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Original Research Article

UV-VISIBLE SPECTROPHOTOMETRIC ANALYSIS FOR SIMULTANEOUS-ESTIMATION OF KAEMPFEROL AND DEXAMETHASONE

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Abstract: ***Background***: Kaempferol, a naturally occurring flavonoid, and Dexamethasone, a synthetic corticosteroid, are known for their broad biological properties, including Reactive oxygen species (ROS) inhibition, anti-inflammatory, anti-cancer, & immunosuppressive properties. While numerous analytical techniques are available for their individual estimation or in multi-drug formulations, no spectrophotometric approach was developed to quantify them simultaneously. ***Aim:*** The research is primarily concerned on developing and validating a simple, accurate, & precise UV-visible spectrophotometric-method for the simultaneous equation approach of Kaempferol & Dexamethasone in bulk & combined pharmaceutical formulations. ***Material and methods:*** The λmax of kaempferol and dexamethasone were determined. The simultaneous equation method was employed for analysis. The proposed analytical method underwent validation for linearity, precision, accuracy, specificity, robustness, & ruggedness according to ICH guidelines. LOD and LOQ values were determined utilising the slope and standard deviation of the calibration plot. ***Results:*** The developed method was derived from the identification of λmax for Kaempferol (366 nm), Dexamethasone (238 nm), and their isoabsorptive point (262 nm) in methanol. The method showed excellent linearity for Kaempferol (5 to 30 microgram/mL) and Dexamethasone (10 to 60 microgram/mL), exhibiting correlation coefficients (R^2) of 0.999 for both. ***Conclusion:*** Validation parameters including linearity, accuracy, precision, ruggedness, & robustness were assessed following ICH Q2(R1) guidelines and observed to be within acceptable criteria. The suggested approach is appropriate for routine quality-control evaluations of those drugs in dosage forms.

Keywords: Kaempferol, Dexamethasone, UV-visible spectrophotometric method, Isoabsorptive point, Robustness.

INTRODUCTION

The most common aglycone flavonoid, kaempferol, is usually found in glycosylated forms. It is a tetrahydroxyflavone, distinguished by its yellow coloring, and contains -OH groups attached at positions 3, 5, 7, & 4′ **[1].** Kaempferol & its glycosylated-derivatives have demonstrated a broad range of bilogical activities, possessing anti-inflammatory, antihyperglycemic, free radical scavenging, anti-microbial, cytotoxic, cardio-protective, neuro-protective, & anti-cancer properties **[2].** Its anticancer potential has been validated in various malignancies such as esophageal, breast, cervical, leukemia, cholangio-carcinoma (CCA), pancreatic, bladder, ovarian, gastric, hepato-cellular carcinoma (HCC), ovarian, gastric, non-small cell lung carcinoma (NSCLC) as well as in benign illnesses including uterine fibroids. Being a non-toxic, cost-effective dietary component, kaempferol holds substantial economic and therapeutic value. As a flavonoid, it is naturally extractable from various plant sources **[3].**

Dexamethasone is a synthetic corticosteroid,

specifically a glucocorticoid, used for its potent immune-suppressive and anti-inflammatory activities. It is widely prescribed for conditions such as severe allergic responses, chronic obstructive pulmonary disease (COPD), rheumatologic conditions, asthma, dermato-logical conditions, intracranial swelling, and used alongside antitubercular agents, co-administered with antibiotics for TB management **[4].** In endocrinology, dexamethasone serves as a Cushing's syndrome diagnostic tool **[5].** Additionally, it is effective in managing chemotherapy-induced vomiting and nausea, and in preventing as well as treating high-altitude illness. In oncology, it is administered to alleviate spinalcord-compression resulting from metastases **[6].** During the COVID-19 pandemic, dexamethasone was recommended for severely ill patients requiring oxygen therapy or mechanical ventilation; however, it is not advised for patients with mild to moderate disease **[7].**

The literature has documented a variety of analytical techniques for the separate quantification of kaempferol and dexamethasone, as well as in combination with other drugs, primarily including RP-HPLC & UV-spectroscopic techniques. As per the available literature, there are no studies describing a spectrophotometric approach for simultaneous estimation of both of these compounds in a combined formulation has been reported. Therefore, the overall purpose of this research is to design & validate a simple, precise, and accurate UV spectrophotometric technique that can be effectively utilized in routine quality control evaluation to determine kaempferol and dexamethasone simultaneously.

