Research Article

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Stability-indicating HPLC-DAD assay for simultaneous quantification of hydrocortisone 21 acetate, dexamethasone, and fluocinolone acetonide in cosmetics

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Abstract: A rapid and specific method was developed for simultaneous quantification of hydrocortisone 21 acetate (HCA), dexamethasone (DEX), and fluocinolone acetonide (FCA) in whitening cream formulations using reversed-phase high-performance liquid chromatography. The effect of the composition of the mobile phase, analysis temperature, and detection wavelength was investigated to optimize the separation of studied components. The analytes were finally well separated using ACE Excel 2, C18 AR column having 150 mm length, 3 mm internal diameter, and 2 µm particle size at 35°C using methanol with 1% formic acid and double-distilled deionized water in the ratio of 60:40 (v/v), respectively, as the mobile phase in isocratic mode. Ten microliters of sample were injected with a flow rate of 0.5 mL/min. The specificity, linearity, accuracy, precision, recovery, limit of detection (LOD), limit of quantification (LOQ), and robustness were determined to validate the method as per International Conference on Harmonization guidelines. All the analytes were simultaneously separated within 8 min, and observed retention times of HCA, DEX, and FCA were 4.5, 5.5, and 6.9 min, respectively. The proposed method showed good linearity with the correlation coefficient, $R^2 = 0.999$ over the range of 1–150 µg/mL for all standards. The linear regression equations were $y = 12.7x + 118.7$ ($r = 0.999$) for HCA, $y = 12.9x + 106.8$ ($r = 0.999$) for DEX, and $y = 12.9x + 96.8$ ($r = 0.999$) for FCA. The LOD was 0.25, 0.20, and 0.08 µg/mL for HCA, FCA, and DEX and LOQ was 2.06, 1.83, and 1.55 µg/mL for HCA, FCA, and DEX, respectively. The recovery values of HCA, DEX, and FCA ranged from 100.7–101.3, 102.0–102.6, and 100.2–102.0%, respectively, and the relative standard deviation for precision (intra- and interday) was less than 2, which indicated repeatability and reproducibility. The novelty of the method was described by forced degradation experimentation of all analytes in the combined form under acidic, basic, oxidative, and thermal stress. The proposed method was found to be simple, rapid, and reliable for the simultaneous determination of HCA, DEX, and FCA in cosmetics.

Keywords: HPLC-DAD, hydrocortisone 21 acetate, dexamethasone, fluocinolone acetonide, cosmetics, forced degradation

1 Introduction

The misuse of topical corticosteroids (TCs) as cosmetics has now become a trend. Corticosteroids are frequently abused as fairness creams. This abuse and addiction of TCs especially on the face as cosmetic cream formulation have developed many skin diseases particularly dermatitis [1]. Brisk whitening effects, easy access, cheapness, inappropriate marketing, ignoring side effects, and society's attitude toward fair skin color are considered as significant reasons for consumers (mostly females) toward the use of whitening cosmetic creams. The TCs are generally safe when used rationally while significant morbidity among people can arise if used excessively. According to the data of the World Health Organization (WHO), most pharmaceuticals are inappropriately prescribed, which leads to misuse of medication among the majority of the world population [2].

It has become evident that TC is being misused by prescribers and people use them in various parts of the globe [3]. The use of TCs over the face for skin lightening is a very common practice in Pakistan. Only after getting approval from official regulator, corticosteroids should be used in drugs. Recently, some prohibited corticosteroids have been detected in commercial cosmetics. To increase the pharmacological efficiency, steroids are being illegally incorporated into cosmetics, and such practice may lead to compromise the health of end-user due to the hazardous side effects of TCs [4]. A study revealed that in Lahore

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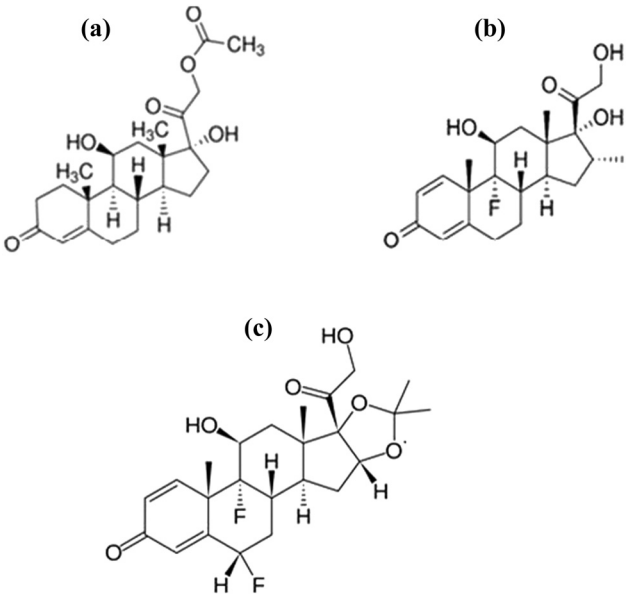

Figure 1: Chemical structure of (a) HCA, (b) DEX, and (c) FCA.

(Pakistan), many patients have suffered from acne due to the use of skin whitening commercial products containing illegal steroids especially glucocorticoids [5]. The extent of skin diseases as a result of the side effects of skin whitening agents depends on the type of chemical, source (cream, ointment, lotion, or gel), and application method [6]. Burning sensation, itching, irritation, dryness, redness at the application site, atrophy of the skin, steroidal rosacea, acne, and perioral dermatitis are commonly observed as adverse effects of the use of TCs [7].

