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Development and Validation Method for Simultaneous Analysis of Retinoic Acid, Hydroquinone and Corticosteroid in Cream Formula by High-Performance Liquid Chromatography

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ABSTRACT

A simple, precise and rapid reverse phase HPLC-PDA method has been developed and validated for the simultaneous analysis of hydroquinone (HYQ), dexamethasone (DEX), triamcinolone acetonide (TSA), hydrocortisone acetate (HYA), betamethasone valerate (BEV) and retinoic acid (REA) in the cream dosage form. The mixture of HYQ, DEX, TSA, HYA, BEV and REA was separated on Waters X Bridge C18 5 µm column (4.6 mm × 250 mm). All separations were performed with a 2998 Photodiode Array (PDA) detector on 210-400 nm wavelength, 400C at column temperature and flow rate at 1.2 ml/min. The mobile phase was acetonitrile (ACN): 0.1% formic acid with gradient system. The retention times of HYQ, DEX, TSA, HYA, BEV, and REA were found to be 3.69; 9.13; 9.47; 9.96; 14.92 and 21.20. The method showed good linearity with correlation coefficient 0.9990; 0.9991; 0.9984; 0.9991; 0.9997 and 0.9991 over the range of 25-150 µg/ml for HYQ, DEX, TSA, HYA, BEV and REA, respectively. The method was mean recoveries in the range of 99.05 to 100.96% for all analytes. The developed method can be used in the routine analysis of HYQ, DEX, TSA, HYA, BEV and REA in the cream formula, as well as for qualitative interest in cosmetic preparations or for quantitative interest in drug preparations.

INTRODUCTION

Hydroquinone (1,4-dihydroxybenzene) is the first line agent in medical therapy of hyperpigmentation. It decreases melanocyte pigment production by inhibiting the conversion of DOPA to melanin through inhibition of the enzyme tyrosinase. Other mechanisms include inhibition of DNA and RNA synthesis, degradation of melanosomes, and destruction of melanocytes. Adverse effects may include dermatitis and ochronosis (Laurence *et al.*, 2011; Charlene, 2014).

Retinoic acid is the organic acid derives from vitamin A and it is also known as all-*trans* retinoic acid. It is a drug commonly used to treat acne vulgaris. Acne vulgaris is a common dermatologic problem which could be treated with systemic or topical drugs. More importantly, the combination therapy of topical retinoic acid is more beneficial for the treatment of mild to moderate stages of acne vulgaris (Laurence *et al.*, 2011; Deswal *et al.*, 2017).

Corticosteroids are highly effective drugs which are used to treat inflammatory skin conditions such as eczema and psoriasis. Side effects of corticosteroids for long-term use including pustular psoriasis, permanent skin atrophy and systemic effects such as hypertension, contact dermatitis, and diabetes. Depigmentation is a side effect of topical steroids, corticosteroids might be used as whitening agents in the topical medical preparations illegally sold as cosmetics. Their absorption through the skin can cause adrenal suppression or even Cushing's syndrome. Local side effects of topical corticosteroids include spread and worsening of untreated infection; irreversible thinning of the skin, dermatitis, acne, and hypertrichosis (Laurence *et al.*, 2011; Desmedt *et al.*, 2014).

To determine hydroquinone, retinoic acid and corticosteroid as single components or simultaneous, many studies have been reported in literature such as analysis of hydroquinone (ACM INO 03, 2005), analysis of retinoic acid

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(ACM 001, 2005), simultaneous analysis of hydrocortisone acetate, dexamethasone, betamethasone, betamethasone 17 valerate and triamcinolone acetonide (ACM MAL 07, 2005), simultaneous analysis of hydroquinone and retinoic acid (Aung Myo Htet *et al.*, 2016), simultaneous analysis of hydroquinone, retinoic acid and mometasone furoate (Sheliya *et al.*, 2014) and simultaneous analysis of hydroquinone, ethers hydroquinone and corticosteroids (Gimeno *et al.*, 2016). From the literature survey, there is no analysis method developed for the simultaneous analysis of HYQ, DEX, TSA, HYA, BEV, and REA in cream. Based on fact that these six components are required to be tested in cream cosmetic preparations, especially in facial skin lightening creams, this method also can later be used for the determination levels in preparation of drug creams. Thus, this study aimed to develop fast, simple, inexpensive, sensitive and validated a new high-performance liquid chromatography (HPLC) method for determination of HYQ, DEX, TSA, HYA, BEV and REA simultaneously in cream. The proposed method was validated with validation parameters, which are specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ), precision, accuracy and in accordance with USP guidelines (USP 40, 2016), and later used to analyze cream whether as cosmetic preparation or medicinal preparation.

