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Spectroscopic determination of hesperidine in ayurvedic formulation madiphal Rasayana

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ABSTRACT

A simple and sensitive spectrophotometric method was developed for the determination of Hesperidine in API and ayurvedic formulation Madiphal Rasayana. Hesperidine with λ_{max} 284.5nm obeyed Beer's law in the concentration range of 10-100µg/ml. An Assay method was developed for Madiphal rasayana and the percentage purity was found to be was 98.94% w/v. The proposed method was successfully validated as per the ICH guidelines for API and ayurvedic formulation containing Hesperidine. The percentage recovery studies were performed and the percentage recovery was found to be 98.27 - 99.70% w/v, the system was found to be linear and the correlation coefficient (r^2) was found to be 0.999, method was found to be precise and the relative standard deviation was found to be 0.197, detection limit was found to 0.03µg/ml and quantification limit was found to be 1µg/ml respectively. The stress degradation studies were performed for the API as per the ICH guidelines, the degradation was observed in hydrolytic degradation under acidic and alkaline conditions, dry heat induced degradation, oxidative and photolytic degradation.

Keywords: Hesperidine, Dimethyl formamide and Stress degradation.

INTRODUCTION

Hesperidine, a polyphenolic bioflavonoid [1], obtained from the fruit peel of Citrus limetta belonging to the family Rutaceae. Hesperidine is the major flavanone glycoside in sweet orange and lemon obtained as an abundant by-product of *Citrus* cultivation [2]. Bioflavonoids are called phytochemicals, Vitamin P, and Vitamin C_2. Vitamin P is not a dietary essential but is known to have the effect on capillary disorders. It increases the strength of the walls of the blood capillaries and regulates their permeability. It may have a contribution to the total antioxidant activity and detoxifiers. It may enhance the action of Vitamin C. It is considered that it lowers down cholesterol levels if it taken with Vitamin C. It is also known to have pharmacological action as an anti-inflammatory, antihistaminic and antiviral agent.

Hesperidine is chemically (S)-7-[[6-O-(6-deoxy-alpha-L-mannopyranosyl)-beta-D-gluco pyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-Benzopyran-4-one, is a white to yellow crystalline powder melting at ca 260^0 C [3]. There are about 2000 known bioflavonoids including catechin, citrin, eriodictin, hesperetin, hesperidin, nobiletin, quercetin, rutin, sinensetin, and tangeretin.

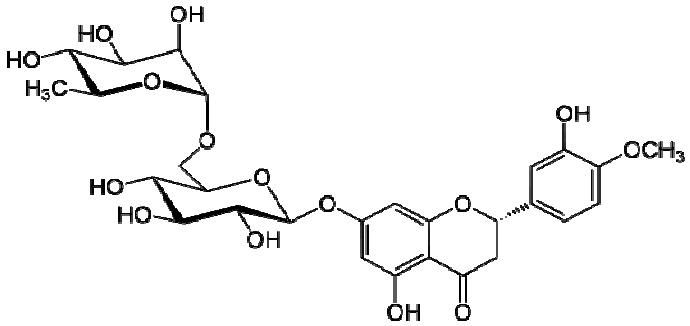

Fig no.1. Structure of Hesperidine

Few analytical methods have been reported for the estimation of Hesperidine, they are UV [4], HPLC [5], TLC [6], GC [7], HPTLC [8], LC-MS [9], GC-MS [10], Fourier transform infrared spectroscopy (FT-IR) [11], Nuclear Magnetic Resonance Spectroscopy (NMR) [12], DSC [12] and X-Ray Diffraction [12] methods. In the present investigation a spectrophotometric method was developed for Hesperidine (API) using Dimethyl Formamide as solvent and the method was validated and stability indicating studies were performed as per ICH guidelines.

MATERIALS AND METHODS

The drug sample Hesperidine was obtained from HIMEDIA Company. All chemicals and reagents were of analytical grade such as Hydrogen peroxide (SD Fine chem. limited), Sodium Hydroxide (Mio chem. Pvt Ltd), and Hydrochloric acid (Merck Chemicals). Madiphal rasayana (Baidyanath) liquid formulation was purchased from local drug store.

