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Development of UV Spectrophotometric Technique for Stability Evaluation of Hesperidin and Diosmin in Pharmaceuticals and Nanoformulations

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Abstract:
Background: From ancient times, India and many other countries have utilised natural products to management of various illnesses. Because they are abundantly available and have fewer adverse effects than synthetic medications, flavonoids are secondary metabolites with diverse biological activities. Diosmin and hesperidin are flavonoids isolated from multiple medicinal plants but are found in citrus plants in large amounts. They have a variety of biological and pharmacological properties. They also improve lymphatic drainage by raising the duration and severity of lymphatic compression and the exact number of fully operational lymphatic capillaries.
Methodology: We have procured Diosmin and hesperidin from Otto Kemi Pvt. Ltd. and other chemicals from SIGMA chemicals. Furthermore, the characterisation of selected flavonoids has been performed through solubility studies and method development for development and validation by the UV method for nano-formulation.
Result: Diosmin and hesperidin are pure and soluble in various solvents and have a melting point between 286 and 259 degrees Celsius, respectively and are more soluble in Acetone, Chloroform, and DMSO, but we have utilised both the compounds for Cancer studies, so we have taken it in 5% DMSO for further studies.
Keywords: Diosmin, Hesperidin, UV Method, Characterization, DSC, XRD, FTIR, NMR

Introduction:

The active ingredient of tangerine peel is hesperidin (Figure 1), which is 3,5,7-trihydroxy-4 methoxy flavanone 7-o-rutinoside, abundant in citrus species. It protects against lead, strontium, & heavy metal toxicity and is also prescribed for people with diabetes and those suffering from gastric reflux illness. Hesperidin is transformed into hesperetin by intestinal bacteria and then absorbed via the intestinal mucosa.

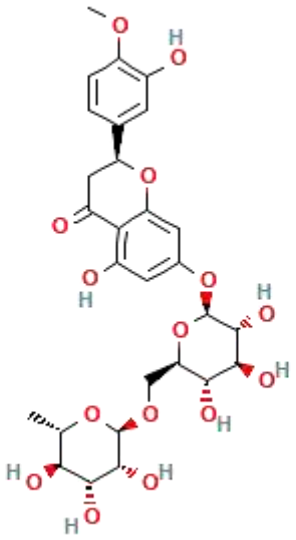

Figure.1. 2D structure of Hesperidin

Diosmin (Figure 2) is a flavonoid-related synthetic medicine (modified Hesperidin), and it is an oral pleiotropic prescription drug used to treat venous illness, namely chronic venous insufficiency (CVI) and haemorrhoid disorders. The IUPAC name for this is 3,5,7-trihydroxy-4 methoxy flavone 7-rutinosode. It may have therapeutic efficacy in the treatment of neurodegenerative illnesses such as Alzheimer's, and anti-inflammatory and antiapoptotic properties in neuronal cells have been reported as lowering venous hypertension that is seen in CVI sufferers. It also enhances lymphatic drainage by increasing the frequency and intensity of the lymphatic contraction and the amount of functioning lymphatic capillaries.

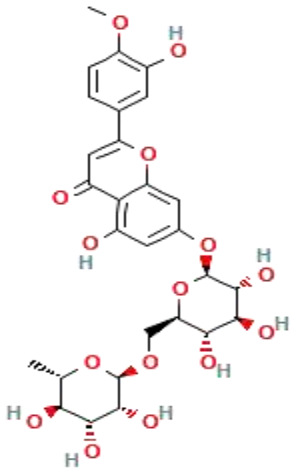

Figure 2. 2D structure of Diosmin

As per the existing literature, a few analytical methods for hesperidin and Diosmin were disclosed that includes UV [1–5] they conclude that they only review the pharmaceutical activity and analytical technique of harmine and also an alternate method to cure diabetes, Fluorometric approach [6, 7], LC-method [8, 9], HPLC technique [9, 10], capillary electrophoresis [11], a simple colourimetric method [12], thin layer chromatography (TLC) [13], as well as adsorptive stripping voltammetry [14]. However, no research on the UV spectrophotometric approach for simultaneous quantification of hesperidin and Diosmin has been published to our knowledge. **As a result, UV spectrophotometric approaches are scarce for measuring hesperidin and Diosmin together.** Therefore, the current investigation was part of an attempt to create and implement a simple, distinctive, efficient, inexpensive, & exact UV spectrophotometric approach for simultaneously measuring two Flavonoids for the nano-formulation.

