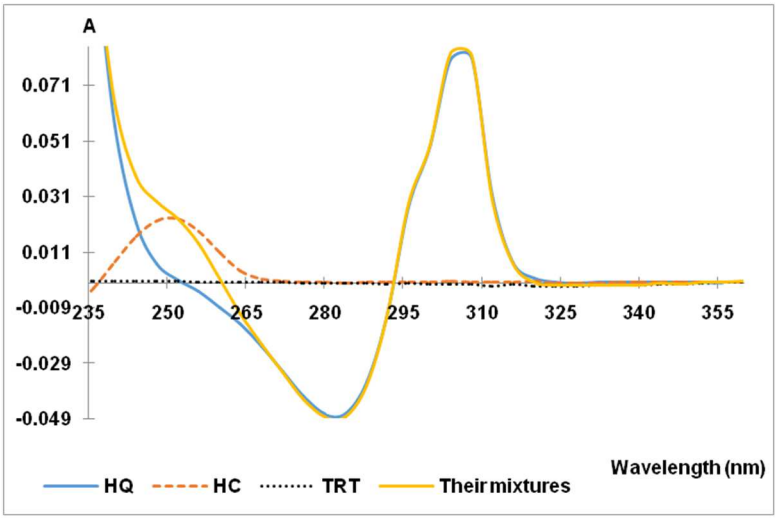

Fig. 5: D_1 spectra of 10 µg. ml^{-1} HC in methanol, 40 µg. ml^{-1} HQ in methanol, 0.5 µg. ml^{-1} in methanol and their mixture

During the RP-HPLC method development, different experimental conditions were studied and optimized using different mobile phases, to maximize the resolution and sensitivity of the analytical procedure. The parameters assessed include the detection wavelength, the type and quantity of organic solvent, pH and composition of the mobile phase and the flow rate. For the selection of the optimal wavelength for measurement, 40 µg/ml HQ, 10 µg/ml HC and 0.5 µg/ml TRT standard solutions were spectrophotometrically scanned in the range of 200-400 nm, all absorbance maxima were tried, when applying the chromatographic run, it was found that 260 nm provided the most suitable wavelength for UV detection of HQ, HC and TRT in the presence of each other.

For the selection of the optimal mobile phase composition, the method development started with 100% methanol, where poor resolution between peaks of the studied drugs was found. The mobile phase composition was then adjusted by mixing acetonitrile with methanol in the ratio of 40:60 % v/v, where the poor resolution was also obtained between the first two peaks and broadening of the third peak occurred. It was found that a mobile phase composed of acetonitrile: phosphate buffer (30:70, % v/v) at a pH of 6.1 contributed to better peak shape, separation and resolution, where buffers are usually added to obtain well-resolved symmetrical peaks.

Flow rate effect was also optimized since it affects both retention time and resolution, where increasing the flow rate, decreases retention time and resolution. Different flow rate values were tried, 0.8 ml/min was chosen as the optimal value at which TRT was eluted at 4.0 min, HQ at 8.2 min and HC at 20.2 min. C_{18} column was used to establish an accurate method for the analysis and separation of HQ, HC and TRT from each other.

Therefore, the proposed method was performed by an isocratic elution using acetonitrile: phosphate buffer (30:70, % v/v) at pH 6.1, using C_{18} column. The overall run time was 20.2 min. The flow rate was 0.8 ml/min. 20 µl of the sample was injected into the HPLC system and all chromatographic conditions were performed at room temperature (25 °C±2 °C). Using the optimized chromatographic conditions, acceptable separation and peak shapes for HQ, HC and TRT were observed (fig. 6).