Spectroscopic conditions:
ELICO-SL 244 UV/ VIS double beam spectrophotometer (spectra treats) with PMT detector, was used for the spectrophotometric estimation of Hesperidine in API and Pharmaceutical preparations.

Reagents:
Hydrochloric acid (1N):
An accurately measured volume of 85 ml of Hydrochloric acid was dissolved in 30 ml of water and the final volume was adjusted to 1000 ml with distilled water.

Hydrogen Peroxide (3%):
An accurately measured volume of 3 ml of Hydrogen Peroxide was dissolved in 30 ml of water and the final volume was adjusted to 100 ml with distilled water.

Sodium Hydroxide (1N):
An accurately weighed quantity of 40 g of Sodium Hydroxide was dissolved in 30 ml of water and sonicated for 10 min for proper dissolution and the final volume was made to 1000ml with distilled water.

Preparation of standard calibration curve:
Accurately weighed 10 mg quantity of API and dissolved in 0.5 ml of dimethyl formamide (DMF) and water was added to make up the volume up to 100 ml to give a concentration of 100µg/ml.

Aliquots of standard solutions of Hesperidine were taken into a series of 10 ml volumetric flasks. The contents in each volumetric flask were finally made upto 10 ml with water to get the final concentration ranging from 10µg/ml – 100µg/ml and the absorbance was measured at 283.5 nm against the water as blank.

Assay of ayurvedic formulation madiphal rasayana:
An accurately measured volume of 2ml of the sample was dissolved in 2ml of DMF then diluted to 10 ml with water. From this 0.4ml was taken and the final volume was made to 10ml with distilled water. Absorbance of the solution was measured against water as blank.

Method Validation:
The method was validated for linearity, accuracy, precision, LOD, LOQ as per the ICH guidelines.

STRESS DEGRADATION STUDIES:
The stress degradation studies such as hydrolytic (in acidic & alkali medium), photolytic, oxidative and dry heat induced degradations studies were performed for API and marketed formulation as per ICH guidelines.

1. Hydrolytic degradation under acidic conditions:
Hydrolytic degradation studies was performed by adding 2ml (100µg/ml) of stock solution, to 1ml of 1N HCL in a 10ml volumetric flask and the volume was made to 10ml with water, and kept aside at normal condition for 90min. 5ml of the above solution was pipetted out into 10ml flask and the volume was adjusted with distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200 - 400nm.

2. **Hydrolytic degradation under alkaline condition:**
Hydrolytic degradation studies was performed by taking 2ml (100µg/ml) of stock solution, to this 1ml of 1N NaOH was added and volume was made upto 10ml with water kept at normal conditions for 90min, from this 5ml of solution was pipetted out into 10ml flask and the volume was adjusted with distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

3. Dry heat induced degradation:
Dry heat induced degradation study was performed by taking drug in a petri plate and subjected to a temperature of 70^{o}C for 48 hrs. After 48 hrs 10mg of the drug was taken and diluted with the distilled water such that to get a final concentration of 5µg/ml, Keeping distilled water as a blank , the resulting solution was scanned from 200-400nm.

4. Oxidative degradation:
An oxidative degradation study was performed by taking 1.5 ml (100µg/ml) of stock solution and to this 1ml of 3% hydrogen peroxide was added and volume is made up to the mark of 10ml of the flask and kept at room temperature for 15 min. A blank solution was prepared with 1 ml of 3% w/v hydrogen peroxide into a 10 ml flask and volume is made up to the mark. The resulting solution was stored over night. Both solutions are boiled to remove excess of hydrogen peroxide. Solutions are kept for 15 min and then diluted to a concentration of 5 µg/ml. Keeping distilled water as a blank , the resulting solution was scanned from 200-400nm.