Hydrocortisone 21 acetate (HCA) is commonly used for treating different skin disorders by its topical application [8]. It is one of those chemicals that are added in skincare cosmetics as illicit agents [9]. Regular or long-term exposure to HCA can cause hypertension and irreversible skin atrophy [10].

Dexamethasone (DEX) as glucocorticoid has been used for the treatment of inflammation, and its treatment can lead to steroid diabetes [11]. Susceptibility of skin cancer is increased due to the use of DEX. Its high concentration in cosmetics can lead to hyperglycemia and hypertension as well as malignancies [12,13].

Fluocinolone acetonide (FCA) is among the highly potent ingredients in cosmetics. It is commonly used for treating eczema in addition to a composite of commercial cosmetics [14]. The chemical structures of the studied components are shown in Figure 1.

Literature review reveals that various analytical methods exist for rapid screening of cosmetic products especially whitening creams; however, stability-indicating high-performance liquid chromatography (HPLC) method based on forced degradation studies has been rarely reported for the quantification of the mixture of glucocorticoids (HCA, DEX, and FCA) in skin whitening creams. Therefore, the objective of this research work was to develop a stability-indicating HPLC method for the simultaneous quantification of HCA, DEX, and FCA in skin whitening commercial creams. Conditions for quick, accurate, and precise separation of analytes were developed, and the effect of forced degradation on studied components in the combined form under acidic, basic, oxidative, and thermal stress was also investigated as per International Conference on Harmonization (ICH) guidelines. The method was practically tested for the detection of HCA, DEX, and FCA in cosmetic samples.

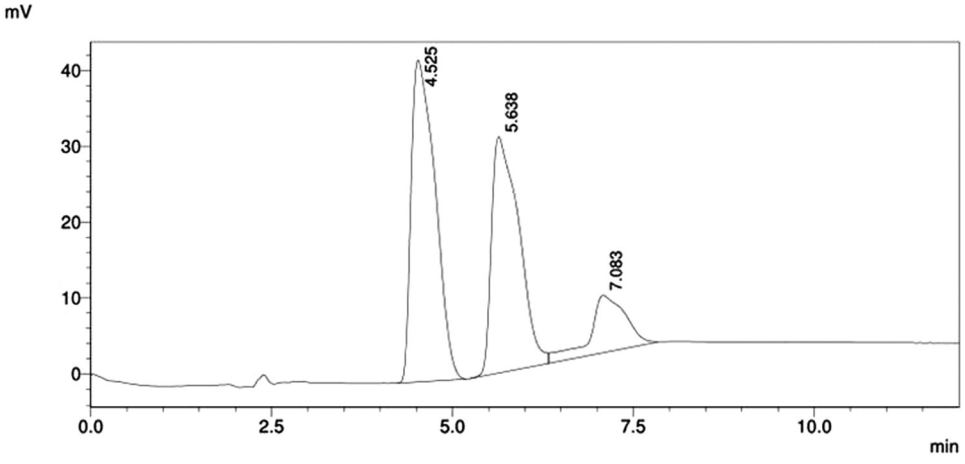

Figure 2: Effect of composition of mobile phase on the separation of standards. The chromatogram is showing broad peaks and distorted baseline using a mobile phase comprising methanol and double-distilled deionized water in the ratio of 60:40 (v/v), respectively, without the addition of formic acid.

