Development and Validation of UV Spectrophotometric Method for the Estimation of Kaempferol in Kaempferol: Hydrogenated Soy PhosphatidylCholine (HSPC) Complex

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ABSTRACT

Introduction: Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a natural flavonoid belongs to a subcategory of flavonol family. The kaempferol – hydrogenated soy phosphatidylcholine (HSPC) complex was obtained by refluxing and freeze drying method. Ultra violet (UV) – visible spectrophotometric method has been developed for the determination of kaempferol in kaempferol – HSPC complex. **Objective:** A validated UV – visible spectrophotometric method for determination of kaempferol in Kaempferol – HSPC complex. **Materials and Methods:** The Kaempferol – HSPC complex (Phytosomes) were prepared by dissolving both kaempferol and HSPC in 1, 4 – dioxane for refluxing up to 2 h and freeze dried. The spectrophotometric detection of kaempferol was done at absorption maximum (λ_{max}) of 365 nm and 265 nm using methanol as solvent. The developed method was validated as per ICH guidelines. **Result:** The kaempferol content in Kaempferol – HSPC complex was found to be 79.32% and 79.19% at 365 nm and 265 nm. Kaempferol demonstrated good linearity in concentration range of 2-12 µg/ml ($r^2 > 0.99$) at 365 nm and 2-14 µg/ml ($r^2 > 0.99$) at 265 nm. Precision and mean recoveries were found to be in the range of (%RSD 0.0957 and 0.0580) and (% RSD 0.1461 and 0.0959) and 99.70% and 91.85% at 365 nm and at 265 nm. Limit of detection and limit of quantification were found to be (0.015 µg/ml and 0.0191 µg/ml) and (0.0457 µg/ml and 0.0579 µg/ml) respectively. **Conclusion:** The developed method was found to be simple, specific, economic, reliable, accurate, precise, reproducible and used as a quality control tool for analysis of Kaempferol.

Keywords: Freeze drying, hydrogenated soy phosphatidylcholine, Kaempferol, method validation, UV – visible spectrophotometer

INTRODUCTION

Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), a yellow compound with a low molecular weight (MW: 286.2 g/mol), is a common natural flavonoid which representative of the subcategory of flavonol. This flavonoid is abundant in many plant-derived foods and traditional medicine.[1] It is widely distributed in the plant kingdom such as onion, kale, endive and tea along with formed as secondary metabolites through the phenylpropenoid biosynthetic pathway.[2] Although it has broad spectrum importance, researcher have been isolated it from different plants like *Asclepias cyriaca* L.,[3] *Crocus sativus* L.,[4] *Cassia alata* L.,[5] *Capsella bursa-patoris* L.,[6] *Leptadenia pyrotechnica* L.,[7] and *broccoli*[8] and also reported its various pharmacotherapeutic effects like anticancer,[7] antioxidant,[9,10] anti-inflammatory[11] and hepatoprotective[12] etc. kaempferol revealed low to moderate absorption, which results poor bioavailability ~2%.[13] It is hydrophobic in nature and freely soluble in methanol, 1, 4 – dioxane, Ethanol and dimethylformamide.[14,15] In a wide range of HPLC and HPTLC techniques were reported for estimation of kaempferol in extracts,[16-20] gingko biloba tablets,[21,22] phytosome formulation[23] (Kaempferol – phospholipids complex) at around 360 nm. Literature survey revealed that no simple UV – visible method has

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been reported for estimation of kaempferol in phytosome formulations.

MATERIALS AND METHODS

Materials

Phospholipion 90 H (Hydrogenated Soy Phosphatidylcholine) was received as gift sample from lipoid GmbH, Germany. Kaempferol standard was purchased from imam international group (Pharmaceutical) Co., Ltd, China. All other chemical used were of either pharmaceutical or analytical grade.

Preparation of kaempferol – HSPC complex

The complex was prepared with Kaempferol and HSPC at a molar ratio of (1:1 w/w). Weighed amount of Kaempferol (mol. wt., 286.2) and HSPC (mol. wt., 790) were taken in 100 ml round bottom flask and 20 ml of 1, 4-dioxane was added. The mixture was refluxed at a temperature not exceeding 50°C for 2 h to get a clear solution. The obtained solution was freeze dried. The resultant Kaempferol complex (yield 92% w/w) was kept in an amber colored glass bottle flushed with nitrogen and stored at room temperature.

Method development

Instrument

Double beam UV – visible spectrophotometer (JASCO V-630).

Preparation of standard stock solution

To find out the wavelength maximum absorption (λ_{max}) of kaempferol, the standard stock solution (1000 µg/ml) of kaempferol was prepared by weighing accurately 5 mg of pure drug into 5 ml volumetric flask and dissolved with a minimum quantity of methanol and final volume was made up to mark with methanol.

Preparation of working stock solution

The working stock solution of kaempferol (100 µg/ml) was prepared by diluting 1 ml of standard stock solution to 10 ml with methanol.