5. Photolytic degradation:
A photolytic degradation study was performed by exposing the sample to near UV light for 30 minutes in a UV chamber. After UV exposure 10 mg of substance was taken and the final dilution was made to get a concentration of 5µg/ml using distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

RESULTS AND DISCUSSION

The absorbance of STD.Hesperidine stock solution (100 was measured from 200 – 800 nm against the corresponding blank. Hesperidine showed a maximum absorbance at 283.5nm spectra shown in **Fig. 2.** The percentage purity of ayurvedic formulation Madiphal rasayana was found to be 98.93% w/v by the proposed method.

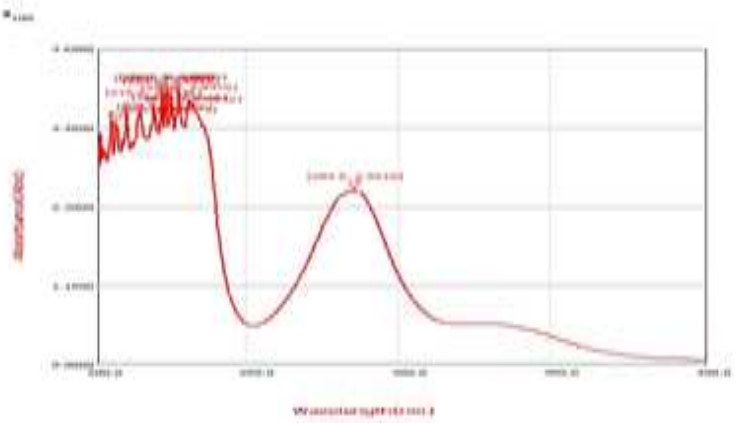

Fig.2. Showing λmax of Hesperidine

Method validation:
The method was validated for linearity, accuracy, precision, LOD, LOQ as per the ICH guidelines.

The linearity was observed to obey the Beer's- Lamberts law in concentration ranging from 10-100µg/ml. The linearity plot plotted with concentration against absorbance as showed in **Fig.3.** Correlation coefficient (r^2) was found to be 0.999; the data's showing linearity are represented in Table 1. The Precision studies were performed for six repeated absorbance of same homogenous solution having the concentration of 10µg/ml and the percentage relative standard deviation was found to be 1.391%, percentage recovery studies was performed for the 80%, 100% and 120% respectively, percentage recovery of Hesperidine was found to be in between 98.27-99.70 %, detection limit and quantification limit was found to be 0.03µg/ml and 1µg/ml. The results of method development and validations were summarized in **Table.2-4.**

Stress degradation Studies:
The stress degradation studies were performed for the Hesperidine (API) as per the ICH guidelines by subjecting the drug to different conditions that stimulate the drug degradation. Spectral studies prove that the Hesperidine shows degradations hydrolytic degradation under acidic and alkaline conditions, dry heat induced degradation, oxidative and photolytic degradation. The observations were represented in **Table.5** and the spectra's were shown in **Fig.4-8.**

Table 1: Showing the linearity studies for Hesperidine

S.No.	Concentration (µg/ml)	Absorbance at 283.5 nm
1	10	0.2925
2	20	0.5423
3	30	0.8168
4	40	1.0739
5	50	1.2548
6	60	1.5384
7	70	1.7932
8	80	2.0237
9	90	2.2651
10	100	2.5395
	Correlation coefficient (r^2)	**0.999**