EXPERIMENTAL

Materials

The reference standard of HYQ, DEX, TSA, HYA, BEV, and REA were kindly gifted by Indonesia National Agency of Drug and Food Control. The chemical structures in Figure 1. Commercial HYQ, TSA, HYA, BEV and REA topical cream were purchased from local market in Indonesia, methanol grade for liquid chromatography (Merck), acetonitrile grade for liquid chromatography (Merck). Formic acid 98-100% pro analysis (Merck), double distilled water used in the experiment was WIDA WI UNICAP 1000 ml (Widatra Bhakti, Indonesia). Cream base oil-in-water purchased from PT. Ikapharmindo Putramas Jakarta, Indonesia.

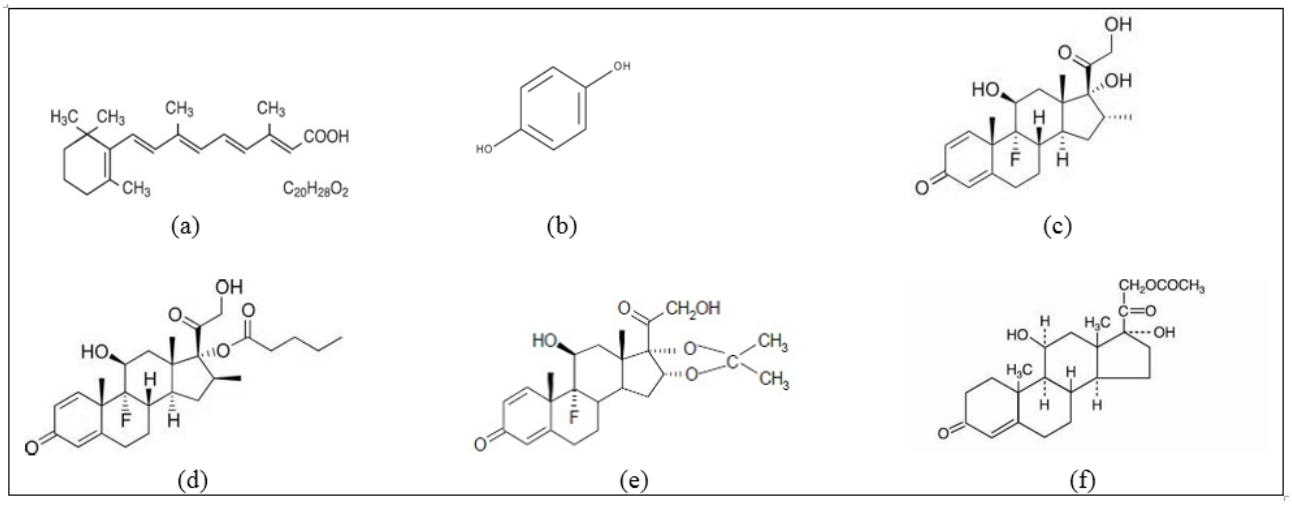

Fig. 1: Chemical structures of (a) Retinoic acid, (b) Hydroquinone, (c) Dexamethasone, (d) Betamethasone valerate, (e) Triamcinolone acetonide, (f) Hydrocortisone acetate.