MATERIALS AND METHODS

Chemicals and Reagents

Sigma-Aldrich (Mumbai, India) provided a gift sample of 97% pure kaempferol. We purchased 98% pure dexamethasone from Sigma-Aldrich in Mumbai, India. We acquired 99.80% pure methanol of analytical reagent grade from Rankem Chemicals in Mumbai, India.

Solvent systems

Based on comprehensive solubility analysis of kaempferol and dexamethasone in multiple solvents, methanol was finalized as the solvent system for the method development.

Preparation procedure for standard-solution:

According to the Indian Pharmacopoeia standards, standard solutions of the APIs were formulated. For this, to make up the solution to a concentration of 100 µg/mL, ten mg of kaempferol was added in 100 mL of methanol. From this stock, 0.5–3 mL was pipetted & diluted to a final volume of ten mL with methanol to prepare working-solutions in the range of 5-30µg/mL. Similarly, hundred mL of methanol was utilized to dissolve 10 mg of dexamethasone (100µg/mL), & from this stock, 1–6 mL was pipetted and diluted to ten mL with methanol to obtain concentrations ranging from 10–60µg/mL.

Determination of Absorption maxima

The 200–800 nm range was used to scan the solutions and to find the drugs' maximum absorbance (λmax), a UV-spectrophotometer was used (UV-1700, Shimadzu) **[8-9].**

Development of calibration plot

Working standard dilutions of kaempferol & dexamethasone were prepared in methanol at concentrations ranging from 5–30 µg/mL & 10–60 µg/mL, respectively. The absorbance of kaempferol and dexamethasone was determined at 366nm & 238nm, respectively, and calibration plots were generated by correlating absorbance with concentration.

Simultaneous Equation Method

From the prepared standard stock solutions of each drug (100 µg/mL), appropriate volumes were taken to prepare final concentrations of 5–30µg/mL for kaempferol & 10–60µg/mL for dexamethasone. Absorbance was assessed at both wavelengths using methanol as the blank. All experiments were conducted three times to ensure accuracy. The absorbance maxima (λmax) of both drugs were recorded at the selected wavelengths. The quantities of the drugs were measured by utilizing the simultaneous equation method **[10]**.

- **Analysis of maximum absorption and isoabsorptive points**

The absorption maxima (λmax) of each drug were measured, & overlapping spectra were recorded to identify the isoabsorptive point. A mixture solution containing 25 µg/mL of each drug was recorded with in the range of 200–800 nm using range-mode to study the spectral characteristics.

- **Simultaneous-equation method formula**

The concentrations in the samples were determined utilizing the equations below:

$$CX = A1ay2 - A2ay1 \,/\, ax1ay2 - ax2ay1 \quad \ldots\ldots$$

$$CY = A1ax2 - A2ax1 \,/\, ay1ax2 - ay2ax1 \quad \ldots\ldots$$

A1 represents sample's absorbance was determined at 366 nm.
A2 represents sample's absorbance was determined at 238 nm.
ax1 is Dexamethasone's absorptivity at 366 nm.
ax2 is Dexamethasone's absorptivity at 238 nm
ay1 is Kaempferol's absorptivity at 366 nm.
ay2 = Kaempferol's absorptivity at 238 nm
Cy is the Kaempferol concentration in µg/ml.
Cx is the dexamethasone concentration in µg/ml

(ICH Q2(R1), 2005) [11].

Method validations:

As per International Council for Harmonisation (ICH) guideline Q2(R1), the developed technique was validated through studies on linearity, range, precision, specificity, accuracy, & determination of LOD & LOQ.