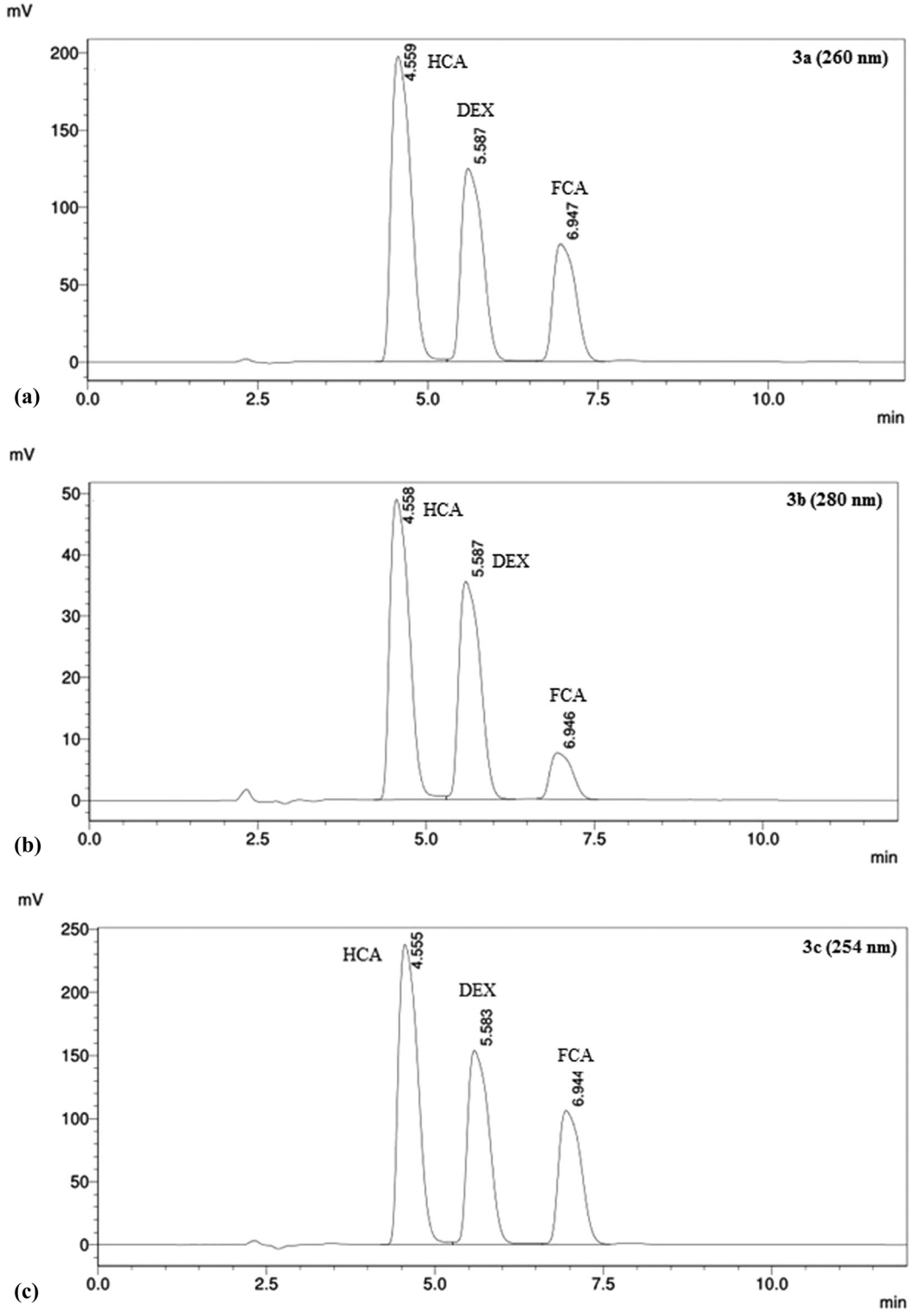

Figure 3: Effect of different wavelengths on detection of standards having concentration of 10 µg/mL, (a = 260 nm, b = 280 nm, c = 254 nm) using DAD detector under the same experimental conditions. More peak height and area were observed at 254 nm wavelength when compared with other wavelengths

Table 1: System suitability parameters for HCA, DEX, and FCA standard solutions (n = 5)

Parameter	HCA	DEX	FCA	Acceptance criteria
Retention time (t_R in min)	4.5	5.5	6.9	—
Resolution (R_s)	6.82	6.94	6.98	>2
Tailing factor (T_f)	0.609	1.223	1.372	<2
Capacity factor (k')	3.86	7.15	6.23	>1.0
Plate count (N)/m	3,56,309	6,48,617	92,482	>1,000

2 Experimental

2.1 Standards and reagents

Reference standards of HPLC grade HCA, DEX, and FCA (99.99% purity) were purchased from Sigma Aldrich, New York, USA. For chromatographic analysis, HPLC grade methanol, acetonitrile, and double-distilled deionized water were also purchased from Sigma Aldrich, New York, USA, and formic acid 98–100% was purchased from Merck, Munich, Germany.

2.2 Sample collection

Thirty skin whitening cosmetic creams belonging to different local and imported brands were purchased from local markets of Lahore (Pakistan) and online. Sample codes were used to ensure the confidentiality of the manufacturers of respective creams.

2.3 Sample preparation

About 500 mg as a uniform amount of each cream was diluted using 10 mL of the solvent mixture comprising methanol, water, and acetonitrile (45:45:10 v/v). The samples were homogenized at 70°C for 20 min on a digital hot plate with stirring at 1,500 rpm. The preparations were stored at room temperature for 1.5 h. Fats and waxes were precipitated and filtered through 0.45 µm polytetrafluoroethylene syringe filters. The filtrate was refiltered and diluted to 10 mL using solvent as described above followed by spinning on the Mini spin plus (4,000 rpm) for 3 min. The supernatants were collected and used as samples for HPLC analysis.

2.4 Standard preparation

The stock solutions of HPLC grade HCA, DEX, and FCA standards were prepared with a concentration of 1,000 µg/mL in a solvent comprising methanol with 1% formic acid and double-distilled deionized water (60:40 v/v). Further serial dilutions were made from the freshly prepared stock solution to make concentrations in the range of 1–150 µg/mL.

2.5 Chromatographic conditions

All separations were carried out on GPC LC-20 AD (Shimadzu, Kyoto, Japan) with the CBM-20A module equipped with autosampler, and chromatographic software (Lab Solutions) was used for acquiring and interpretation of results. A reversed-phase column (ACE Excel 2, C18 AR) having 150 mm length, 3 mm internal diameter, and 2 µm particle size was used for the separation of analytes.

Two different combinations of mobile phase were tested to separate the sample mixtures. Initially, methanol and double-distilled deionized water were used as mobile phases in the ratio of 60:40 (v/v), respectively, and the separation result was compared with the second mobile phase comprising methanol with 1% formic acid and double-distilled deionized water in the ratio of 60:40 (v/v), respectively. The effect of temperature on the separation efficiency of the column was studied by using a different range of column incubation temperatures (25, 30, 35, 40, 45, and 50°C). The detection wavelength was also varied in the range of 210–300 nm using a diode array detector to observe maximum absorption (lambda max) of a mixture of three standards followed by their separation in the column.

After optimization of chromatographic conditions, standard and sample analysis was performed for 12 min using isocratic mode; however, the run time was decreased in accordance with the observed maximum retention time of the analytes. The flow rate was set to 0.5 mL/min, and 10 µL of the sample was injected into the HPLC system for simultaneous detection of analytes in standards and samples.

