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Rapid UV/Visible Spectrophotometric Method for Hesperidin Estimation in Pharmaceutical Dosage Forms and Proniosomes

Research Article

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Abstract

A sensitive accurate UV/Visible spectrophotometric method was developed to determine hesperidin concentration through simple detection at low cost using rapid analysis. The analytical system reached ICH guideline standard validation which delivered both precision and accuracy results. The defined solution of methanol and phosphate buffer at pH 6.8 (30:70) established the best conditions for drug analysis. A method for determining maximum absorption wavelength demonstrated a value of 285 nm with its linear relationship represented by the equation y = 0.253x + 0.0138. The method displayed linear performance from 6-30 µg/ml with an R^2 value of 0.9987 which verifies its precise functioning. The analytical method achieved ICH guideline compliance for linearity, precision, specificity, robustness, ruggedness, accuracy and the determination of limit of detection (LOD-0.25 µg/ml) and limit of quantification (LOQ-0.78 µg/ml). Analysis of Hesperidin in proniosomes through the coacervation phase separation method was achieved successfully using UV/Visible spectrophotometry. The validation results showed that this method stands out for its high selectivity in addition to its precise and reproducible approach which makes it appropriate for hesperidin quality control in pharmaceutical monitoring of both bulk substances and formulated preparations. Through proniosomal formulation hesperidin gains higher stability along with improved delivery capabilities when applied topically. The validated approach provides medical laboratories with a both pragmatic and precise method to analyze hesperidin content in pharmaceutical settings.

Keywords: Hesperidin, UV- spectroscopic method validation, Proniosomes, ICH guidelines.

Introduction

Acne vulgaris is an inflammatory skin disorder of pilosebaceous unit with moderately high prevalence in adolescents and adults. The Global Burden of Disease study reveals that 85 percent of the population are hit by this disease. Moreover, the research findings of Global burden of disease state that acne ranks as the eighth most common disease worldwide (1).

Hesperidin exists naturally as a plant-based bioflavonoid substance which primarily occurs in citrus fruits originating from oranges and lemons and grape fruits. Hesperidin represents a natural phytochemical which resides within the flavonoid group and researcher teams obtain it primarily from citrus peel materials. The production process includes isolation and purification of natural hesperidin which serves both pharmaceutical and cosmetic industries (2).

Hesperidin (2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)7[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one (Figure 1).

Hesperidin has been studied for their antioxidant and anti-inflammatory activities since its isolation in 1920 and has been identified and isolated in it for the first time (3).

The use of hesperidin can cause skin irritation, redness and rash. One of the herbal phytoconstituent Hesperidin helps to maintain and repair the skin barrier, and also inhibits breakouts of acne. Flavonoids are free radical scavengers that help protect the skin's fragile, delicate layers from oxidative damage found in this multi active substance (4).

Dry formulations of water soluble carrier particles coated with surfactant, proniosomes, have been studied. Proniosomal gels are liquid crystalline (gel) vesicular structures consisting of non-ionic surfactant with the entrapment capacity of both hydrophilic and lipophilic drugs. Produced from non-ionic surfactants having the ability to entrap both hydrophilic and lipophilic drugs (5). Formulating medication proniosomes has the advantage of increasing bioavailability while minimizing adverse effects, as well as improving drug penetration through the skin (6). Topical treatment of acne was improved utilizing an innovative drug delivery system of proniosomes. This allows for an improved encapsulation of, and release of, active ingredients to promote skin penetration and efficacy (7).

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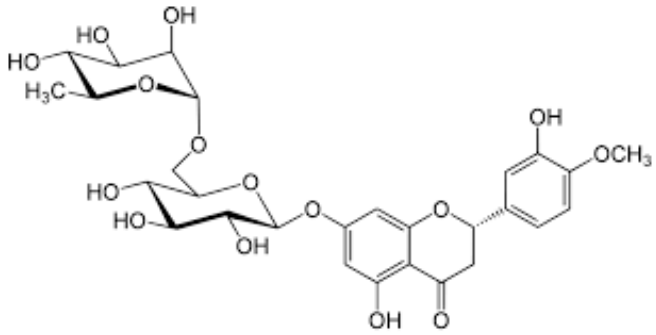
It has been published in the literature using different methods for estimation hesperidin such as UV spectrophotometric (8), HPLC (9), and HPTLC (10), LC-MS/MS (11). The available methods in the literature have their own drawbacks e.g. it takes more time, is very expensive, has high operational costs, and lower sensitivity as compared to the listed methods. In this study an attempt was made to develop and validate a simple, rapid, sensitive, accurate, and precise UV spectrophotometric technique for Hesperidin (12).