Methodology:

Chemicals and Instruments:

Otto Kemi Pvt. Ltd. in Mumbai, India, provided the Hesperidin and Diosmin operating standards. Merck Chemicals supplied the methanol, acetonitrile, ethanol, Chloroform, DMSO, Acetone, N-hexane, Ethyl acetate, and N-octanol. A water purification system was used to prepare the Distilled water. A Shimadzu Model 1800 UV-Visible twin-beam spectrophotometer was used.

Method:

Firstly, we procure the Flavonoids from Otto Chemo. Pvt. Ltd., And then we characterise them by employing FTIR, DSC, NMR and XRD. Further, the solubility check of both flavonoids in Solvents includes methanol, ethanol, Acetonitrile, Chloroform, DMSO, Acetone, N-hexane, Ethyl acetate, distilled water and N-octanol (Shown in Table 1). Following the solubility test, we determine the partition co-efficient and their lambda max (Figures 5 and 6). After that, we use UV spectroscopy to determine absorbency. The UV spectra of Hs and Ds were obtained in ethanol at various concentrations. (Results shown in Figures 3 and 4).

The synthesis procedure of Hesperidin and Diosmin:

Several investigators presently employ that approach for DM production, which involves Hesperidin biochemical transformation [14], [15]. According to Victor et al. 2021, we fixed a method for retrieving hesperidin using the Citrus plant *Sinesis L. Osbeck*. Our goal was to

increase yields by using their peel waste for extraction by separating it with methanol, then crystallising it in aqua by adding dichloroethane, reducing the solvent quantity, and transferring it to hot retrieval for fresh citrus albedo with methanol. Further, For Diosmin, the ionic liquids are used to formulate Diosmin from hesperidin.

Table 1: Solubility studies of Diosmin and Hesperidin

List of Solvents	**Quantity of Compound Soluble (In mg) in Diosmin**	**Quantity of Compound Soluble (In mg) in Hesperidin**
Chloroform	20	20
Ethanol	15	10
Methanol	10	25
DMSO	20	20
Acetone	15	15
N-hexane	10	15
Ethyl acetate	15	15
Acetonitrile	10	5
N-octanol	10	10

After the solubility checks, we move forward to find out the partition coefficient. After that, we utilise the UV spectroscopy technique for absorbance. The UV spectra of Hesperidin and Diosmin were recorded in ethanol at different concentrations (Results shown in Figures 3 & 4).

Hesperidin (2–100 µg/mL), and Diosmin (2–100 µg/mL), were made and examined in various concentrations. The two highest absorbances were observed at the two wavelengths used for the investigation. At all wavelengths, the absorbance of Hesperidin and Diosmin was measured, and absorptivity ratios E (1 per cent 1 cm) were calculated. The following equations can be used to compute the concentration of two medicines in a mixture:

$$C^{\text{Hesperidin}} = (A2ay1 - A1ay2)\ ax2ay1 - ax1ay2,$$

$$C^{\text{Diosmin}} = (A1ax2 - A2ax1)\ ax2ay1 - ax1ay2, \quad (1)$$

where $C^{\text{Hesperidin}}$ and C^{Diosmin} are the concentrations of Hesperidin and Diosmin in sample solutions. $A1$ and $A2$ are the absorbances of the Diosmin sample at 275.5 and the Hesperidin sample at 286.5nm. $ax1$ and $ax2$ are the absorptivities of Hesperidin at given wavelengths; $ay1$ and $ay2$ are the absorptivities of Diosmin at given wavelengths, respectively. (Rewrite and give the outcome of it.)

Results and Discussion:

The analytical technique's linearity, precision, accuracy, the limit of detection (LOD), quantification (LOQ), and ruggedness were all verified.