Table 2: Linearity, correlation coefficient, LOD, and LOQ of standards

Standards	Linear equation	Correlation coefficient	Slope	SE of Slope	Intercept	Standard error of intercept	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
HCA	$y = 12.7x + 118.7$	0.999	12.74	0.172	118.7	16.20	1–150	0.25	2.06
DEX	$y = 12.9x + 106.8$	0.999	12.92	0.209	106.8	19.70	1–150	0.08	1.55
FCA	$y = 12.9x + 96.81$	0.999	12.92	0.190	96.81	17.90	1–150	0.20	1.83

2.6 Method validation

The specificity, accuracy, precision, reproducibility, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness were determined to validate the method as per ICH guidelines [15]. The linearity of method was studied for all standards by the analysis of solutions with different concentrations (1–150 µg/mL) in triplicate. The values of LOD and LOQ were determined through the signal-to-noise ratio.

For determination of recovery and accuracy, 5, 15, and 25 µg/mL solution of HCA, DEX, and FCA standards were added in the cosmetic samples, and analysis was performed with three replicates to observe percentage recovery and relative standard deviation (RSD). Accuracy was calculated as the difference between the theoretically added amount and the practically obtained amount. While for the determination of precision, five injections of different concentrations (20, 40, and 60 µg/mL) of all standards were administered, and precision was calculated through observation of found concentrations of analytes on the same day of injection and other days (days 1, 2, and 3). Moreover, flow rate, wavelength, mobile phase composition, and column temperature were the determining factors of the robustness of the method [15].

2.7 Forced degradation studies

Forced degradation studies were performed to evaluate the stability of the proposed method by treating the studied components with various conditions of stress including acid, alkali, chemical oxidant, and heat stress. The main purpose of the stability testing was to look over how the quality of a drug product changes with respect to time under the influence of environmental factors [16]. The interference produced by the degradation products was noted. Forced degradation study in the basic medium was performed by addition of 0.1 N NaOH into 5 mL of stock solution of HCA, DEX, and FCA in a 25 mL volumetric flask and stored at ambient temperature for a period of 6 h. Then, the solution was neutralized with an acid and further diluted to a final concentration of 10 µg/mL of a mixture of HCA, DEX, and FCA. Similarly, degradation experiments were performed in the acidic medium by using 0.1 N HCl. For the purpose of oxidative degradation, the prepared standard solution was treated with 3% hydrogen peroxide (H_2O_2) for 6 h. For heat degradation, the standard solution was subjected to heat in the oven at 80°C for 6 h. Finally, all

Table 3: Recovery and accuracy results for detection of HCA, DEX, and FCA

Studied component	Concentration after spiking[a] (µg/mL)	Concentration found[b] (µg/mL), mean ± SD; RSD	Recovery (%)
HCA	10	10.08 ± 0.19; 1.88	100.8
	20	20.15 ± 0.40; 1.98	100.7
	30	30.40 ± 0.10; 0.32	101.3
DEX	10	10.26 ± 0.10; 0.97	102.6
	20	20.40 ± 0.38; 1.86	102.0
	30	30.70 ± 0.25; 0.81	102.3
FCA	10	10.20 ± 0.15; 1.47	102.0
	20	20.05 ± 0.33; 1.64	100.2
	30	30.30 ± 0.20; 0.66	101.0

[a] Actual concentration of each standard was 5 µg/mL. [b] Three replicates were run for each sample. The value of the RSD was less than 2%.

solutions were diluted to obtain a 10 µg/mL solution of a mixture of HCA, DEX, and FCA and injected into the system.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Optimization of chromatographic conditions

Among different compositions of the mobile phase, the first mobile phase comprising methanol and double-distilled deionized water without formic acid showed poor separation of analytes as distortion was observed in baseline along with broad peaks (Figure 2). However, the best separation was achieved using the second combination of solvents as the mobile phase comprising methanol with 1% formic acid and double-distilled deionized water with a ratio of 60:40 (v/v). This composition of the mobile phase exhibited more polarity-based compatibility for the separation of analytes leading to sharp and more symmetrical peaks (Figure 3c). Among various ranges of temperatures (25, 30, 35, 40, 45, and 50°C), the best separation was observed at 35°C with 0.5 mL/min flow rate and 10 µL injection volume. Among different tested wavelengths in the range of 210–300 nm, three values

Table 5: Robustness results for HCA, DEX, and FCA

	RSD (%)		
Conditions	**HCA**	**DEX**	**FCA**
Flow rate (0.6 mL/min)	1.50	0.83	1.52
Flow rate (0.4 mL/min)	1.20	1.04	1.00
Wave length (256 nm)	1.75	0.25	1.25
Wave length (252 nm)	1.02	0.33	0.35
Mobile phase (65:35)	0.33	0.67	0.66
Mobile phase (55:45)	1.51	0.50	0.50
Column temperature (40°C)	1.23	0.99	1.47
Column temperature (30°C)	0.15	0.51	0.15

Table 4: Precision, repeatability, and reproducibility results of HCA, DEX, and FCA