Selection of (λ_{max})

The working stock solution was further diluted with methanol to get (10 µg/ml) concentration (1ml to 10 ml). This solution was scanned between the wavelength regions of 200-400 nm against methanol as blank. The UV spectra were shown in [Figure 1] and absorption curve showed two characteristics absorption maxima at 365 nm and 265 nm. Hence both (λ_{max}) were selected for analysis of kaempferol.

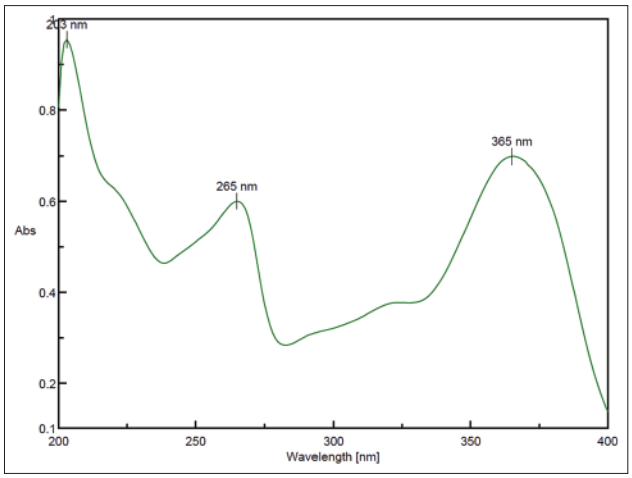

Figure 1. UV spectrum of kaempferol in methanol.

From working stock solution, a series with a concentration range of 2-12 µg/ml at 365 nm and 2-14 µg/ml at 265 nm for preparation of the calibration curve were obtained by further dilution of stock solution with methanol.

Percent drug content

To determine the content of kaempferol in kaempferol – HSPC complex, the complex equivalent to 5 mg of kaempferol was weighed and transferred it to 5 ml volumetric flask; 2 ml of methanol was added, sonicated for 10 min and volume was adjusted to 5 ml using methanol. The solution was filtered through Whatman filter paper No.1; from this filtrate 1 ml was transferred to 10 ml volumetric flask and diluted with methanol to 10 ml to get the concentration of (100 µg/ml). The (100 µg/ml) solution was further diluted with methanol to get the final concentration of (10 µg/ml). The absorbance was measured at 365 nm and 265 nm. The same procedure was followed for making the dilution of HSPC and it is used as blank. The analysis was repeated six times. The absorbance was mentioned in [Table 1].

Validation of the analytical method

The developed analytical method was validated as per ICH guidelines and prepared different series of diluted solutions (2-12 µg/ml) and (2-14 µg/ml) were analyzed for linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The linearity for this method at various concentrations of the range between 2-12 µg/ml and 2-14 µg/ml were analyzed at 365 nm and 265 nm [Tables 2, 3]. The absorbance v/s concentration plot for kaempferol was found to be linear in [Figures 2, 3].[24]

Table 1: Estimation of Kaempferol in Standard Kaempferol, Kaempferol-HSPC complex

λ_{max}	Formulation	Concentration (µg/ml)	Absorbance (nm)	Practical yield	% purity
365	Standard kaempferol	10	0.7422	9.8567	98.56
265	Kaempferol-HSPC complex	10	0.5938	7.9244	79.32
Standard kaempferol	Standard kaempferol	10	0.6791	9.9143	99.14
Kaempferol-HSPC complex	Kaempferol-HSPC complex	10	0.5424	7.8241	79.19

HSPC: Hydrogenated soy phosphatidylcholine

Table 2: Data for calibration curve of kaempferol at 365 nm

Concentration (µg/ml)	Absorbance at 365 nm
2	0.1328
4	0.2969
6	0.4552
8	0.5977
10	0.7422
12	0.9126

Table 3: Data for calibration curve of kaempferol at 265 nm

Concentration (µg/ml)	Absorbance at 265 nm
2	0.1542
4	0.2949
6	0.4334
8	0.5551
10	0.6791
12	0.8126
14	0.9480

Precision

The precision studies were performed by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was determined by analyzing 10 µg/ml of kaempferol at three different time points within a day. Inter-day precision was determined by analyzing same concentration of solutions at three different points for three days, and average % RSD was calculated.[24]

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy was evaluated by performing recovery studies by spiking pre – analyzed sample solutions of of kaempferol with three different concentrations of 8 µg/ml, 10 µg/ml and 12 µg/ml and % recovery was computed.[24]

LOD and LOQ

LOD is the lowest concentration of analyte in a sample that an analytical process can reliably differentiate from background levels, and LOQ is the lowest concentration of the calibration curve that can be measured with an acceptable accuracy and precision. In this method, LOD and LOQ were based on a standard deviation of the response and slope of the calibration curve using following equations.[24]

