International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 832-837
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Stability Indicating HPTLC Method for Hesperidin

Rasika A. Bhalerao, Divya R. Mehta, Neha K. Raka, Akansha M. Markad, Aishwarya R. Pawar, Pratiksha S. Undre, Mrinalini C. Damle*.

All India ShriShivaji Memorial Society's College of Pharmacy, (Affiliated to Savitribai Phule Pune University) Kennedy Road, Near RTO, Pune-411001.

Received: 12 Mar 2019 / Accepted: 14 Apr 2019 / Published online: 1 Jul 2019
Corresponding Author Email: mcdamle@rediffmail.com

Abstract

A stability indicating assay method was developed and validated according to the ICH guidelines for estimation of Hesperidin using HPTLC. **Objective**-Hesperidin is flavonoid with anti-inflammatory, anti-oxidant properties. The objective was stability-indicating method development and validation for Hesperidin by HPTLC. **Method** – HPTLC method was developed and validated using Mobile phase consisting of Ethyl acetate: Methanol: Water (7:2:2 v/v/v) and detected at wavelength 283 nm. Various forced degradation conditions were used to check degradation of drug. **Results** - The method showed a good linear relationship (r^2= 0.9855) in the concentration range 200-1000 ng/band. It was found to be linear, accurate, precise and specific. **Conclusion**–The proposed HPTLC method for Hesperidin can be applied for quality control as well as for stability testing of Hesperidin. The developed method was validated as per ICH guideline Q2(R1).

Keywords

Hesperidin, HPTLC, Stability indicating. ICH guidelines.

*****

INTRODUCTION

Chemically, Hesperidin is (2S)-5-hydroxy-2-(3hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5trihydroxy-6-{[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6methyloxan-2-yl]oxymethyl}oxan-2-yl]oxy-2,3dihydrochromen-4-one[1]. Hesperidin is a flavonoid. Highest concentrations are found in citrus fruit peels. For instance, peels from tangerines contain hesperidin the equivalent of 5-10 % of their dry mass [2]. Hesperidin plays a protective role against fungal and other microbial infections in plants. These flavonoids have been detected in human plasma after orange and grapefruit diets. Decades of research revealed its many therapeutic applications in prevention and treatment of many human disorders. Hesperidin shows different activities such anti-inflammatory, anti-oxidant, anti-carcinogenic, cardiovascular, anti-diabetic, anti-allergic, etc. It is used clinically for the treatment of Rheumatoid Arthritis [3]. The objective was development of stability indicating method for Hesperidin by HPTLC. The method was validated as per ICH Q2(R1) guidelines. The stability indicating assays are important to determine the shelf life of the products. It also helps to determine the storage conditions by knowing the process of degradation. Literature survey reveals that, there are some reported quantitative estimation methods [4-8] and stability indicating methods reported for Hesperidin

but result of stress degradation do not match [9-10]. Hence the objective was to check result under stress conditions. The drug was exposed to different stress conditions to understand the inherent stability characteristics of the active substance and to develop the stability indicating HPTLC assay method (ICH, Q1A(R2).

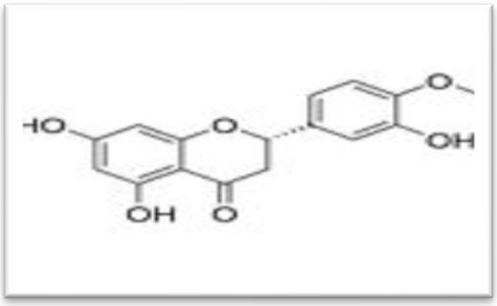

Fig 1. Structure of Hesperidin

MATERIAL

CHEMICALS -Dimethyl sulfoxide (DMSO) LR grade, Methanol (HPLC grade), Ethyl acetate(AR) were purchased from LOBA Chemicals (Mumbai).

INSTRUMENTS-HPTLC CAMAG Linomat5 (Applicator), CAMAG Twin trough development chamber (10 x10 cm), CAMAG TLC Scanner-3, CAMAG WINCATS Software (version 1.4.3.6336), Hamilton Syringe (100µl). All weighing was done on electronic analytical balance. (Shimadzu AY 120).

METHOD

Optimised HPTLC Chromatographic Conditions: Aluminium sheets precoated with silica gel G60 F254(10 x10 cm) of layer thickness 0.2mm were used. TLC plates were prewashed with Methanol & activated in oven at 60°C for 20 minutes. Mobile phase consisted of Ethyl acetate: Methanol: Water (7:2:2 V/V/V). The detection wavelength 283nm was used. TLC chamber was saturated for 20 minutes.

Preparation of Solutions: Preparation of Hesperidin standard stock solution (1000µg/ml)

Accurately weighed 10mg of Hesperidin was transferred into 10ml volumetric flask, dissolved it in 1ml of Dimethyl sulfoxide (DMSO) and diluted up to the mark with methanol to get stock solution having concentration 1000µg/ml.