Instrumentation and chromatographic conditions

All separations were performed on the HPLC Waters Alliance e2695 with Waters 2998 photo array (PDA) detector. The data were acquired and analyzed using Empower 3 software. Chromatographic separation was carried out on Waters X Bridge C18 5 µm column (4.6 mm × 250 mm). The component of the mobile phase used for gradient elution was ACN: 0.1% formic acid solution. Before use, the mobile phase was filtered through 0.45 µm membrane filter and degassed by ultrasonication. The flow rate was maintained at 1.2 mL/min with a column temperature of 40°C. The injection volume was 20 µL, and detection was performed at 210-400 nm using a PDA detector.

Preparation of standard stock solutions

The standard stock solutions of HYQ, DEX, TSA, HYA, BEV, and REA were prepared by accurately weighing 25 mg of each into a 50 ml volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. Further dilutions were made from this stock solution become 25, 50, 75, 100, 125 and 150 µg/ml.

Preparation of spike stock solutions

An aliquot (500 mg) of the matrices (cream base oil in water) were weighed into 15 ml tubes and spiked with 5 ml standard stock solution and get 6 concentration of spike solution 25, 50, 75, 100, 125, 150 µg/ml respectively.

Method validation

Specificity

The specificity of this HPLC method was determined by complete separation of HYQ, DEX, TSA, HYA, BEV and REA without any interference of excipients peak with analyte peaks by analyzing the resolution (R), tailing factor (TF) and plate count (PC) parameters of each peak, besides the purity plots from PDA detector.

Linearity and range

Determining linearity, calibration curves were plotted over a concentration range of 25-150 µg/mL for HYQ, DEX, TSA, HYA, BEV and REA respectively. A 20 µL of sample solution was injected into the chromatographic system. Chromatograms were recorded. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration. Vxo ≤ 5.0 and R-value (≥ 0.999) confirmed the good linearity of the method.

Limits of detection and quantification

Using a sample from linearity parameter, the limit of detection (LOD) and limit of quantification (LOQ) were calculated with the formula. The value was calculated from the standard deviation (SD) of response and the slope of the curve (S) by means of the equations: LOD = 3.3 (SD/S) and LOQ = 10 (SD/S), where SD: standard deviation of the detector response; S: slope of the calibration curve.

Precision

The precision of the proposed method was assessed by preparing three different sample solutions at low, medium, and high concentrations, which were freshly prepared and analyzed. The precision % relative standard deviation (% RSD) was assessed by analyzing standard drug solutions within the calibration range. The precision of the method was expressed as RSD % and Horwitz value.

Accuracy

Accuracy was calculated as the difference between theoretical added amount and the practically achieved amount. To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100%, and 150%. The known amount of standard HYQ, DEX, TSA, HYA, BEV, and REA were added to pre-analyzed samples and were subjected to the proposed method (Petrova *et al.*, 2013).

Analysis of marketed formulation

The validated method, used for testing cream samples on the market. The sample used in this test is a drug cream, which is; HYQ 2 %, DEX not tested, TSA 0.1 %, HYA 1%, BEV 0.1%, and REA 0.1%. A portion of the cream was weighed about 500 mg into the appropriate tube for getting 100 µg/ml concentration. The sample extracted with methanol with the aid of ultrasonication for 15 minutes, then filtered using 0.45 µm PTFE filter.

RESULT AND DISCUSSION

Optimization of procedures

The HPLC procedure was optimized for simultaneous determination of HYQ, DEX, TSA, HYA, BEV and REA. Good resolution of both components was obtained with the gradient system 0.1% formic acid in water as solvent A and Acetonitrile as solvent B. The gradient was, in 0 min 8% A, 5 min 8% A, 16 min 50% A, 23 min 95% A, 28 min 8% A, and then return to initial condition. The flow rate of 1.2 mL/minutes was optimum, with oven temperature 40°C. Using PDA detector the chromatograms was read with Waters Empower 3 Chromatography software by using time wavelength procedure. HYQ were read at 289 nm, corticosteroid DEX, TSA, HYA and BEV were read at 240 nm, and REA was read at 321 nm. The six component already read on their optimum wavelength (Table 1). The system suitability parameters for HPLC chromatogram are as follows (Table 2). The average retention times for HYQ, DEX, TSA, HYA, BEV and REA was found to be 3.693; 9.130; 9.470; 9.964; 14.919 and 21.202 respectively (Figure 2).