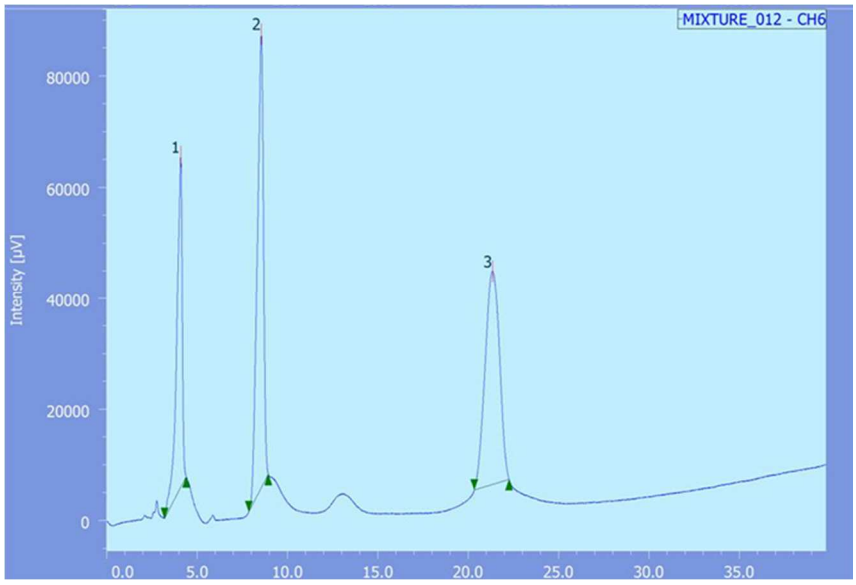

Fig. 6: HPLC chromatogram of a 20 µl injection of a standard mixture of 0.1 µg/ml TRT, 8 µg/ml HQ and 1 µg. ml^{-1} HC using the optimized chromatographic conditions

System suitability

According to FDA 1994 (20), system suitability tests are an integral part of any liquid chromatographic method. Various parameters, including capacity factors (k′), selectivity (α), resolution (Rs), asymmetry factor (Af) and the number of theoretical plates (N) are listed in table 1. All these parameters were found to be satisfactory and within the reported acceptance criteria listed in the reference. As shown in table 1, the tailing factor was<2, acceptable retention time along with good resolution, are indicative of the good efficiency and selectivity of the method for separation of HQ, HC and TRT ternary mixture.

Table 1: HPLC system suitability parameters for the determination of HQ, HC and TRT, using the proposed RP-HPLC method

Analyte	Retention time (t_r)	Capacity factor (k′)	N ° of theoretical plates (N)	Asymmetry factor (A_F)	Selectivity (α)	Resolution (R_s)
TRT	4.04	0.393	995	1.01	4.65	7.3
HQ	8.21	1.83	2689	0.793	3.16	12.13
HC	20.2	5.78	3886	0.885		

Method validation and statistical analysis

Under the described experimental conditions, the graphs obtained by plotting the A, D_1 values (in the case of spectrophotometric method) or the peak area (in case of the RP-HPLC method) each versus concentration of HQ, HC and TRT gave linear relationships over the concentration ranges stated in table 2. The slopes, intercepts and correlation coefficients obtained by the linear least-squares treatments of the results are also given in table 2. The correlation coefficients (r) obtained were higher than 0.999 for the drugs with high values of F (low significant F) which confirmed the linearity of the calibration curves. An important statistical parameter for indicating the random error in the estimated values of y is the standard deviation of the residuals $S_{y/x}$ (21). In addition, the importance of $S_{y/x}$ originates from being used to calculate S_a and S_b, the standard deviation of the intercept (a) and the slope (b). These values showed the good linearity of the calibration graphs and the compliance to Beer's law.

Linearity and concentration range

Under the described experimental conditions, the graphs obtained by plotting the signals of the proposed methods versus concentration of HQ, HC and TRT gave linear relationships over the concentration ranges stated in table 2.

Accuracy

The accuracy of the proposed methods was assessed by analyzing five synthetic mixtures with different ratios of pure HQ, HC and TRT by the standard addition method using the proposed spectrophotometric and RP-HPLC. The accuracy expressed as percentage relative error (Er %) was shown in tables 3 and 4. The results indicate that the proposed methods could be applied for the simultaneous determination of HQ, HC, and TRT in pharmaceutical formulation with high accuracy.

Precision

The precision of the method was verified by repeatability studies (intra-day precision) and intermediate precision studies (inter-day precision). Repeatability studies were performed by analyzing the mixture composed of 10 µg/ml HC, 40 µg/ml HQ and 0.5 µg/ml TRT five times on the same day (intra-day analysis) and on three different days (inter-day analysis). The High value of percentage RSD obtained, proved the high precision of the method (table 5).