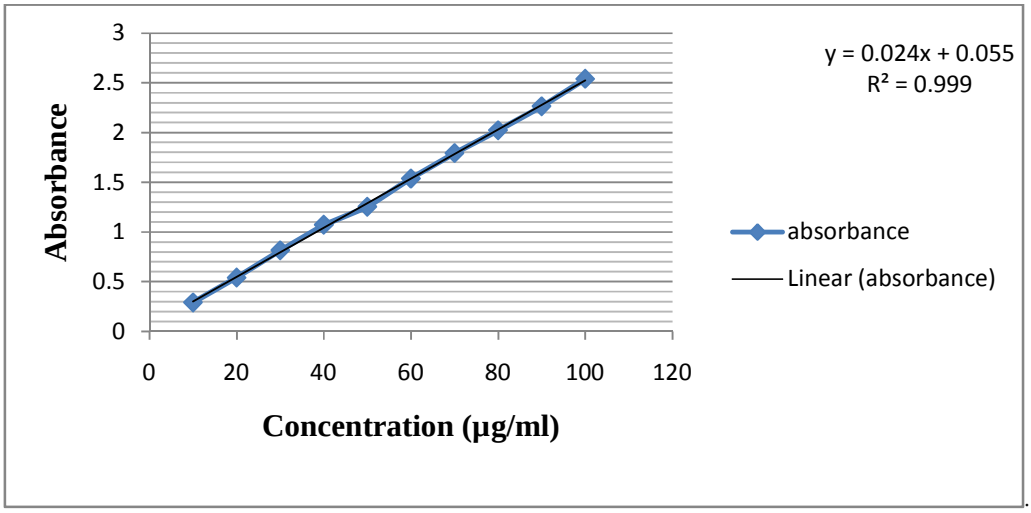

Fig.3. Linearity plot of Hesperidine

Table 2: Showing the precision studies of Hesperidine

S. No	Conc. (µg/ml)	Absorbance
1	10	0.0829
2	10	0.0825
3	10	0.0828
4	10	0.0829
5	10	0.0826
6	10	0.0827
7	**Mean**	**0.08273**
8	**Standard deviation**	**0.000163**
9	**%RSD**	**0.197**

Table 3: Showing the accuracy studies of Hesperidine

S. No	Accuracy	Amount of Standard added(mg)	Absorbance	Amount recovered (mg)	% Recovery	Mean
1	**80%**	10	0.0340	1186.9	98.20	98.27
		10	0.0342	1187.5	98.27	
		10	0.0343	1188.0	98.33	
2	**100%**	10	0.0342	944.5	98.80	98.85
		10	0.0344	950.00	98.85	
		10	0.0346	955.5	98.90	
3	**120%**	10	0.0345	790.58	99.61	99.70
		10	0.0347	791.66	99.70	
		10	0.0349	790.74	99.79	

Table 4: Optical density characterization of Hesperidine

Parameter	Observations
λmax(nm)	283.5
Beer's law limits (mg/ml)	10-100 µg/ml
Molar absorptivity (Liter/mole^{-1}cm^{-1})	249.8
Correlation Coefficient (r^2)	0.999
Regression Equation (Y)*	Y= 0.024x + 0.055
Slope (b)	0.024
Intercept(a)	0.055
%RSD	0.197
LOD	0.3 µg/ml
LOQ	1 µg/ml

Table 5: Showing the Stress Degradation Studies of Hesperidine

S. No.	STRESS DEGRADATION STUDIES	TIME PERIOD	STANDARD OBSERVATION
1.	HYDROLYTIC DEGRADATION UNDER ACIDIC CONDITIONS	90 min.	DEGRADATION WAS OBSERVED
2.	HYDROLYTIC DEGRADATION UNDER ALKALI CONDITIONS	90 min.	DEGRADATION WAS OBSERVED
3.	PHOTOLYTIC DEGRADATION	30 min.	DEGRADATION WAS OBSERVED
4.	OXIDATIVE DEGRADATION	15 min.	DEGRADATION WAS OBSERVED
5.	DRY HEAT INDUCED DEGRADATION	48 hrs	DEGRADATION WAS OBSERVED