Preparation of standard solution (100µg/ml)

1 ml of standard solution stock solution of Hesperidin was pipetted out and transferred to 10 ml volumetric flask and diluted to up to the mark with methanol to get working standard solution having 100µg/ml.

Preparation of working standard solution (40µg/ml)

4 ml of standard solution (100µg/ml) of Hesperidin was pipetted out transferred to 10 ml volumetric flask and diluted to up to the mark with methanol to get working standard solution having 40µg/ml.

Forced degradation studies [11-12]

Degradation conditions were optimised to get 10-30% degradation, by varying strength of reagent, time of exposure and temperature.

The forced degradation studies were performed by using conditions such as acidic, alkaline & neutral, oxidative, thermal and photolytic. The optimised conditions were follows-

Acid degradation- Standard stock solution of Hesperidin 1000 ug/ml was prepared. From that 1 ml of sample was pipetted out. 1ml of 0.01 N HCL was added. Volume was made up to 10 ml & sample was kept for 1 Hour for degradation & examined.

Alkaline degradation- From 1000 ug/ml stock solution, 1 ml of sample was pipetted out and 1ml of 0.01 N NaOH was added. Volume was made up to 10 ml & sample was kept for 1 Hour for degradation & examined.

Oxidative degradation from 1000 ug/ml stock solution, 1 ml of sample was pipetted out. 1ml of 6% v/v H_2O_2 was added. Volume was made up to 10 ml & sample was kept for 1 Hour for degradation & examined.

Neutral degradation- From 10000 ug/ml stock solution, 1 ml of sample was pipetted out. 1ml of distilled Water was added. Volume was made up & sample was kept for 1 Hour for degradation & examined.

Thermal Degradation-Drug Hesperidin was exposed to 50 degrees Celsius for 1 hour. Then 10 mg of sample was weighed & dissolved in DMSO and diluted with methanol up to 10 ml and examined it.

Photolytic degradation- Hesperidin was exposed to UV light for 200-watt hours and to cool white

fluorescent light for 1.2million Lux-hours. Then 10 mg of Hesperidin was weighed and dissolved it in 1 ml of DMSO and volume was made up with methanol.

Method Validation [13]- The method was validated as per the ICH guidelines in term of Linearity, range, precision, accuracy, specificity, limit of detection, limit of quantitation and robustness.

Specificity-The peak purity of Hesperidin was examined by comparing the spectra at peak start, peak middle and peak end positions of the bands. This facility is available in WINCAT software.

Linearity: Linearity responses for Hesperidin were taken by using the five different volumes (5, 10, 15, 20, 25µl) from 40 µg/ml solution. Table No 3 shows the data of calibration curve and Figure No 4 shows the calibration curve (200-1000ng/band) and the linear equation for the calibration plot was y = 7.5418x + 959.91 with correlation coefficient r^2= 0.985.

Precision: The precision was evaluated with respect to both Intra Day and Inter Day precision. Precision was evaluated by spotting one concentration for six times. This studies were repeated for three different days to determine inter day precision. Peak areas of the drug were determined and % RSD was calculated.

Limit of detection (LOD) and Limit of Quantitation (LOQ): The limit of detection and quantitation was calculated by using the formula

LOD = 3.3 σ/S

LOQ = 10 σ/S respectively.

where,

σ=standard deviation of the y-intercept of linearity equations.

S=slope of the calibration curve of the analyte

Robustness: Robustness was evaluated by deliberate variation in parameters like mobile phase, chamber size, saturation time. The proposed method was found to be robust under given conditions.

Assay: To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample. The assay is determined by spotting working solution of citrus peels extract.

Preparation of standard solution of citrus peels extract

Take 100 mg of citrus peels extract, dissolve it in 1 ml of DMSO make up the volume up to 10 ml with methanol (10000 ug/ml).

Preparation of working solution

Pipette out 1 ml from standard solution of citrus peels extract, further make up the volume up to 10 ml (1000 ug/ml).

Accuracy-The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Prepared the working solution of Hesperidin 50 µg/ml. Prepared the sample solutions of citrus peels extract. Spotted the sample solution of citrus peels extract as 20µl, then 3, 6, 9µl of working standard solution was over spotted for recovery studies at 80%, 100%, 120%.

Summary of validation parameters for HPTLC method

The HPTLC method for determination of Hesperidin was developed and validated. The results confirmed linearity, accuracy, precision and selectivity of the developed analytical method. The method showed good linearity over the selected range. The summary validation parameters for the developed analytical method are shown in Table no 6.