Standards	Intraday (n = 5)		Interday (n = 5) conc. found (µg/mL) ± SD; RSD		
	Conc. (µg/mL)	Conc. found (µg/mL) ± SD; RSD	Day 1	Day 2	Day 3
HCA	20	19.93 ± 0.30; 1.51	19.98 ± 0.03; 0.15	19.96 ± 0.30; 1.50	19.98 ± 0.20; 1.00
	40	39.96 ± 0.70; 1.75	39.92 ± 0.40; 1.00	39.92 ± 0.60; 1.50	40.05 ± 0.50; 1.24
	60	59.91 ± 0.20; 0.33	59.94 ± 0.30; 0.50	59.96 ± 0.30; 0.50	60.03 ± 0.02; 0.03
DEX	20	19.97 ± 0.30; 1.50	19.95 ± 0.10; 0.50	19.93 ± 0.20; 1.00	19.98 ± 0.05; 0.25
	40	39.94 ± 0.60; 1.50	39.97 ± 0.40; 1.00	39.98 ± 0.10; 0.25	39.95 ± 0.40; 1.00
	60	59.95 ± 0.50; 0.83	59.97 ± 0.20; 0.33	59.98 ± 0.40; 0.66	60.02 ± 0.20; 0.33
FCA	20	19.92 ± 0.30; 1.51	19.93 ± 0.03; 0.15	19.96 ± 0.20; 1.00	20.04 ± 0.10; 0.49
	40	39.95 ± 0.40; 1.00	39.96 ± 0.20; 0.50	39.95 ± 0.50; 1.25	39.99 ± 0.20; 0.50
	60	59.96 ± 0.20; 0.33	59.98 ± 0.40; 0.66	59.94 ± 0.30; 0.50	59.98 ± 0.30; 0.50

Table 6: Results of forced degradation of HCA, DEX, and FCA by HPLC analysis; each analysis was performed in three replicates

Nature of degradation	Time (h)	Remaining amount mean ± SD (%)			Extent of degradation		
		HCA	DEX	FCA	HCA	DEX	FCA
0.1 N HCl	6	93.62 ± 0.22	89.47 ± 0.12	70.36 ± 0.29	Slight	Non-significant	Non-significant
0.1 N NaOH	6	35.49 ± 0.16	26.28 ± 0.73	20.44 ± 0.52	Significant	Significant	Significant
3% H_2O_2	6	10.27 ± 0.12	96.57 ± 0.28	15.82 ± 0.17	Significant	Slight	Significant
80°C	6	92.84 ± 0.31	83.72 ± 0.56	90.39 ± 0.28	Slight	Non-significant	Slight

(254, 260, and 280 nm) were selected for final testing based on maximum absorption of standards through DAD detector; however, 254 nm was found to be the optimum wavelength for the simultaneous detection of HCA, DEX, and FCA as maximum peak height and area were observed at 254 nm wavelength when compared with 260 and 280 nm wavelength detection (Figure 3a–c). The ACE Excel 2 C18 AR (150 mm × 3 mm, 2 µm) column yielded well-defined sharp peaks of both standards and sample analytes. The mixture of standards of HCA, DEX, and FCA was simultaneously eluted at 4.5, 5.5, and 6.9 min, respectively, using the above-mentioned optimized chromatographic conditions with a reduced run time of 8 min (Figure 3c).

3.2 Method validation

3.2.1 Specificity

The system suitability parameters including retention time (t_R), number of theoretical plates (N), capacity factor (k'), tailing factor (T_f), and resolution (R_s) were determined, and their values indicated good specificity of the analytical method for the determination of stability of HCA, DEX, and FCA standard solutions, as per acceptance criteria listed in Table 1. The proposed method was found specific as the results of all validating parameters were in accordance with expectations for the determination of all the three analytes. HCA, DEX, and FCA were completely separated without the formation of interfering excipient peaks with the peaks of analytes, and chromatograms were free from baseline noise as shown in Figure 3c.

3.2.2 Linearity, LOD, and LOQ

A linear dynamic range of six concentrations in the range of 1–150 µg/mL of standard solutions were analyzed for the determination of linear regression values. The value of the correlation coefficient, R^2, was 0.999, which shows the linearity of the developed method for respective ranges of standards. A linear calibration curve in the form of $y = ax + b$ was obtained by plotting the peak area (y) in triplicate against the concentration (x) at different concentrations of HCA, DEX, and FCA, whereas b is the intercept and a is the slope of the calibration curve. The values of resultant parameters of regression analysis are listed in Table 2. The LOD and LOQ were determined through signal-to-noise ratio 3:1 and 10:1, respectively. The LOD of HCA, DEX, and FCA was found to be 0.25, 0.08, and 0.20 µg/mL, respectively, while LOQ of HCA, DEX, and FCA was found to be 2.06, 1.55, and 1.83 µg/mL, respectively (Table 2).

3.2.3 Recovery and accuracy

Different concentrations of standard solutions were added to the cosmetic samples to test the recovery and accuracy. The values of recovery were determined at different concentrations of standards by mean recovery and RSD as listed in Table 3. The detected amounts of standards (added in samples) were found to be significant as RSD was less than 2 which is in accordance with ICH guidelines. HCA, DEX, and FCA were recovered in the range of 100.7–101.3, 102.0–102.6, and 100.2–102.0%, respectively (Table 3). The sufficient yield of recovery in an overall range of 100.2–102.6% indicates the accuracy of the method.

3.2.4 Precision, repeatability, and reproducibility

The observed results of intra- (same day) and interday (Day 1, 2, and 3) injections at different concentrations of 20, 40, and 60 µg/mL (n = 5) are listed in Table 4 indicating precision, repeatability, and reproducibility. The significant concentrations of injected standards showed reliable repeatability (intraday) and reproducibility (interday) of the developed method. The value of