Figure 1: molecular structure of Hesperidin



Materials and Methods

Chemicals and Solvents

Otto Chemie Pvt Ltd., of Mumbai, Maharashtra, was the source of Hesperidin (Pure drug). The experiment was carried out in analytical purity and all the chemicals and reagents used were from a storehouse maintained at the KLE college of Pharmacy in Belagavi. HPLC grade methanol was used.

Instrumentation and apparatus

The Shimadzu UV/Visible spectrophotometer with software UV-Probe, model 1900, and the Shimadzu UV-1800 with UV probe were used to measure Hesperidin.

Method Development

At the first stage of developing the UV spectrophotometric method we select the solvent system and measured the wavelength of maximum wavelength (λ max) of the solvent for absorption of UV light. A thorough review of the literature was followed by practical solubility studies of Hesperidin. Expression in several solvents (methanol, ethanol, Phosphate buffer pH 6.8, dimethyl sulfoxide) as well as on the mobile phase was investigated. Finally, a solvent mixture combining Methanol and Phosphate buffer pH 6.8 (30:70% v/v) was completed. For single peak UV spectra of solutions containing analytes, a solution search was made in the 400 - 200 nm region (13).

Method Validation

An UV Spectrophotometric technique for Hesperidin development and validation according to the ICH criteria was done considering factors of linearity, specificity and selectivity, accuracy, robustness, ruggedness, solution stability, LOD, and LOQ (14,15,16).

Preparation of Solvent system

Mobile phase

Phosphate buffer pH 6.8 was prepared by dissolving 0.69g of potassium dihydrogen phosphate and 1.75g of sodium hydrogen phosphate in 100 mL distilled water. It was prepared that 30 ml of methanol had to be measured correctly and dissolve into 70 ml of prepared phosphate pH buffer.

Stock Solution Preparation

Hesperidin was weighed carefully at 10mg and placed into a 10 mL volumetric flask. The volume in flasks was adjusted to the mark using methanol: phosphate buffer, resulting in a concentration of 1000µg/ml for the primary stock solutions. 1 ml of Hesperidin solution was drawn into 10 ml volumetric flasks from the aforementioned primary stock solution and adjusted to the mark with methanol : phosphate buffer. This method produced a secondary stock solution containing 100 µg/ml of the drug.

Selection of wavelength

Hesperidin at 400-200 nm were tested for in a stock solution of 10µg/ml in methanol and buffer as blanks.

Specificity and selectivity

UV spectra between 400-200 nm were produced by scanning both the mobile phase and drug solutions in order to determine the identity of an analyte among a sample's combination of similar components.

Linearity and range response

Hesperidin linearity ranges were measured in triplicate over the 6–30 µg/ml dose in which absorption was measured at 285 nm using the standard stock solution. Abundance was plotted against absorbance and a linear regression equation was fit (17).

Precision

- System precision: The concentration of 18 (µg/ml). Hesperidin concentrations of 18µg/ml were created in six duplicates from the stock solution, analysed, and %RSD was determined (18).
- Intraday precision: A same day study involving the generation of Hesperidin concentrations of 6, 18 and 30 µg/ml, analysis, and %RSD calculations at various time intervals was carried out.
- Interday precision: Hesperidin concentrations of 6, 18, and 30µg/ml from stock solutions were made, analysed, and %RSD was computed over three days.

Ruggedness

Ruggedness was determined by changes in the analyst as well as the UV instrument. Stock solution of 2.24mg/ml of hesperidin was prepared and used to prepare concentrations of 6, 18, and 30 µg/ml, which were measured absorption at 285 nm by different analysts. The same concentration was performed on a different instrument (UV Spectrophotometer) Analysed and % RSD were calculated (19).

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Robustness

The robustness parameter was accomplished by changing the wavelength. Hesperidin concentrations of 6, 18, and 30µg/ml were generated in triplicate from the stock solutions. Absorption at 283nm and 287nm was measured and analysed, and %RSD was determined (20).