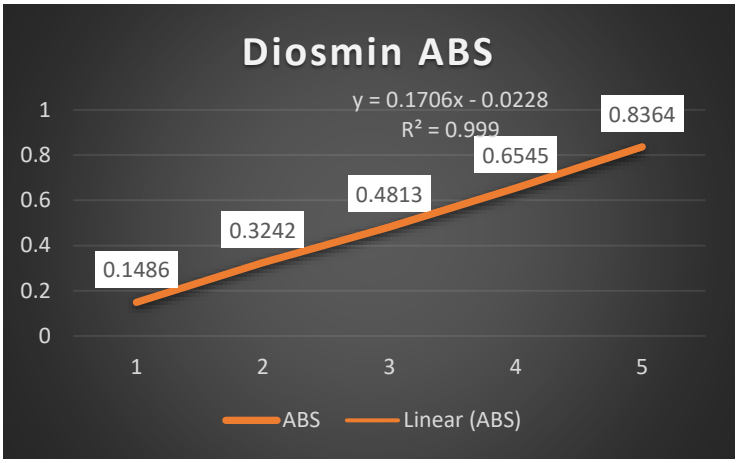

Figure 3. Calibration curve of DM. It is a way to identify the concentration of an unknown substance. These curves use data points of known substances at varying concentrations, and researchers or developers can use these curves to find where an unknown substance plot.

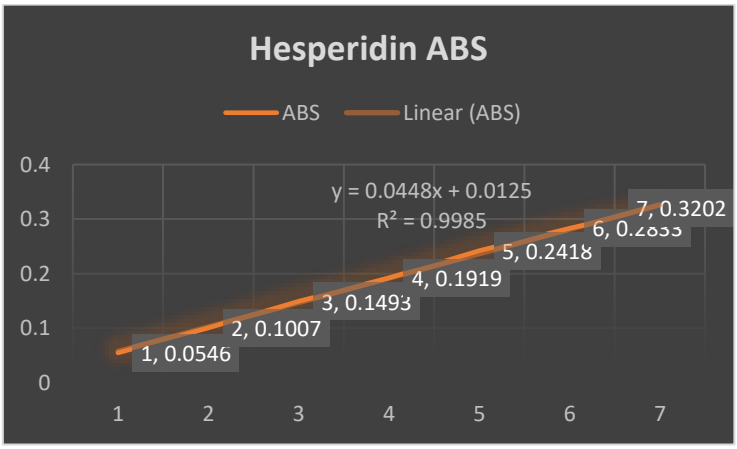

Figure 4. Calibration curve of HS.

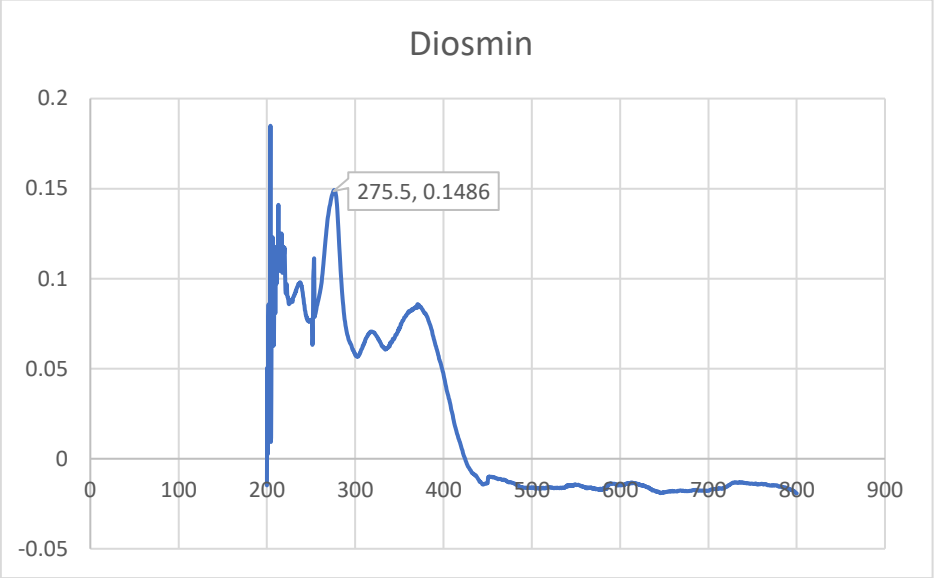

Figure 5. showing Lambda max of DM. Lambda max (λ_{max}): The wavelength at which a substance has its most substantial photon absorption (highest point along the spectrum's y-axis). This ultraviolet-visible spectrum for DM has λ_{max} = 275.5 nm.