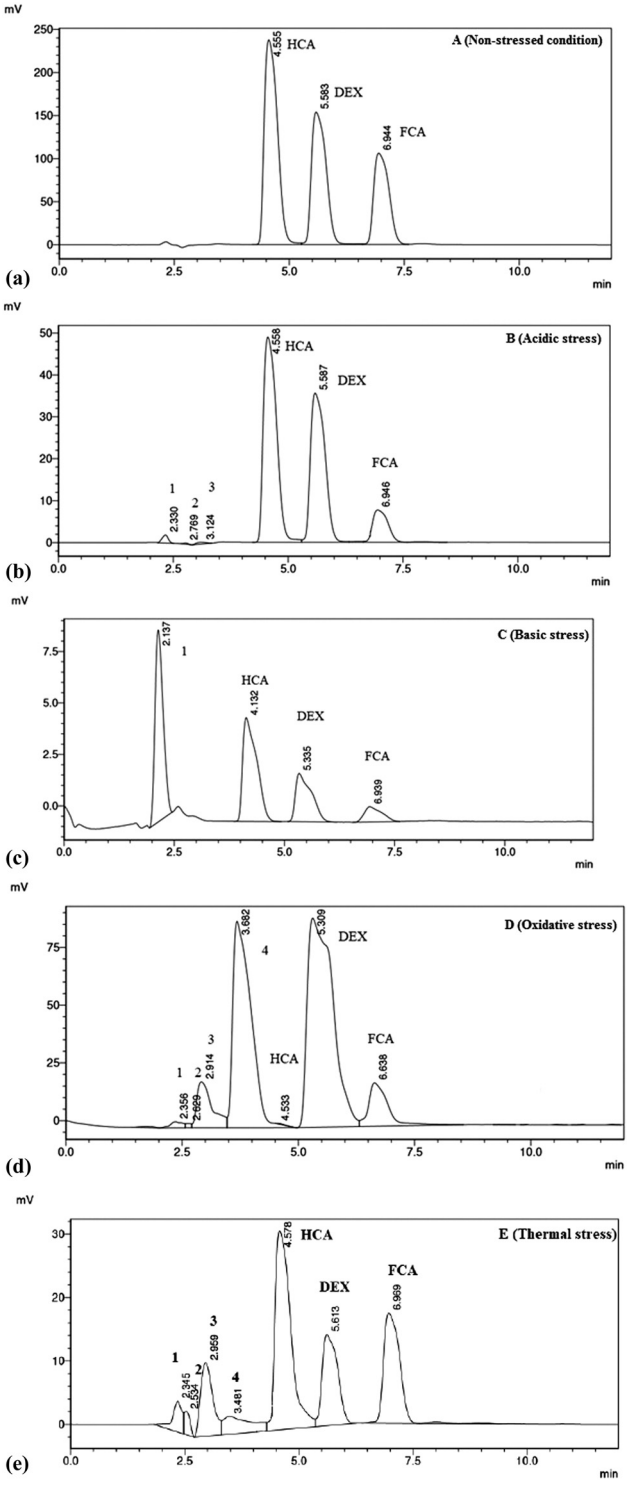

Figure 4: Chromatograms of HCA, DEX, and FCA under (a) non-stressed condition, (b) acidic stress, (c) basic stress, (d) oxidative stress, and (e) thermal stress.

RSD was also acceptable for each run as per ICH guidelines.

3.2.5 Robustness

Experiments for robustness were performed by inducing careful changes in the chromatographic conditions. The newly developed method was found to be robust as no significant effects were observed by variation in the flow rate (±0.1 mL/min), wavelength (±2 nm), mobile phase composition (±5.0 mL), and column temperature (±5°C) as listed in Table 5.

3.3 Forced degradation studies

The stability-indicating capacity of the proposed method was assessed by the forced degradation experimentation performed under different conditions of stress. The extent of degradation of HCA, DEX, and FCA by different stress conditions is tabulated in Table 6. When compared with the control (Figure 4a), under acidic stress, HCA was very slightly degraded and non-significant degradation was observed for DEX and FCA with the formation of three degradation products at the retention time of 2.33, 2.76, and 3.12 min (Figure 4b). However, alkaline treatment significantly degraded HCA, DEX, and FCA with the remaining concentration of 35, 26, and 20%, respectively, and with one degradation product observed at 2.13 min (Figure 4c). The effect of oxidative stress was significant on the degradation of HCA and FCA while DEX was slightly degraded and four degradation products were observed at the retention time of 2.35, 2.62, 2.91, and 3.68 min (Figure 4d). During thermal stress, HCA and FCA were slightly degraded when compared with the degradation of DEX, and the formation of four degradation products was observed at the retention time of 2.34, 2.53, 2.95, and 3.48 min (Figure 4e). As per the results of degradation studies, the proposed method was found to be specific for the determination of HCA, DEX, and FCA.

Although different methods have been described by researchers for the determination of studied components, forced degradation studies of the reported work describe its novelty in comparison to the existing procedures (Table 7).

3.4 Quantification of HCA, DEX, and FCA in cosmetic samples

The applicability of the proposed method was checked by the evaluation of commercial cosmetic creams for the presence of

Table 7: Comparison of some existing analytical methods for quantification of HCA, DEX, and FCA in cosmetic samples with reported research work