Table 1: The Final optimized condition for the analysis.

No	Parameter	Specification		
1	Column	C18		
2	Particle size	5 µm		
3	Detector	PDA		
4	Wavelength	Time wavelength		
		Time	Wavelength	
		0	289	
		8	240	
		18	321	
5	Mobile phase	Gradient		
		Time	Formic Acid 0.1%	Acetonitrile
		0	8	92
		5	8	92
		16	50	50
		23	95	5
		28	8	92
6	Flow rate	1.2 ml/min		
7	Injection volume	20 µl		
8	Column temp	40°C		

Table 2: System suitability parameters.

System Suitability Parameters	HQ	DM	TA	HA	BV	RA	Limits
Retention time	3.697	9.130	9.47	9.964	14.919	21.202	-
Resolution	-	25.3039	3.2606	4.2133	23.3667	29.7337	>2
Tailing	0.807	1.248	1.352	1.222	1.273	1.229	<2
Plate Count	1950	132392	112438	99335	37808	5286214	>1000

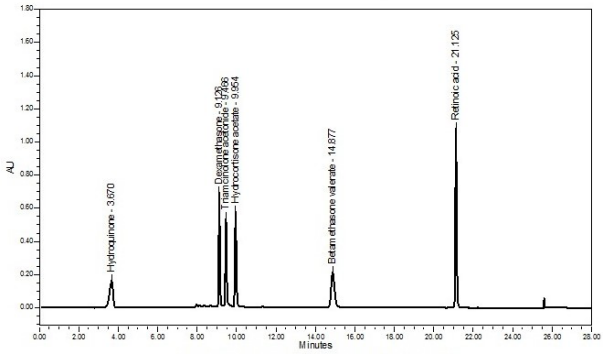

Fig. 2: Chromatogram of hydroquinone, dexamethasone, triamcinolone acetonide, hydrocortisone acetate, betametasone valerate and retinoic acid (reference standard).

Specificity

This HPLC method was specified, determined by complete separation of HYQ, DEX, TSA, HYA, BEV and REA without any interference of excipients peak with analyte peaks (Figure 2, Figure 3). The resolution (R), tailing factor (TF) and Plate Count (PC) parameters of each peak meet the requirement. It was less than 2 for TF, more than 2 for R and more than 1000 for PC (Table 2). Beside that the purity plot shows that the six component was pure (Figure 4).

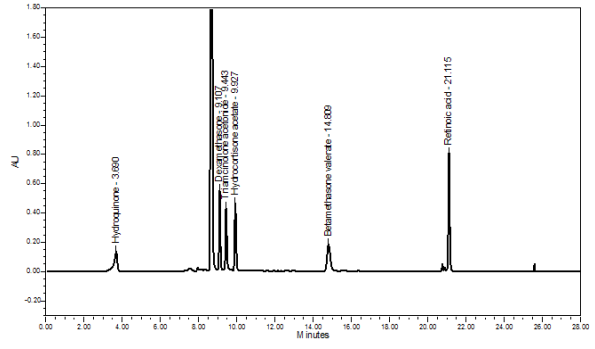

Fig. 3: Chromatogram of hydroquinone, dexamethasone, triamcinolone acetonide, hydrocortisone acetate, betametasone valerate and retinoic acid reference standard in cream bases oil in water.

Linearity and range

From the calculation were got that Vxo of each component ≤ 5.0, and R value ≥ 0.999 confirmed the good linearity of the method (Table 3). Linear regression data for the calibration plots revealed good linear relationships between response and concentration. The linear regression equations were Y = 17766.04X + 19823.77 (r = 0.9990) for HYQ, Y = 26943.51X + 98029.48 (r = 0.9991) for DEX, Y = 23602.22X + 256560.22 (r = 0.9984) for TSA, Y = 28540.38X + 39937.17 (r = 0.9991) for HYA, Y = 21217.47X + 290070.95 (r = 0.9997) for BEV, and Y = 41552.68X + 298920.30 (r = 0.9991) for REA (Table 5).