1. Linearity and range

Standard solutions of kaempferol (15–35 µg/mL) and dexamethasone (10–60 µg/mL) were examined to evaluate the linearity and range of the proposed method.

2. Precision Study (validation for method during analysis)

Both intra-day & inter-day precision were conducted in accordance with the statistical standard to support the method's reproducibility **[12]**. The intraday & interday precision were performed by analyzing the samples (kaempferol and dexamethasone) solutions at 20 µg/mL concentrations.

- Intra Day Assay (validation for method during analysis)

The assay process was performed at a fixed concentration for two to three hours on the same day, and the outcomes were compared.

- Inter Day Assay (validation for method during analysis)

After a 24-hour period at a fixed concentration, the test process was conducted, and the outcomes were compared.

3. Ruggedness study (validation for method during analysis)

The assay process was performed the next day for a total of twenty-four hours at a fixed concentration, and the obtained results were evaluated and compared **[12]**.

4. Robustness study (validation for method during analysis)

By changing the temperature, the same process was repeated to assess the robustness, and the outcome was compared to the same earlier methods **[12].**

5. Specificity:

The specificity of the analytical method was assessed by recording the spectra of both the standard & kaempferol and dexamethasone solutions used in the experiment.

6. Accuracy:

The accuracy assessment of the proposed spectrophotometric approach was evaluated using the standard addition technique. Precisely weighed amounts of pure kaempferol & dexamethasone (80%, 100%, and 120% of the prequantified concentration) were spiked into the previously analyzed sample solutions containing 8, 10, and 12 µg/ml, respectively. The absorbance data were recorded at 366 nm for kaempferol and 238 nm for dexamethasone. The % recovery and %RSD were measured to assess method accuracy. The results demonstrated satisfactory recovery values, confirming the reliability and reproducibility of the developed analytical procedure **[13-14].**

7. Limit of detection (LOD)

The Limit of Detection (LOD) represents the minimum analyte concentration that can be produces a detectable response but not be accurately quantified with precision **[11]**.

According to ICH guideline Q2 (R1), the LOD was assesses using the signal-to-noise ratio method, as per the following equation:

$LOD = 3.3 \times (\sigma / S)$

where σ represents the standard deviation of the y-intercepts of the regression line, and S denotes the slope of the calibration curve.

8. Limit of quantification (LOQ)

The Limit of Quantitation (LOQ) represents the minimum analyte concentration that can be quantitatively determined with acceptable accuracy & precision **[11]**. According to ICH guideline Q2 (R1), the LOQ was determined using the following equation:

$LOQ = 10 \times (\sigma / S)$

Where

σ represents the standard deviation of the y-intercepts of the regression line

S denotes the slope of the calibration curve

RESULTS

Absorption maxima of Kaempferol and dexamethasone

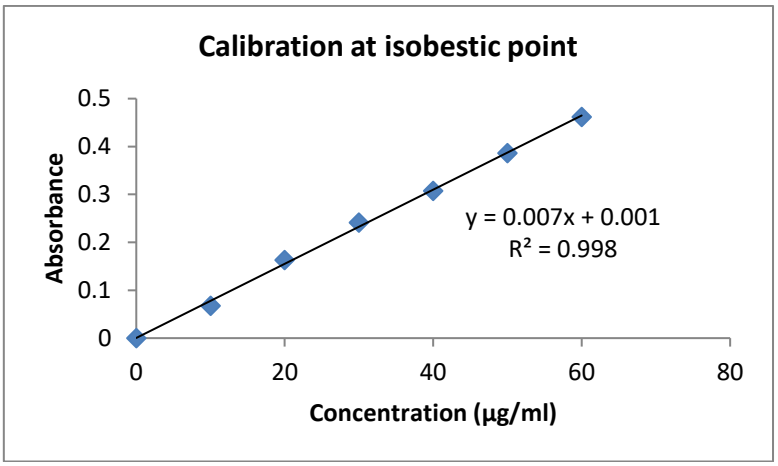
A UV-visible spectrophotometer (Model 1700, Shimadzu) was utilised to measure the λmax (absorption maximum) of the substances. The λmax of kaempferol was found to be 366.0 nm, which falls within the specified drug's standard limits. Similarly, the λmax of dexamethasone was recorded at 238.0 nm, also conforming to the drug specification requirements. These values ascertain the identity, chemical integrity, & purity of the respective compounds.