Solvent and standard stock solution stability

The stability of solvent and standard stock solutions was demonstrated by preparing a fresh stock solution. To analyse, a fresh stock solution was produced, followed by dilutions of 6, 18, and 30µg/ml. The absorbance was compared to that of the old stock dilutions, and the percent RSD was computed.

Accuracy

An analysis was evaluated by a recovery study to determine accuracy. It involved analyzing three concentration levels of the standard solutions of the drug (50%, 100% and 150%). drug at three distinct concentration levels (50%, 100%, and 150%) (21).

Limit of Detection (LOD) and Limit of Quantification and (LOQ)

LOD and LOQ were derived from linear curve with the following formulae (22).

$$LOD = \frac{3.3 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}} \quad \text{Equation -1}$$

$$LOQ = \frac{10 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}} \quad \text{Equation-2}$$

Preparation of Hesperidin Loaded Proniosomal gel

Different surfactants from span and tween were used with a coacervation phase separation method with some modification for topical application to prepare proniosomes. The surfactant and cholesterol dissolved with absolute ethanol in a beaker and 20mg Hesperidin was then added to the mix. The beaker was then put in a water bath (55–60°C) for a few min while shaking until the complete dissolution of cholesterol. In the water bath, a solution was obtained by warming up in the hot distilled water (55-60°C) for 3-5 min till clear or translucent solution. It was allowed to cool to room temperature to allow the dispersion to convert to gel (23,24).

Hesperidin loaded Proniosome characterization

The mean particle size, poly dispersibility index and zeta potential values for Hesperidin were characterized using Nano ZS ZS90 (Malvern Instruments, UK).

Encapsulation efficiency

The Hesperidin samples were centrifuged (High Speed Refrigerated Centrifuge, Floor Model, 7000 Kubota, Japan; at 19000 rpm for 60min. Hesperidin content in proniosomes was determined by UV/Visible spectroscopy analysis of supernatant and the supernatant was collected, diluted and run through UV / Visible spectroscopy analysis. Following equation was used to calculate % EE.

$$\% EE = \frac{\text{Total entrapped drug} - \text{Amount of the drug in supernatant}}{\text{Total entrapped drug}} \times 100$$

Results and Discussion

Method development

In order for analytes to be able to dissolve in both solvents, they must accomplish simultaneous estimation. This technique was performed in analytical grade methanol and phosphate buffer pH 6.8 solvent. table 1 shows illustrative results of method development parameters.

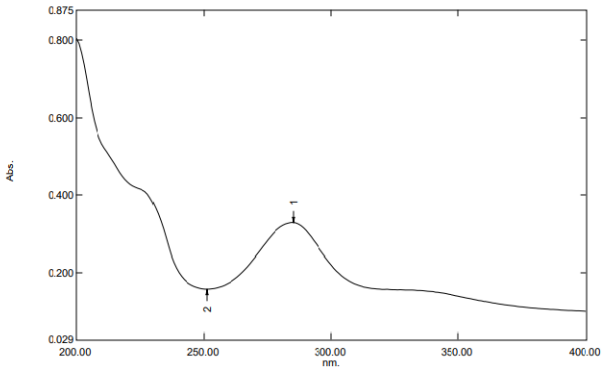
Table 1: Method development parameters

Sr.no	Parameters	Specifications
1	Analytes	Hesperidin
2	Solvent	Methanol and Phosphate Buffer PH (6.8) (30:70)
3	Maximum absorbance in solvent	285nm
4	Instrument	UV Spectrophotometer Shimadzu 1800

Method Validation

Hesperidin showed maximum absorption at 285 nm, respectively.

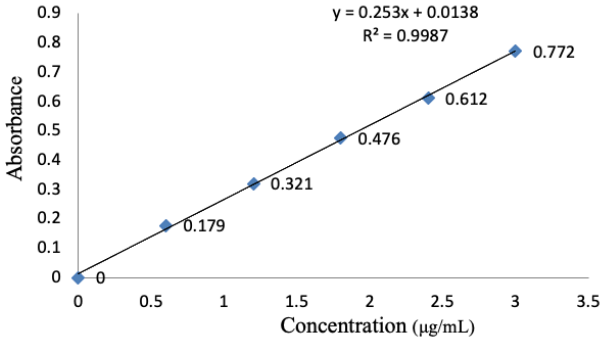
Figure 2: UV Spectrum of Hesperidin



Linear range response

The investigation established a linear connection between drug concentration and absorbance for Hesperidin, with values ranging from 6 to 30µg/ml. This was true for the analysed wavelengths (285 nm). The finding is backed by a strong correlation coefficient (R^2) of 0.9987 for Hesperidin in figure 3.