Technique	Sample preparation/ analysis time	Mobile phase (v/v)	Column/stationary phase	Analytes/matrix	LOD	LOQ	Forced degradation studies	Reference no.
HPLC-UV	Solvent extraction/ 50 min	Methanol:water (1:1)	Reversed-phase zorbax phenyl (250 mm × 4.6 mm, 5.0 µm)	43 analytes including HCA, DEX, FCA/oil, creams, milk, and soaps	(%) 0.01 to 0.05	Not reported. Qualitative analysis	No	[17]
UHPLC	Solvent extraction/12 min	Water with 0.025 M ammonium borate buffer (pH = 10): acetonitrile	Waters acquity BEH shield RP18 (2.1 mm × 100 mm, 1.7 µm)	12 analytes including HCA, DEX, FCA/creams, lotions, and soaps	(ng/mL) HCA = 55.76 DEX = 65.55 FCA = 49.72	(ng/mL) HCA = 111.53 DEX = 157.32 FCA = 132.60	No	[18]
HPTLC	Solvent extraction/ 30 min	*n*-Hexane:ethyl acetate (1:9)	Silica gel aluminum plate $60F_{254}$, (10 × 10 cm)	2 analytes including FCA/ ointment	(ng/spot) FA = 11.54	(ng/spot) FA = 34.97	Yes	[19]
LC/DAD/MS	Solvent extraction/ 27 min	Acetonitrile:methanol: water (gradient mode)	Phenomenex prodigy ODS-3, (100 mm × 2 mm, 3 µm)	13 analytes including HCA, DEX, FCA/phytocosmetics, and ointments	No data	No data	No	[20]
LC-ESI-MS/MS	Solvent extraction/ 10 min	Water:acetonitrile (both with 0.1% formic acid	Thermo scientific hypersil gold PFP RP-UHPLC (100 mm, 2.1 mm, 1.9 µm)	10 analytes including DEX, FCA/Gels, ointments, and creams	(mg/kg) DEX = 0.109 FCA = 0.093	(mg/kg) DEX = 0.117 FCA = 0.107	No	[21]
HPLC-PDA	Solvent extraction/ 28 min	0.1% Formic acid in water:acetonitrile (gradient mode)	Waters X bridge C18 column (4.6 mm × 250 mm, 5 µm)	6 analytes including HCA, DEX/creams	(µg/mL) HCA = 2.003 DEX = 1.96	(µg/mL) HCA = 6.676 DEX = 6.54	No	[22]
LC/MS/MS	Solvent extraction/ 25 min	0.1% formic acid in water:acetonitrile (60:40) gradient mode	Atlantis T3 column (4.6 mm × 150 mm, 3 µm)	11 analytes including HCA, DEX, FCA/creams	(ng/mL) HCA = 1 DEX = 0.25 FCA = 1	(ng/mL) HCA = 3 DEX = 0.75 FCA = 3	No	[23]
UPLC-MS/MS	Solvent extraction/ 25 min	0.1% formic acid in distilled water:0.1% formic acid in acetonitrile gradient mode	Waters acquity UPLC BEH C18 column (2.0 mm × 100 mm, 1.7 µm)	43 analytes including HCA, DEX, FCA/lotions, creams, solutions, gels, and powders	(ng/mL) HCA = 5 DEX = 10 FCA = 7.50	(ng/mL) HCA = 15 DEX = 30 FCA = 22.50	No	[24]
UPLC-TOF-MS	Solvent extraction/6 min	Aqueous ammonia 0.01%:acetonitrile	Column C18 (10 cm × 2.1 mm, 1.7 µm)	43 analytes including HCA, DEX, FCA/cream, lotion, soap, oil, gel, and serum	(pg/mL) HCA = 732 DEX = 102 FCA = 102	No data	No	[25]
HPLC-DAD	Solvent extraction/ <8 min	Methanol with 1% formic acid:double-distilled deionized water (60:40)	ACE Excel 2, C18 AR column (150 mm × 3 mm, 2 µm)	3 analytes HCA, DEX, FCA/ cosmetics, creams	(µg/mL) HCA = 0.25 DEX = 0.08 FCA = 0.20	(µg/mL) HCA = 2.06 DEX = 1.55 FCA = 1.83	Yes	This work

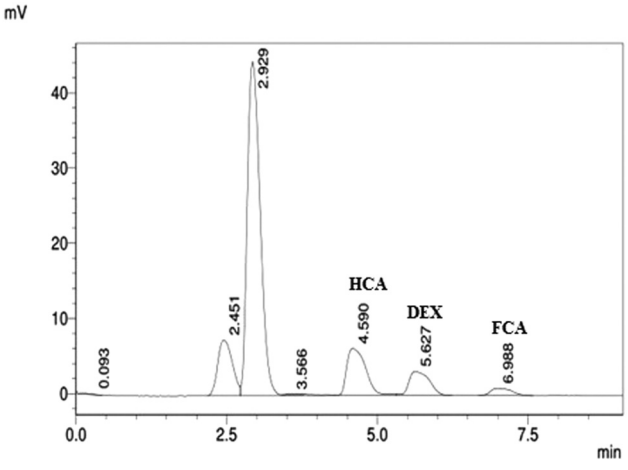

Figure 5: Chromatogram of a typical sample (cosmetic cream) for the detection of HCA, DEX, and FCA under optimized chromatographic conditions as described in Section 3.1.

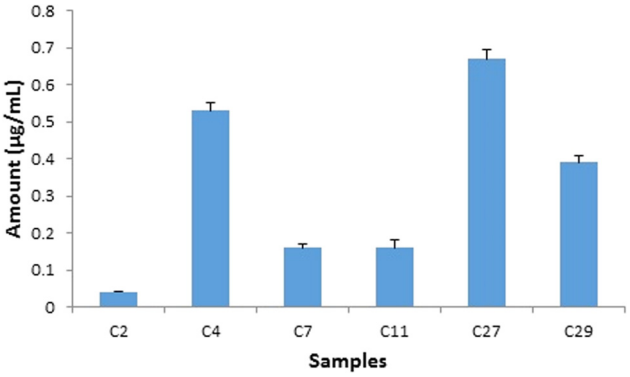

Figure 6: Assay result of HCA in cosmetic creams by HPLC analysis. HCA was detected in six samples (n = 30) with a maximum amount of 0.67 µg/mL (C27).

studied components. Sharp and well-separated peaks of HCA, DEX, and FCA were obtained in sample mixtures at 254 nm. A typical chromatogram of sample analysis has been shown (Figure 5) in which all of the three analytes have been well-separated and eluted at their specific retention times in accordance with standards under the proposed optimized conditions. HCA as one of the undeclared illicit whitening agents was detected in six samples (Figure 6) with a maximum amount of 0.67 µg/mL found in C27 while the least amount was detected in C2 (0.04 µg/mL). DEX was present in 16 samples (Figure 7) with a maximum concentration of 56.38 µg/mL in C17 and a minimum concentration (0.03 µg/mL) in C9. FCA was found in 13 samples with a maximum concentration of 5.24 µg/mL (C20) and the least amount (0.09 µg/mL) in C29 (Figure 8).