Selectivity

Selectivity was ascertained by analyzing different mixtures containing the studied drugs in different ratios within the linearity

range. Satisfactory results were shown in tables 3 and 4. Good percentage recoveries with acceptable standard deviations were obtained in all cases, which proves the negligible effect of the excipient in the determination of the active ingredients.

Table 2: Assay parameters for the determination of HQ, HC and TRT using the applied spectrophotometric and RP-HPLC methods

Parameters	HQ		HC		TRT	
Method	Spectrophotometry	RP-HPLC	Spectrophotometry	RP-HPLC	Spectrophotometry	RP-HPLC
Conc. Range (µg/ml)	10-50	2-10	2-10	0.1-1	0.5-5	0.05-2
Signal	D_1	Peak Area	D_1	Peak Area	A	Peak Area
$\lambda_{(nm)}$	300	260	252	260	362	260
r	0.9993	0.9999	0.9998	0.9993	0.9990	0.9992
$S_{y/x}$	$1.938x\ 10^{-6}$	$9216\ x10^{4}$	$2.58\ x\ 10^{-7}$	$3588\ x10^{6}$	$0.603\ x10^{-2}$	$4714\ x10^{4}$
F	2456	76769	10328	3189	164	$4816\ x10^{2}$
Significance F	$1.81\ x\ 10^{-5}$	$1.03\ x10^{-7}$	$2\ x\ 10^{-6}$	$5.8\ x10^{-7}$	$0.102\ x10^{-2}$	$6597\ x10^{-6}$
a (intercept)	$-0.707\ x10^{-3}$	416	$0.481\ x10^{-3}$	-20324	$-0.166\ x10^{-1}$	-3381
b (slope)	$0.125\ x10^{-2}$	$2428\ x10^{2}$	$0.221\ x10^{-2}$	$2168\ x10^{2}$	0.167	$1625\ x10^{3}$
S_a	0.000843	5813	0.000277	2330	0.0373	2109
S_b	$2.54\ x\ 10^{-5}$	876	$2.01\ x\ 10^{-5}$	38400	0.0131	234
LOD (µg/ml)	2.00	0.0718	0.375	0.0322	0.666	$0.389\ x10^{-2}$
LOQ (µg/ml)	6.692	0.239	1.25	0.107	2.22	$0.129\ x10^{-1}$
a/S_a	-0.839	$0.716\ x10^{-1}$	1.73	-0.872	-0.446	-1.60
$(S_b)^2$	$6.4\ x\ 10^{-}$[10]	$7680\ x10^{2}$	$4.7\ x\ 10^{-}$[10]	$1474\ x10^{6}$	$0.171\ x10^{-3}$	$5484\ x10^{3}$
S_b %	$2.54\ x\ 10^{-3}$	$876\ x10^{2}$	$2.01\ x\ 10^{-3}$	$3840\ x10^{3}$	1.31	2340 x10

Table 3: Accuracy for the simultaneous determination of HQ, HC and TRT in laboratory-made mixtures using the proposed spectrophotometric method

HQ: HC: TRT µg/ml	Mean recovery±SD[a]	RSD%[b]	Er%[c]
	A (357 nm)	D_1 (252 nm)	D_1 (300 nm)
10:2:3	101.52±0.69	100.91±0.54	99.14±1.45
	070	0.54	1.46
	1.52	0.61	-0.86
20:4:2	100.07±0.97	100.25±0.86	99.99±0.74
	0.97	0.86	0.74
	0.07	0.25	-0.01
30:6:1	98.56±0.90	98.51±0.56	101.51±1.42
	0.91	0.57	140
	-1.44	0.17	1.51
40:8:4	101.31±0.87	98.71±0.98	98.59±0.82
	0.86	0.99	0.83
	1.31	0.23	-1.41
50:10:0.5	101.12±0.96	101.00±0.89	101.12±0.96
	0.95	0.88	0.95
	1.12	1.00	1.12

[a]mean±SD for the three determinations, [b]% Relative standard deviation, [c]% Relative error

Table 4: Accuracy for the simultaneous determination of HQ, HC and TRT in laboratory-made mixtures using the proposed RP-HPLC method