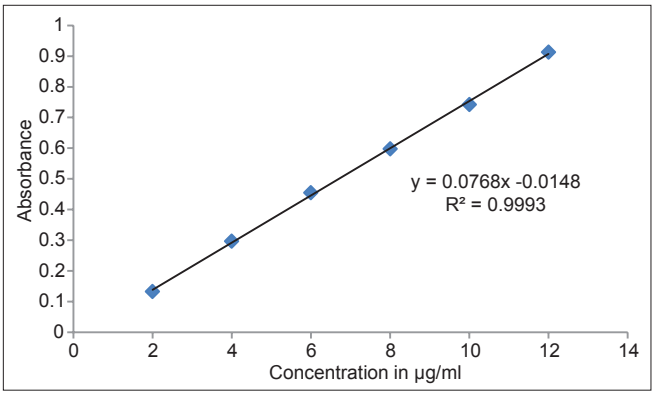

Figure 2. Calibration curve of kaempferol at 365 nm.

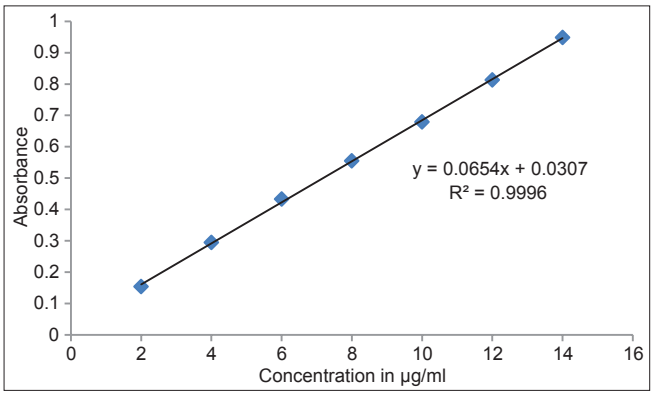

Figure 3. Calibration curve of kaempferol at 265 nm.

$$LOD = 3.3\ S/M;\ LOQ = 10\ S/M$$

Where S is the standard deviation of the absorbance of the sample and M is the slope of the calibration curve.

RESULT AND DISCUSSION

The kaempferol was found to be soluble in methanol. The absorption maximum (λ_{max}) was found to be 365 nm and 265 nm as shown in [Figure 1]. The content of kaempferol in Kaempferol – HSPC complex was found to be 79.32% and 79.19% at 365 nm and 265 nm. The good linearity was found to be within concentration range of 2-12 µg/ml

Table 4: Results of intra- and inter-day precision (n=6)

λ_{max}	Concentration (µg/ml)	Intra-day		Inter-day	
		Absorbance (nm)±SD	% RSD	Absorbance (nm)±SD	% RSD
365 nm	10	0.7796±0.0002	0.0957	0.7796±0.0002	0.1461
	10	0.7807±0.0014		0.8328±0.0024	
	10	0.7993±0.0005		0.8917±0.0010	
265 nm	10	0.7166±0.0003	0.058	0.7166±0.0003	0.0959
	10	0.7203±0.0006		0.7552±0.0011	
	10	0.7115±0.0002		0.8080±0.0007	

RSD: Relative standard deviation

Table 5: Recovery study of kaempferol

λ_{max}	Level of recovery (%)	Amount spiked recovery (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean recovery
365 nm	80	8	7.85	98.12	99.70
	100	10	10.15	101.50	
	120	12	11.94	99.50	
265 nm	80	8	7.17	89.20	91.85
	100	10	9.35	93.50	
	120	12	11.09	92.42	

Table 6 Validation parameters

Parameters	Results	
λ_{max}	365 nm	265 nm
Beer's law range (µg/ml)	2-12	2-14
Correlation coefficient (r^2)	0.9993	0.9996
Slope (m)	0.0768	0.0654
Intercept (c)	0.0148	0.0307
Accuracy	98.12-101.5%	89.62-93.5%
Precision (%RSD)		
Intra-day	0.0957	0.0580
Inter-day	0.1461	0.0959
LOD (µg/ml)	0.015	0.0191
LOQ (µg/ml)	0.0457	0.0579

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

and 2-14 µg/ml [Tables 2, 3] with correlation coefficient (r^2) >0.99 and regression equation of the curve was found to be y = 0.0768x − 0.0148 at 365 nm and y = 0.0654x + 0.0307 at 265 nm. The precision (intra-day and inter-day) data represents [Table 4] good reproducibility with % RSD lower than 2.0% which assured that method is précised. Mean recovery value [Table 5] at different concentrations was found to be higher than 90%, indicates accuracy of the method. LOD and LOQ for Kaempferol were reported [Table 6] and were found to be 0.015 µg/ml and 0.0457 µg/ml and 0.0191 µg/ml and 0.0579 µg/ml at 365 nm and 265 nm respectively.

CONCLUSION

The developed method was found to be simple, specific, economic, reliable, accurate, precise, and reproducible used as a quality control tool for analysis of Kaempferol in phytosomes formulations.

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