Determination of Iso-bestic point and selection of suitable Wavelength

From the overlain spectra, dexamethasone exhibited a λmax at 238 nm, while kaempferol showed a λmax at 366 nm. An isoabsorptive point was identified at 262 nm was determined from the overlapping spectra and was used for the simultaneous assessment of both analytes.

Table 1: Calibration curve at isobestic point

Concentration (µg/ml)	Absorbance at 262 nm
10	0.067
20	0.163
30	0.241
40	0.307
50	0.386
60	0.461



Graph 1: Calibration graph at isobestic point (262nm)

Simultaneous-estimation:

Table 2: Absorptivity of Kaempferol and dexamethasone

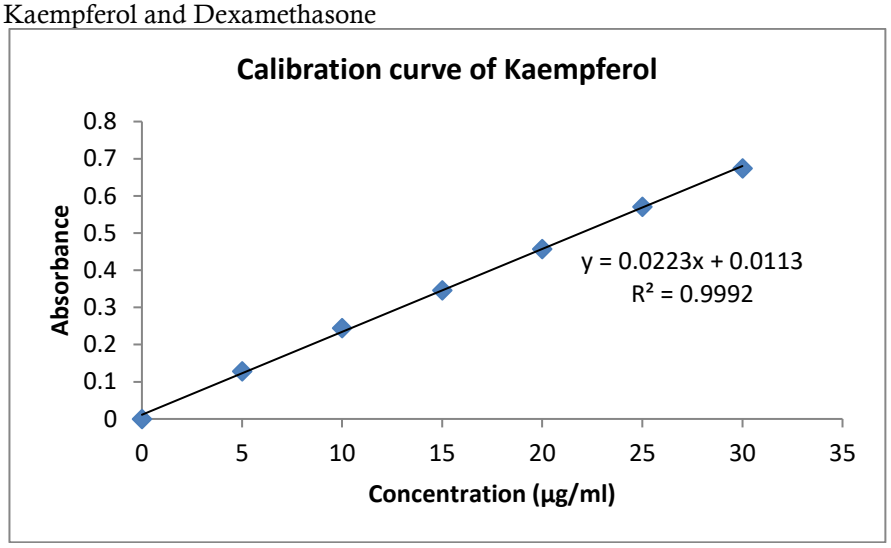
Name of drug	Concentration (ppm)	Absorbance		Absorptivity (Abs/Conc)			
		ʎ = 366 nm	ʎ = 238 nm	ʎ 1 =366 nm		ʎ 2 = 238 nm	
Kaempferol	5	0.128	0.116	0.025		0.023	
	10	0.244	0.237	0.024		0.023	
	15	0.345	0.363	0.023		0.024	
	20	0.457	0.429	0.022		0.021	
	25	0.571	0.538	0.022		0.021	
	30	0.674	0.645	0.022		0.021	
	Mean			ax1	0.023	ax2	0.022
Dexamethsone	10	0.121	0.155	0.012		0.015	
	20	0.23	0.295	0.011		0.014	
	30	0.326	0.42	0.010		0.014	
	40	0.473	0.549	0.011		0.013	
	50	0.631	0.683	0.012		0.013	
	60	0.717	0.852	0.011		0.014	
	Mean			ay1	0.011	ay2	0.014

Linerity and range:

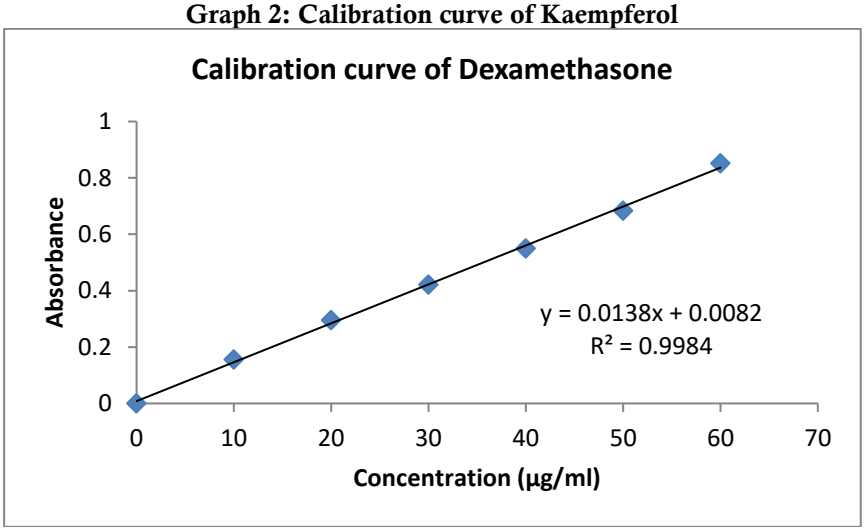
The linearity of kaempferol and dexamethasone was evaluated using the least-squares regression method, demonstrating acceptable accuracy in compliance with ICH-guidelines. Linearity for both drugs was confirmed through regression analysis across the tested concentration range. The correlation coefficients (r^2) for kaempferol and dexamethasone were found to be 0.998 and 0.999, respectively, indicating excellent linearity and reliability of

the developed method. The regression analysis found were y=0.022x + 0.011 for kaempferol & y=0.013x + 0.008 for dexamethasone, as shown in the calibration graph.

Standard curve of Kaempferol and Dexamethasone



Graph 2: Calibration curve of Kaempferol



Graph 3: Calibration curve of Dexamethasone

Precision study

- Intraday & Interday Precision study

The precision of the proposed analytical approach was established by calculating the percentage RSD of replicate measurements. Results from three separate days were compared to evaluate intermediate precision. Intra-day (repeatability) and inter-day (intermediate) precision were evaluated and found to have average percentage RSDs of 1.98% and 1.8%, respectively. These numbers fall within acceptable bounds, demonstrating the precision and reproducibility of the process under normal operating circumstances.

Table 3: Intraday & Interday Precision of Kaempferol

Concentration (µg/ml)	Intraday Precision of Kaempferol		Inter-day Precision of Kaempferol	
	Mean±SD (n=3)	% RSD	Mean±SD (n=3)	% RSD
20	0.419±0.008	1.928	0.436±0.003637	1.813

The %RSD was utilised to express the developed method's precision. Comparing the outcomes from three separate days allowed for the evaluation of intermediate precision. Intra-day (repeatability) & inter-day (intermediate) precision were found to have experimental %RSD values of 1.02% and 0.960%, respectively. The method's great precision and reproducibility under the studied conditions are indicated by these low percentage RSD values.

Table 4: Intraday & Interday Precision of Dexamethasone

Concentration (µg/ml)	Intraday Precision of dexamethasone		Inter-day Precision of dexamethasone	
	Mean±SD (n=3)	% RSD	Mean±SD (n=3)	% RSD
20	0.294±0.00102	1.0288	0.294±0.002128	0.960

Ruggedness

By comparing the absorbance values obtained by two separate analyzers in the same lab, the ruggedness of the suggested procedure was assessed. The study was conducted at a concentration of 20µg/mL for both kaempferol & dexamethasone.

For kaempferol, the SD values were 0.002483 for Analyst 1 and 0.001633 for Analyst 2, with corresponding %RSD values of 0.55% and 0.366%, respectively.

For dexamethasone, the SD values were 0.002658 for Analyst 1 and 0.003189 for Analyst 2, with %RSD values of 0.90% and 1.07%, respectively.