Figure 3: Standard calibration curve of Hesperidin



Limit of Detection and Limit of Quantification (LOD and LOQ)

This study assessed two critical parameters for our analytical method: Limit of detection and Limit of quantification. The LOD and LOQ for Hesperidin were determined to be **0.25 µg/ml** and **0.78 µg/ml** respectively.

Precision

The analysis approach showed good precision, as the percent relative standard deviation (%RSD) computed for three repeated measurements (replicates) of Hesperidin at three distinct doses was consistently less than 2%. Detailed findings are reported in table 2 &3 and 4.

Table 2: System precision (*n=6)

Sr. no	Concentration (µg/ml) Hesperidin	Absorbance at 285
1	18	0.469
2	18	0.470
3	18	0.471
4	18	0.476
5	18	0.475
6	18	0.475
%RSD		0.63%

Table 3: Interday Precision (*n=3)

Concentration (µg/ml)	Hesperidin (285 nm)	Hesperidin (285 nm)	Hesperidin (285)
	Day 1	Day 2	Day 3
Hesperidin	**Absorbance**		
6	0.197	0.190	0.187
%RSD	0.50%	1.04%	0.61%
18	0.478	0.483	0.479
%RSD	0.12%	0.41%	0.20%
30	0.773	0.786	0.790
%RSD	0.12%	0.12%	0.12%

Table 4: Intraday precision (*n=3)

Concentration (µg/ml)	Hesperidin (285nm)	Hesperidin (285 nm)	Hesperidin (285 nm)
	Morning	Afternoon	Evening
Hesperidin	**Absorbance**		
6	0.184	0.188	0.192
%RSD	0.31%	0.30%	0.52%
18	0.477	0.475	0.470
%RSD	0.31%	0.21%	0.21%
30	0.773	0.780	0.791
%RSD	0.21%	0.07%	0.07%

Ruggedness

The Ruggedness of Method was determined using the %RSD on Hesperidin under variations in different Analysts and Instruments. Data for the %RSD ranged from 0-2% for all replicates signifying that the present method is precise and can easily be replicated. These findings are described in the table 5.

Table 5: Ruggedness (Change in analyst and Change in Instrument)

Concentration (µg/ml)	Hesperidin (285 nm)	
	Change in Analyst	Change in Instrument
Hesperidin	**Absorbance**	
6	0.210	0.223
%RSD	1.91%	1.34%
18	0.477	0.508
%RSD	0.20%	0.39%
30	0.786	0.819
%RSD	0.25%	0.14%

Robustness

Varying the detector wavelength was done for the estimation of method robustness table 6. Change of Mobile phase have developed the estimation of Hesperidin at 283nm and 287nm table 7.

Table 6: Change in Wavelength (*n=3)

Concentration (µg/ml)	Hesperidin (283 nm)	Hesperidin (287 nm)
Hesperidin	**Absorbance**	
6	0.217	0.214
%RSD	0.92%	0.22%
18	0.476	0.473
%RSD	0.32%	0.24%
30	0.791	0.790
%RSD	0.38%	0.19%

Table 7: (Change in Mobile phase)

Concentration	Methanol : Buffer (28:72)	Methanol : Buffer (32:68)
6	0.254	0.260
%RSD	0.22%	0.58%

Solvent and standard stock solution stability

Solution stability experiments were carried out to demonstrate that the working solution remained stable during analysis and did not degrade in the diluent media. The %RSD values, estimated with regression coefficients, were all less than 2%.Table 8 results show that the working solutions have undergone little modifications, indicating that they will remain stable during analysis

Table 8: Solution stability (*n=3)

Concentration (µg/ml)	Hesperidin (285 nm)	
	Old	Fresh
	Absorbance	Absorbance
6	0.197	0.215
%RSD	0.29%	0.46%
18	0.475	0.492
%RSD	0.21%	0.42%
30	0.734	0.771
%RSD	0.07%	0.14%

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International Journal of Ayurvedic Medicine, Vol 16 (2), 2025; 525-530

Accuracy

The proposed method was confirmed to be an accurate method for the estimation of Hesperidin by the recovery experiments where mean recovery of the sample was within limits of 98.4 – 99.79 %. The data are shown in table 9.