4 Conclusion

A selective, sensitive, and rapid HPLC method was developed and validated for the simultaneous determination of HCA, DEX, and FCA in commercial cosmetics. All of the analytes were quantified with precision, accuracy, and robustness within 8 min. The separation of analytes was obtained with good resolution under optimized chromatographic conditions. Forced degradation behavior of the studied components in the combined form as per ICH guidelines further confirmed the stability of assay. The method was found to be suitable for the routine analysis of HCA, DEX, and FCA in

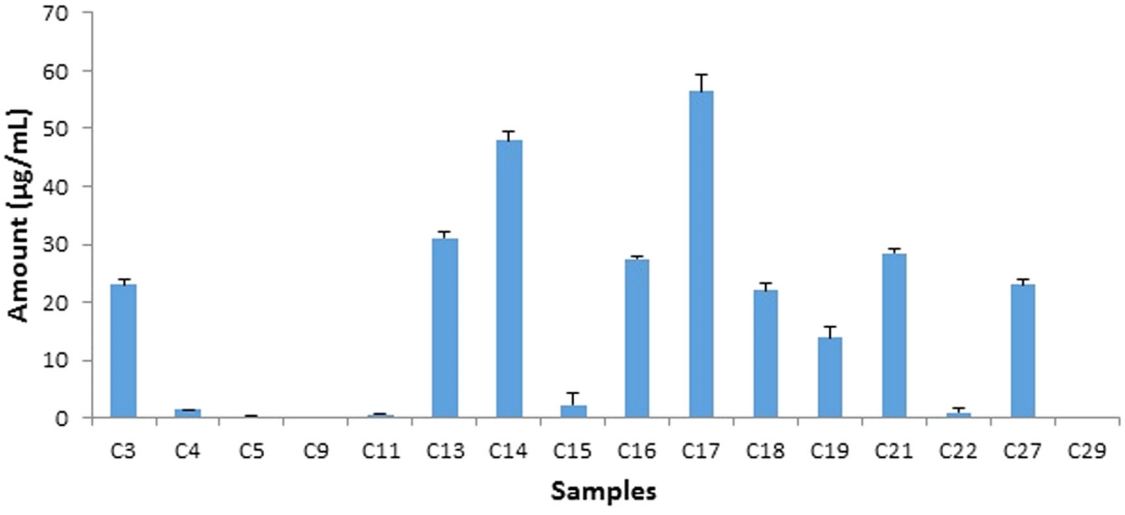

Figure 7: Assay result of DEX in cosmetic creams by HPLC analysis. DEX was detected in 16 samples (n = 30) with a maximum amount 56.38 µg/mL (C17).

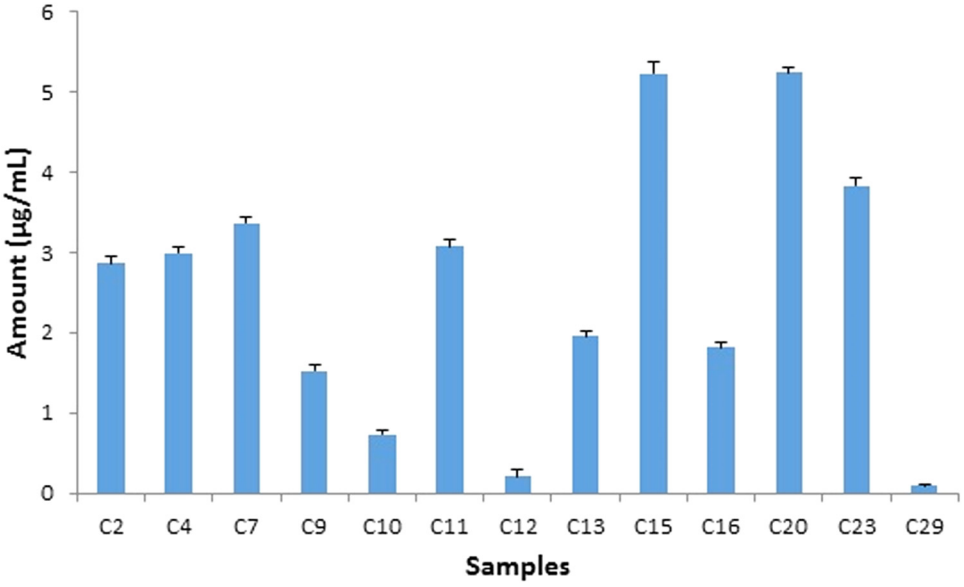

Figure 8: Assay result of FCA in cosmetic creams by HPLC analysis. FCA was detected in 13 samples (n = 30) with a maximum 5.24 µg/mL (C20).

commercial cosmetics as RSD of all parameters was observed within limits (<2).

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Conflict of interest: The authors declare no conflicts of interest.

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