Table 3: Specificity, Range, LOD and LOQ parameters.

No	Parameter	HQ	DM	TA	HA	BV	RA	Limits
1	Specificity	No interference of excipients peak with analyte peaks						
2	Range							
	- Linearity range	25-150 µg/ml						
	- Target concentration	100 µg/ml						
	- Vxo	1.670	1.545	2.051	1.576	0.935	1.526	≤ 5.0
	- R	1.000	1.000	0.999	1.000	1.000	1.000	≥ 0.999
3	LOD	2.123	1.964	2.607	2.003	1.188	1.940	-
4	LOQ	7.077	6.545	8.689	6.676	3.960	6.465	-

Table 4: Regression.

Agents	Regression Equation	R2
HYQ	y = 17918.84x + 19823.77	0.9990
DEX	y = 26835.74x + 98029.49	0.9991
TSA	y = 23408.69x + 25656.23	0.9984
HCA	y = 28243.56x - 39937.17	0.9991
BMV	y = 21340.53x + 290070.95	0.9997
REA	y = 41785.38x - 298920.31	0.9991

Limits of detection and quantitation

The LOD and the LOQ for this simultaneous analysis were found to be 2.14 µg/mL and 7.14 µg/mL for HYQ, 1.96 µg/mL and 6.52 µg/mL for DEX, 2.59 µg/mL and 8.62 µg/mL for TSA, 1.98 µg/mL and 6.61 µg/mL for HYA, 1.19 µg/mL and 3.98 µg/mL for BEV, and 1.95 µg/mL and 6.50 µg/mL for REA, respectively (Table 3).

Precision

The repeatability of the method was carried out and the high values of the mean assay and low values of % RSD and the Horrat value for HYQ, DEX, TSA, HYA, BEV and REA revealed the proposed method is precise. The results showed in (Table 5).

Accuracy

The recovery of the method was 99.05–100.96%. The values of recovery in Table 5 indicate the method is accurate.