Table 5: Ruggedness of Kaempferol and dexamethasone

Concentration (µg/ml)	Ruggedness of Kaempferol			Ruggedness of Dexamethasone		
	Analyst	Mean±SD (n=6)	% RSD	Analyst	Mean±SD (n=6)	% RSD
20	Analyst-1	0.446167±0.002483	0.557	Analyst-1	0.294667±0.002658	0.9021
20	Analyst-2	0.446±0.001633	0.366	Analyst-2	0.295833±0.003189	1.0778

Robustness

In order to assess the robustness of the suggested procedure, the impact of minor deliberate changes to the analytical temperature was examined. The analysis was performed at a drug concentration of 20µg/mL, with measurements taken at 25°C and 30°C.

For kaempferol, the standard deviation values were 0.001871 at 25°C and 0.001169 at 30°C, with corresponding percentage RSD values of 0.42% & 0.26%, respectively.

For dexamethasone, the standard deviation values were 0.00258 at 25°C and 0.00288 at 30°C, with percentage RSD values of 0.87% & 0.97%, respectively.

Table 6: Results showing robustness of Kaempferol and dexamethasone

Concentration (µg/ml)	Robustness of Kaempferol			Robustness of Dexamethasone		
	Temperature °C	Mean±SD (n=6)	% RSD	Temperature °C	Mean±SD (n=6)	% RSD
20	Temperature 25^0C	0.4455±0.001871	0.42	Temperature 25^0C	0.29367±0.00258	0.879
20	Temperature 30^0C	0.445833±0.001169	0.262	Temperature 30^0C	0.29467±0.00288	0.976

Accuracy:

The accuracy of the proposed approach was assessed by performing recovery studies at three concentration levels—80%, 100%, & 120%. The percentage recovery of kaempferol and dexamethasone was calculated at each level. The recoveries for kaempferol were found to be 95.125%, 99.6%, and 99.388%, while those for dexamethasone were 99.458%, 94.066%, and 99.333%, respectively. The obtained results are summarized in the table below.

Table 7: Accuracy data of developed method

Drug	Level %	Amount added (µg/ml)	Total amount recovered (µg/ml)	% RSD	% Recovery ± SD
Dexamethsone	80	8	7.61	1.167	95.125 ± 0.088
	100	10	99.6	1.416	99.6 ± 0.147
	120	12	11.926	1.383	99.388 ± 0.165
Kaempferol	80	8	7.956	1.805	99.458 ± 0.143
	100	10	9.406	1.166	94.066 ± 0.109
	120	12	11.92	1.235	99.333 ± 0.147

Table 8: Following table summarizes the data employed for the validation of the proposed parameters:

Parameter	Kaempferol	Dexamethasone
λmax (nm)	366 nm	238 nm
Linearity range (µg/mL)	5-30 µg/mL	10-60 µg/mL
Specificity	Specific	Specific
Linearity equation	Y = 0.022x = 0.011	Y = 0.013x + 0.008
Correlation coefficient	0.999	0.0998
Slope (b)	0.022	0.013
Intercept (a)	0.011	0.008
Intra day (% RSD)	2.559	1.028
Inter day (% RSD)	1.813	0.960
Accuracy (% recovery)	95-99.4	94-99.5
LOD	0.750	1.015
LOQ	2.272	3.076

As per ICH recommendations, the LOD & LOQ were estimated for both analytes. Kaempferol exhibited a detection limit of 0.750 µg/mL & a quantification limit of 2.272µg/mL, while dexamethasone showed respective values of 1.015µg/mL & 2.076µg/Ml

These findings demonstrate that the developed analytical approach is reliable and effective for the quantitative estimation of kaempferol and dexamethasone in their respective concentration ranges.

CONCLUSION

In this work, a novel UV–Visible spectrophotometric analytical procedure for simultaneous-estimation of dexamethasone & kaempferol is developed and validated. The method is simple, cost-effective, & does not require sophisticated instrumentation or complex sample preparation. The results revealed good linearity, precision, accuracy, and robustness, in compliance with ICH Q2(R1) guidelines.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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