RESULTS

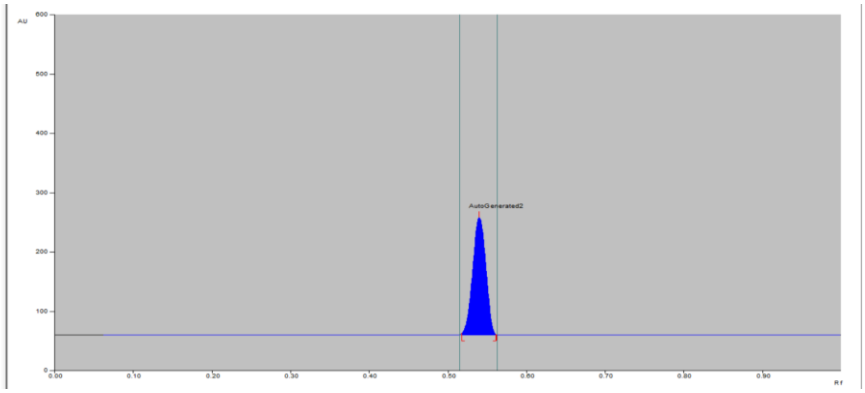

Figure No.2 Standard densitogram of Hesperidin (200 ng/band)

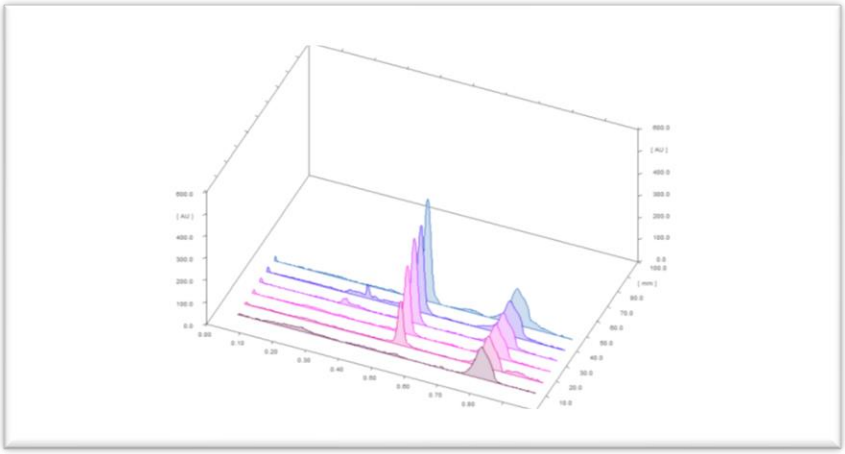

Fig 3. 3D Densitogram of Hesperidin linearity (200-1000ng/band)

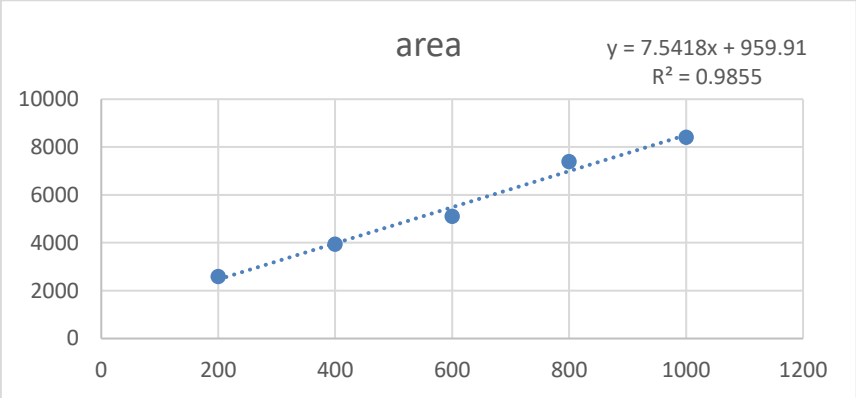

Figure No 4. Calibration curve of Hesperidin (200-1000ng/band)

No 1: Results of degradation study

Stress Condition	Recovery%
Acidic (0.01 N Hcl-1 hour)	88.0%
Alkali (0.01 N NaOH-1 hour)	85.0%
Oxidative H_2O_2 (6 % V/V-1 hour)	85.0%
Neutral (1 hour)	87.27%
Thermal (50^0C-1 hour)	83.10%
UV (200-watt hours)	101%
Fluorescence(1.2million Lux-hours)	99.43%

Table No 2: Result of Calibration curve for Hesperidin

Concentration Area (ng/band)	SD % RSD
200 2593.6	51.45 2.0
400 3934.5	48.04 1.2
600 5098.5	76.90 1.5
800 7391.2	95.31 1.2
1000 8407.0	86.45 1.0

Table No 3: Intra-Day, Inter-Day and Repeatability study of Hesperidin

Sr.no	Concentration	Precision Type	Average	SD	% RSD
1.	200	Inter Day	2583.56	47.57	1.84%
2.	200	Inter Day	2559.2	33.291	1.30%