HQ: HC: TRT µg/ml	HQ	HC	TRT
	RP-HPLC (260 nm)		
2:1:2	99.09±1.01	101.43±0.76	100.27±0.89
	1.02	0.75	0.89
	-0.91	1.43	0.05
4:0.8:0.5	102.22±0.73	99.89±1.08	99.83±0.85
	0.71	1.08	0.85
	2.22	-0.11	-0.97
6:0.6:0.05	101.17±0.63	101.17±0.63	100.14±1.13
	0.62	0.62	1.13
	1.17	1.17	-0.26
8:0.4:1	98.39±0.85	98.39±0.85	101.38±1.51
	0.86	0.86	1.50
	-1.61	-1.61	1.38
10:0.1:1.5	101.18±0.89	98.02±0.53	101.47±0.89
	0.88	0.54	0.88
	1.18	-1.98	1.47

[a]mean±SD for the three determinations, [b]% Relative standard deviation, [c]% Relative error

Table 5: Intra-day and inter-day precision for the simultaneous determination of a mixture of HQ, HC and TRT in laboratory-made mixtures using the proposed spectrophotometric and RP-HPLC method

Analytical method	Intra-day precision mean recovery±SDa RSD%b Er%c			Inter-day precision mean recovery±SD[a] RSD%[b] Er%[c]		
	HQ	HC	TRT	HQ	HC	TRT
A or D_1	100.73±0.46	99.99±0.79	100.05±0.89	100.82±0.62	99.74±1.13	100.17±0.56
	0.15	0.79	0.89	1.32	1.13	0.56
	0.26	-0.01	0.05	1.90	-0.26	0.17
RP-HPLC	100.86±0.35	100.10±0.52	100.23±0.98	101.24±0.57	101.01±0.98	100.65±0.73
	0.22	0.52	0.98	0.54	0.42	0.73
	0.59	0.10	0.23	0.20	0.50	0.65

[a]mean±SD for the five determinations, [b]% Relative standard deviation, [c]% Relative error

Assay of the pharmaceutical preparation

The suggested methods were successfully applied for the determination of HQ, HC and TRT in its pharmaceutical ointment formulation provided by Pharmadex-Lebanon. The results, shown in table 6, were satisfactory and agreed with the labeled amounts. Since no analytical method (reference) is available for the assay of this triple mixture, statistical comparison of the results obtained by the proposed spectrophotometric and RP-HPLC techniques were compared to each other and proved to be valid and selective where the excipients did not adversely affect the results (table 6). The calculated t and F values [19] were less than the theoretical ones indicating that there was no significant difference between the proposed methods with respect to accuracy and precision.

Table 6: Assay results for HQ, HC and TRT in their pharmaceutical tablets using the proposed spectrophotometric and RP-HPLC method

HQ+HC+TRT	RP-HPLC			Spectrophotometry		
Mean recovery±SD[a]	**HQ**	**HC**	**TRT**	**HQ**	**HC**	**TRT**
RSD %[b]	99.90±0.24	99.20±0.53	98.60±1.55	99.60±0.41	100.06±0.83	99.11±0.99
Er %[c]	0.24	0.53	1.57	0.41	0.83	0.99
****t-test**	-0.10	-0.42	-1.40	-0.40	0.06	-0.89
****F-test**	––	––	––	1.42	1.95	1.22
	––	––	––	2.92	2.45	0.41

[a] mean±SD for the five determinations, [b]% Relative standard deviation, [c]% Relative error, **Theoretical values of t-and F-at P = 0.05 are 2.13 and 6.93, respectively

CONCLUSION

The proposed methods were very simple with minimum manipulation steps, very sensitive, precise, do not need any sophisticated apparatus or a special program and could be easily applied in quality control laboratories as they are having equal accuracy and precision compared to the reported methods for the simultaneous determination of HQ, HC and TRT. Thus, the proposed methods could be successfully applied for the routine analysis of the studied drugs either in their pure bulk powders or in quality control laboratories without any preliminary separation step.