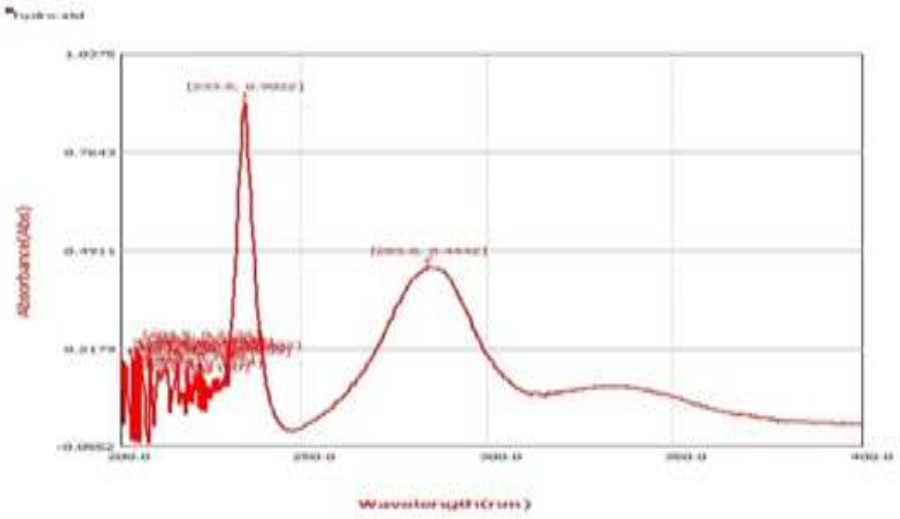

Fig.4. Acidic degradation of standard

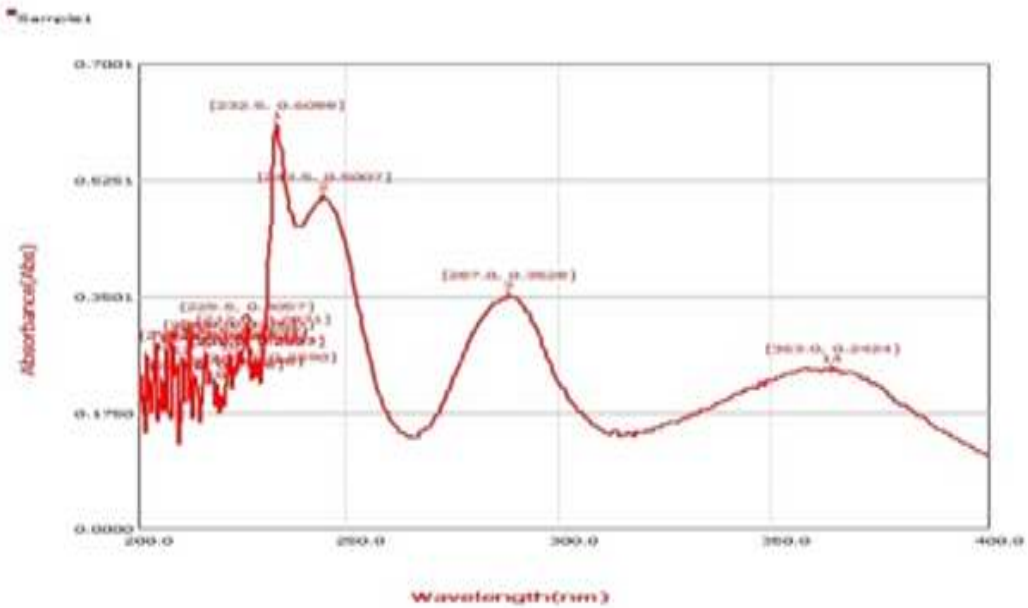

Fig.5. Alkali degradation of standard

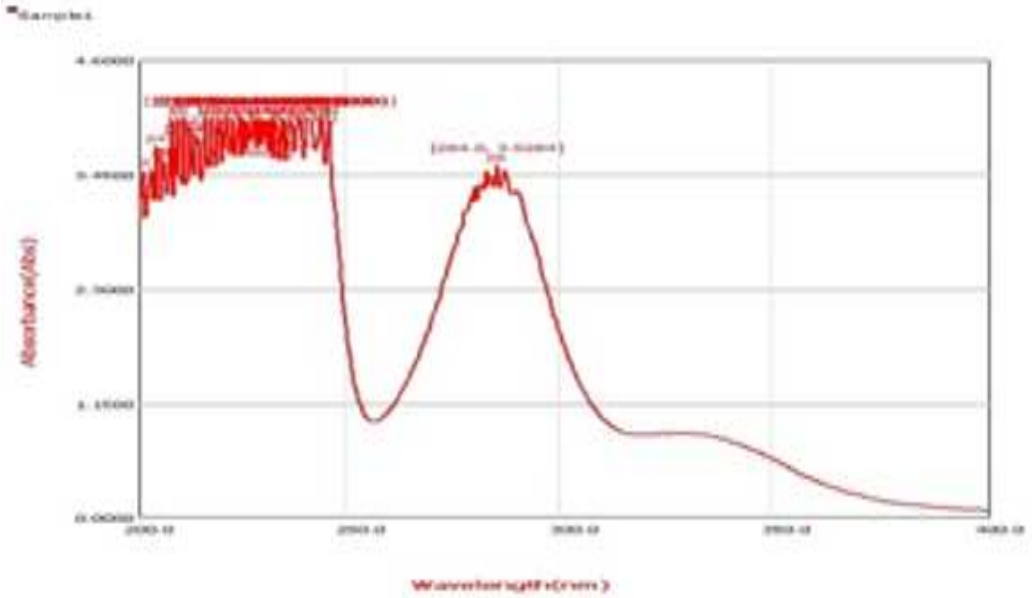

Fig.6. Dry heat degradation of standard

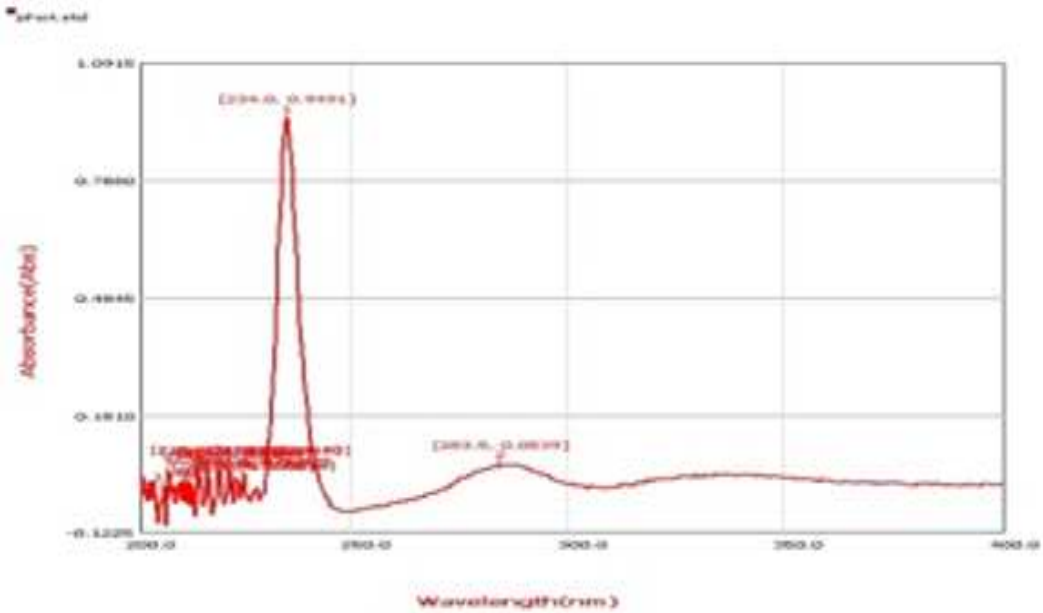

Fig.7. Photolytic degradation of standard

Fig.8. Oxidative degradation of standard

CONCLUSION

The proposed spectroscopic method for the estimation of Hesperidine in API, and ayurvedic formulation (*Madiphal Rasayana*) was successfully developed & validated as per the ICH guidelines; the results observed for the proposed method were within the standard limit. Stability indicating stress degradative studies were performed for the API as per ICH guidelines indicated degradation of the drug in various conditions such as hydrolytic degradation under acidic and alkaline conditions, dry heat induced degradation, oxidative and photolytic degradation. Hence, the proposed assay method can be used for the routine analysis of Hesperidine in the bulk drug and ayurvedic formulation (*Madiphal Rasayana*).

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