Table 9: Accuracy (recovery studies) (*n=3)

Drug	Level	Absorbance	Mean Absorbance	Measured concentration (mg/L)	% Recovery
Hesperidin	50%	0.121	0.123	0.57	98.4%
		0.125			
		0.124			
	%RSD	1.69%			
	100%	0.211	0.214	1.0	99.67%
		0.215			
		0.217			
	%RSD	1.39%			
	150%	0.332	0.334	1.56	99.79%
		335			
		0.336			
	%RSD	0.59%			

Characterization of Proniosomes

Particle size and zeta potential were selected in the appropriate range for topical administration using the appropriate proniosomal batch. The optimized batch size was 254.8nm, suggesting that particles are Nano sized and zeta potential was found to be -29.07mV which indicated high anionic nature of the formulation and was stable enough not to form aggregation after signed to zeta potential. The data are visually displayed in table 10 and shown on Figure 4a and 4b**.** The ability of a formulation to hold the drug and prevent premature release or degradation is driven by entrapment efficiency, which needs to be measured. Prepared Hesperidin loaded proniosomes had %EE of 97.82%. Better formulation performance is evidenced with higher formulation entrapment efficiency, implying more effective retention of drug within the delivery system for controlled and targeted release.

Table 10: Characterization of Hesperidin Loaded proniosomes

Formulation	Particle size (nm)	Entrapment efficiency (%)	Zeta potential (mV)	PDI
Hesperidin Loaded proniosomes	254 ±3.41	97.82±1.3	-29.07 ±0.25	0.467 ±1.3

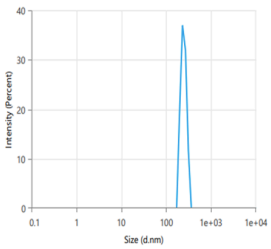

Figure 4a: The particle size distribution

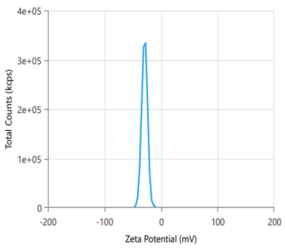

Figure 4b: Zeta potential distribution

Discussion

The investigated UV/visible spectrophotometric approach delivered precise quantification of Hesperidin with reliable accuracy and precision. Pharmaceutical quality control testing benefited from fulfilling International Conference of Harmonization standards. Hesperidin achieved optimal analysis conditions through its maximal absorbance wavelength at 285 nm while utilizing the stable combination of methanol and phosphate buffer solution at pH 6.8. The UV analytical method minimizes organic solvent usage (methanol), reducing environmental impact and operator exposure, while also offering a quick, straightforward analysis suitable for routine quality control and being relatively inexpensive with minimal sample preparation compared to other methods. The strong analytical performance was confirmed through the correlation coefficient value of 0.9987 which established linearity within the 6–30 µg/ml concentration range. The analytical method demonstrated its sensitivity through LOD and LOQ measurements of 0.25 µg/ml and 0.78 µg/ml respectively. A robust framework made the method suitable for regular use since the robustness and ruggedness testing demonstrated minimal variations. The proniosomal gels containing hesperidin exhibited optimal encapsulation efficiency as well as good zeta potential and particle size values which reflects their potential benefits for improved skin medication administration through topical routes. The research provides a cost-effective efficient method to detect hesperidin in defined dosage units and bulk material.

Conclusion

This method is referred as a straightforward, dependable and selective method that provides satisfactory accuracy, precision with lower limits of detection, more specific quantification and sensitivity. In all cases, the good recoveries were obtained and the reliable agreement with the reported procedure showed that the proposed method can be used efficiently for the determination of Hesperidin in topical dosage form with acceptable precision, and the shorter duration of Hesperidin analysis makes the proposed method suitable for daily routine analysis in pharmaceutical dosage forms.

Abbreviations

API: Active Pharmaceutical Ingredient; **UV**: Ultra-violet; **ICH**: International Council on Harmonization; **LC**: Liquid chromatography; **MS**: Mass Spectrometry; **MS/MS**: Tandem Mass Spectrometry; **HPLC**: High-Performance Liquid Chromatography.

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Conflict of interest

The authors indicated their absence of any conflicting interests.

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