Table 4: LOD & LOQ for Hesperidin

Sr no	LOD	LOQ
1.	55.87 ng/band	169.29 ng/band

Table 5: Summary of validation parameters for Hesperidin

Sr no	Parameter	Result
1.	Specificity	Specificity
2.	Linearity	0.9855
3.	Range	200-1000 ng/band
4.	Precision (Intra Day)	1.8 %
	(Inter Day)	1.3 %
5.	Limit of Detection	55.50 ng/band
6.	Limit of Quantification	168.19 ng/band
7.	Assay	1.4%

DISCUSSION

While developing stability indicating method, in the current work, the stress conditions were optimized to achieve 10-30% degradation. In the literature survey, it observed that the degradation conditions reported in Reference no 9 and 10 do not match at all. Reported paper (ref no-9) do not have mention of neutral, thermal, and photo degradation studies. In our work all stress condition, as recommended by ICH guidelines are optimised.

CONCLUSION

Hesperidin was found be prone to degradation in acidic, alkaline, oxidation, neutral & photolytic conditions. The developed method is validated as per ICH guideline Q2(R1). The proposed method was found to be highly reproducible and reliable. Hence, the developed HPTLC method is simple and can be used for determination of hesperidin stability.

ACKNOWLEDGEMENT

Authors are thankful to Principal and the management of AISSMS College of Pharmacy, Pune for providing the necessary facilities for research work.

REFERENCES:

[1] Maryadele J. Neil, Patricia E. Heckelman, Peter H. Dobbelaar, Kristin J. Roman, Catherine M. Kenny, Linda S. Karaff, The merck index, An encyclopedia of chemicals, drugs, and biologicals, RSC publication, Fifteenth edition, 4707(2013)

[2] CornaraC.'Arrigo, F. Piol, B. Borghesi, C. Bottino, E. Patron, & M. G. Mariotti, Micro morphological investigation on the leaves of the rock samphire: Occurrence of hesperidin crystals, Plant Bio systems - An International Journal Dealing with all Aspects of Plant Biology. 11(12):524-528, (2009)

[3] Garg A, Garg S, Zaneveld L, Singla A. Review article: chemistry and pharmacology of the citrus bioflavonoid hesperedin. Phytother12:655–669, (2011)

[4] Aftab Alam1, Md. Khalid Anwer, Saleh I Alqasoumi. Quantitative estimation of hesperidin by HPTLC in different varieties of citrus peel. Asian Pacific Journal of Tropical Biomedicine, Asian Pac J Trop Biomed 4(4):262-26, (2014)

[5] Prawezalam, Simultaneous Determination of Flavonoids in Different Parts of Citrus reticulata 'Chachi' Fruit by by High Performance Liquid Chromatography.15(16):5378-5388, (2013)

[6] Azza M El-Shafae, Maher M El-Domiaty, Improved LC methods for the determination of diosmin and/or hesperidin in plant extracts and pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis26(27):539-54, (2001)

[7] Feras Imad Kanaze, ChrysiGabrieli, Eugene Kokkalou, ManolisGeorgarakis, IoannisNiopa, Simultaneous reversed-phase high-performance liquid chromatographic method for the determination of diosmin, hesperidin and naringin in different citrus fruit juices and pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis 8(9):243-249, (2012)

[8] AveenNozadAdham, Qualitative and Quantitative Estimation of Hesperidin in Peel and Juice of Citrus Fruits by RP-HPLC Method Growing in Kurdistan Region, Iraq., International Journal of Pharmacy. Sci. Rev. Res., 33(2):220-224, (2015)

[9] Anwer M.K.1, Jamil S, Ansari M. J, Al-Shdefat R. and Abdel-Kader M.SA new improved Stability-Indicating

RP-HPLC method for Determination of diosmin and hesperidin in combination. International Research Journal of Biological Sciences.3(6):41-46, (2010)

[10] Loba R, Richa Agrawal, Prabhu, K. S. Shirwaikar, A. A.MamthaBallal, NipunDashora , Vijay Sodde, Annie Shirwaikar. Development and validation of a stability-indicating HPTLC method for analysis of Hesperidin in pharmaceutical dosage form. Journal of Pharmacy Research 2010 3(7):1648-1651,(2010)

[11] Bakshi M, Sing S. Development of validated Stability-indicating assay methods critical review, Journal of pharmaceutical and Biomedical Analysis. 28:1011-1040, (2002)

[12] ICH guidelines, Q1A (R2); Stability Testing of New Drug Substances and Products, International council for Harmonization, Geneva.(http://www.ich.org/products /guidelines/quality/article/quality-guidelines/html), (2 003)

[13] ICH guidelines, Q2(R1): Validation of Analytical Procedures: Text and Methodology, International conference on harmonization, Geneva. ⟨http://www .ich.org/products/guidelines/quality/article/qualityg uidelines/html), (2005)