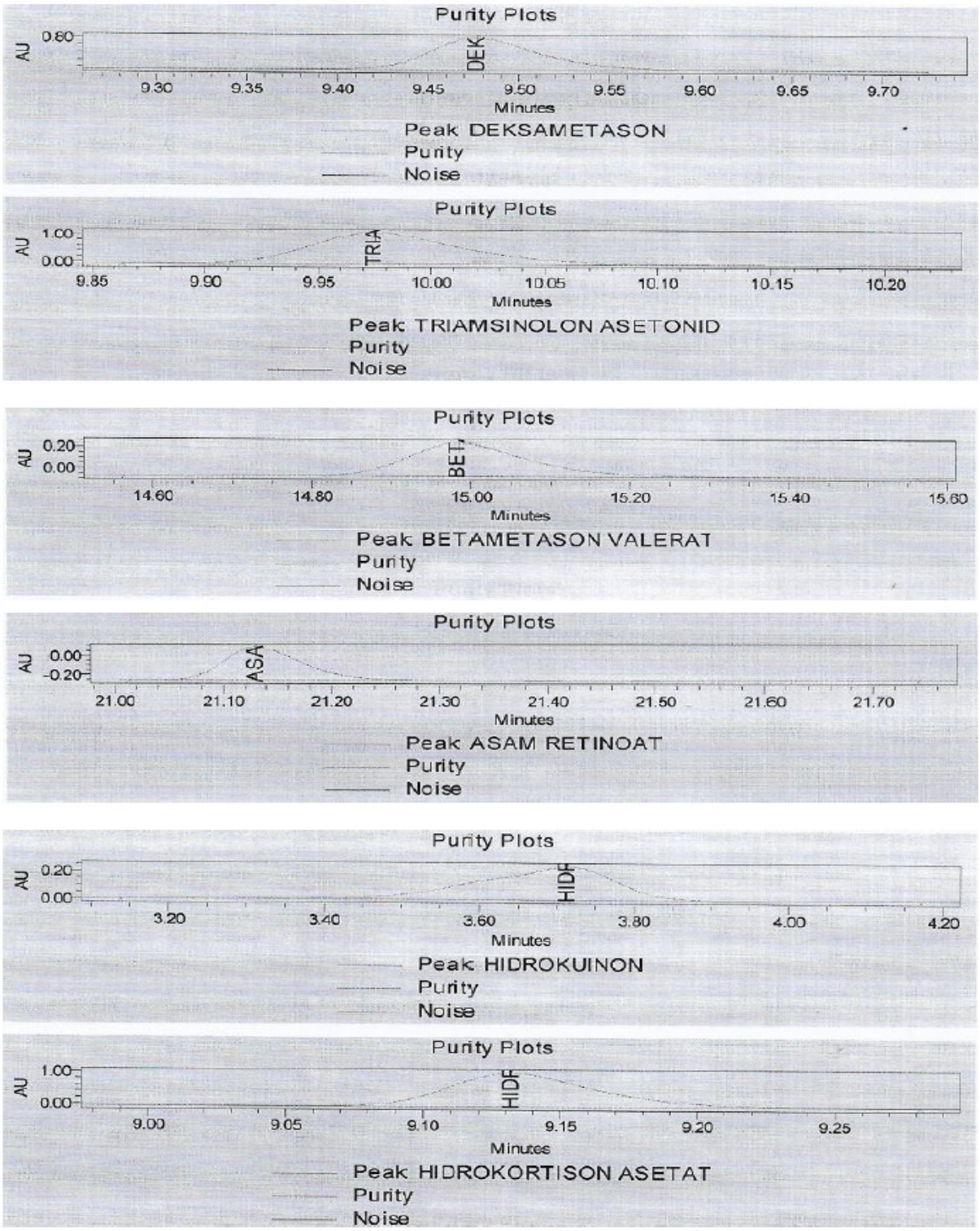

Fig. 4: The purity plots of hydroquinone, dexamethasone, triamcinolone acetonide, hydrocortisone acetate, betametasone valerate and retinoic acid.

Analysis of marketed formulation

In this market sample analysis, DEX sample was not tested. Analysis was done to commercial HYQ (2%), TSA (0.1%), HYA (0.1%), BEV (0.1%), and REA (0.1%) cream. Sharp and well-defined peaks for HYQ, TSA, HYA, BEV, and REA were obtained respectively when scanned with PDA detector at 210-400 nm. The result of the measurement is presented in Table 6.

CONCLUSION

A simple, linear, accurate and precise HPLC method was

developed and validated for the simultaneous analysis of HYQ, DEX, TSA, HYA, BEV and REA in the cream topical formulation. The validation results revealed that the methods are precise, linear and accurate, which proves the reliability of the proposed method. The method could be successfully applied for routine analysis and quality control of pharmaceutical dosage forms.

Table 5: Precision and accuracy parameters.

Concentration		Parameter		
		Precision		Accuracy
		RSD (%)	Horrat	% Recovery
HQ	50	1.289	0.606	99.70
	100	0.289	0.151	100.96
	150	1.656	0.918	99.28
DM	50	1.331	0.626	99.76
	100	1.621	0.846	99.05
	150	0.992	0.551	100.80
TA	50	1.417	0.667	99.79
	100	2.535	1.323	99.14
	150	1.816	1.009	100.84
HA	50	1.187	0.559	100.07
	100	2.011	1.050	99.34
	150	1.905	1.058	100.63
BV	50	1.300	0.612	100.27
	100	0.932	0.486	99.28
	150	0.118	0.621	100.43
RA	50	0.232	0.109	99.83
	100	0.912	0.476	99.27
	150	0.517	0.287	100.14

Table 6: Result from the assay of marketed formulation.

Sample	Component	% Mean Amount	SD	% RSD
1	Hydroquinone	99.86	0.8600	0.8612
2	Triamcinolone acetonide	100.13	0.3022	0.3018
3	Hydrocortisone acetate	97.02	0.1760	0.1814
4	Betamethasone valerate	102.50	0.7606	0.7420
5	Retinoic acid	104.87	0.9222	0.8795

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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