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AUTHORS CONTRIBUTIONS

Marwa k. Al Jamal designed and performed the experiments and the measurements. Azza A. Gazy was involved in planning and supervised the work. Marwa k. Al Jamal and Azza A. Gazy processed the experimental data along with the calculations, drafted the manuscript, designed the fig. and interpreted the results.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. https://www.forhers.com/blog/hydroquinone-for-melasma-does-it-work. [Last accessed on 05 Jul 2019].
2. https://www.aocd.org/page/Hydroquinone. [Last accessed on 05 Jul 2019].
3. https://pubchem.ncbi.nlm.nih.gov/compound/hydrocortisone. [Last accessed on 05 Jul 2019].
4. https://pubchem.ncbi.nlm.nih.gov/compound/retinol. [Last accessed on 05 Jul 2019].
5. Kaur S, Kaur T, Kaur G, Verma SV. Development and validation of UV-spectrophotometric method for estimation of hydroquinone in bulk marketed cream and prepared NLC formulation. Int J Appl Pharm 2017;9:102.
6. Jogarami R, Jain P, Sharma S. Validated UV spectrophotometric method development for simultaneous estimation of tazarotene and hydroquinone in gel preparation. JPR 2012;5:2273–5.
7. Kaur S, Kaur T, Kaur G, Verma S. Development and validation of a UV-spectrophotometric method for estimation of hydroquinone in bulk marketed cream and prepared NLC formulation. Int J Appl Pharm 2017;9:102–8.
8. Garcia PL, Santoro MIRM, Singh AK, Kedor Hackmann ERM. Determination of optimum wavelength and derivative order in spectrophotometry for quantitation of hydroquinone in creams. Braz. J Pharm Sci 2007;43:397–404.
9. Htet AM, Thin EE, Saw MM, Win S. Chemical analysis of hydroquinone and retinoic acid contents in facial whitening creams. Afr J Plant Sci 2016;11:89–90.
10. Lin YH, Yang YH, Wu SM. Experimental design and capillary electrophoresis for the simultaneous analysis of arbutin, kojic acid and hydroquinone in cosmetics. J Pharm Biomed Anal 2007;44:279–82.
11. Abbas SS, Elghobashy MR, Bebawy LI, Shokry RF. Stability-indicating chromatographic determination of hydroquinone in combination with tretinoin and fluocinolone acetonide in pharmaceutical formulations with a photodegradation kinetic study. RSC Adv 2015;5:43178–94.
12. Elghobashy MR, Bebawy LI, Shokry RF, Abbas SS. Successive ratio subtraction coupled with constant multiplication spectrophotometric method for determination of hydroquinone in a complex mixture with its degradation products, tretinoin and methylparaben. Spectrochim Acta A 2016;157:16–123.
13. Zanwar As, Sen Db, Sen Ak, Seth Ak. Simultaneous estimation of mometasone furoate and formoterol fumarate by HPLC method in rotacaps. Int J Pharm Pharm Sci 2019;11:12–6.
14. Sheliya K, Shah K, Kapupara P. Development and validation of analytical method for simultaneous estimation of mometasone

furoate, hydroquinone and retinoic acid in the topical formulation by RP-HPLC. J Chem Pharm Res 2014;6:934–40.

15. Zayed MA, Abdel Basset MH. Spectrophotometric micro determination of tretinoin, isotretinoinusing iodine and tazarotene micro determination via reaction with rose-bengal reagent. Egypt J Chem 2018;61:143-53.
16. Rahmayuni E, Harmita H, Suryadi H. Development and validation method for simultaneous analysis of retinoic acid, hydroquinone and corticosteroid in cream formula by high-performance liquid chromatography. J Appl Pharm 2018;8:87-92.
17. Maggadani B, Harmita H, Harahap Y, Hutabalian H. Simultaneous identification and quantification of hydroquinone, tretinoin and betamethasone in cosmetic products by isocratic reversed-phase high-performance liquid chromatography. Int J Appl Pharm 2019;11:181-5.
18. ICH Harmonized Tripartite guideline, Validation of Analytical Procedures text and methodology Q2 (R1) current step 4 version, parent guideline dated 27 November (Complementary guideline on Methodology. Geneva; 1996. p. 1-13.
19. JN Mileer JCM. Statistics and chemometrics for analytical chemistry. 5th ed. London, Pearson Prentice Hall; 2005.
20. FDA Center for Drug Evaluation Research (CDER), Reviewer Guidance: Validation of Chromatographic Methods, Washington, USA; 1994.
21. ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Text and Methodology Q2 (R1); 2005.