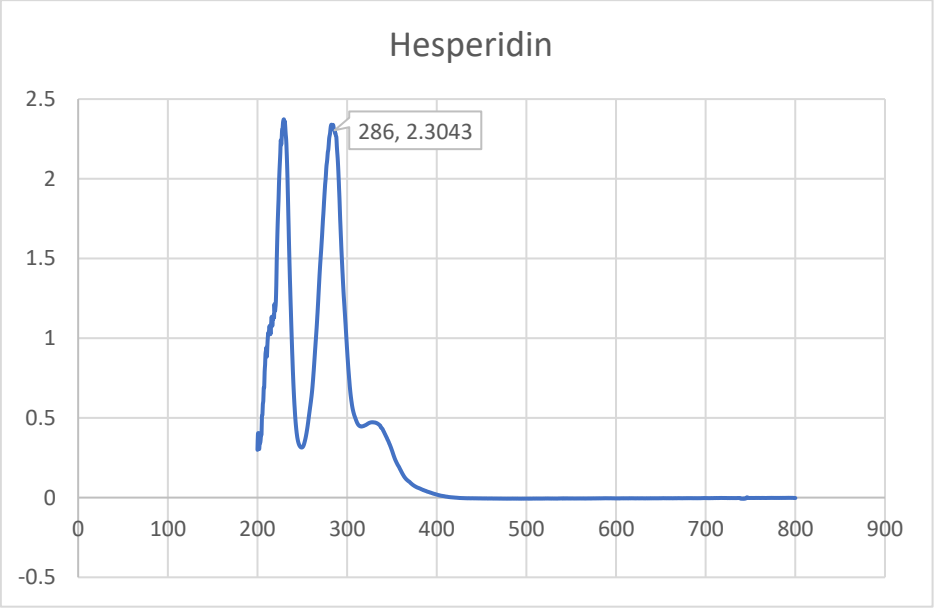

Figure 6. shows Lambda max of HS. Lambda max (λ_{max}): The wavelength at which a substance has its strongest photon absorption (highest point along the spectrum's y-axis). This ultraviolet-visible spectrum for DM has λ_{max} = 286 nm.

Linearity:

The calibration curves were linear throughout the range of concentrations of 2–100 µg/mL for hesperidin and 2–100 µg/mL for Diosmin, as determined by least squares linear regression analysis.

Table 2: Linearity values of Hs and D

Parameter	**DM and HS**
Regression equation	y = 0.0565x + 0.138
Linearity µg/mL	2–100
Correlation coefficient	0.999

Absorbances were plotted vs concentrations, and linear regression analysis was conducted on the resulting curves; correlation coefficients for hesperidin and Diosmin were determined to be 0.999 and 0.999, respectively (Table 2).

Precision:

A statistical examination was performed to determine the degree of repeatability of the procedure. The concentrations of the two Flavonoids were tested five times on the same day at one-hour intervals and three times on separate days for intra- and inter-day studies by three individuals. We estimated the standard deviation (SD) and meant (Table 3).

Table.3. Precision value of DM and HS.

Drug	**Conc.(µg/mL)**	**Inter-day(%RSD)**	**Intra-day (%RSD)**
DM	8	0.45	0.11
HS	8	0.43	0.12

Accuracy:

Recovery trials were conducted by applying the procedure to a drug sample with a known standard quantity. Hesperidin and Diosmin, corresponding to label claims 50, 100, and 150, have been added. Three determinations were made at each level of the amount.

LOD and LOQ:

The LOD for hesperidin and diosmin was 0.139 g/mL and 0.048 g/mL, respectively, while the LOQ was 0.042 g/mL and 0.147 g/mL, respectively.

Characterisation of Compounds:

To characterise Desired compound, i.e., DM, we use various methods such as DSC, FTIR, and XRD.

FTIR (Fourier Transform Infrared)

The FTIR method was employed to identify the materials' inorganic and organic components based on the readings of several peaks in the IR radiation area. Whenever the sample of chemicals was put through the FTIR, the constituents were distinguished based on their peak frequency. The existence of several inorganic and organic components of the substance was verified by FTIR analysis, and results are shown in 7 and 8. However, it has significant drawbacks, such as not displaying the metallic details of any chemical, for which we can use NMR.

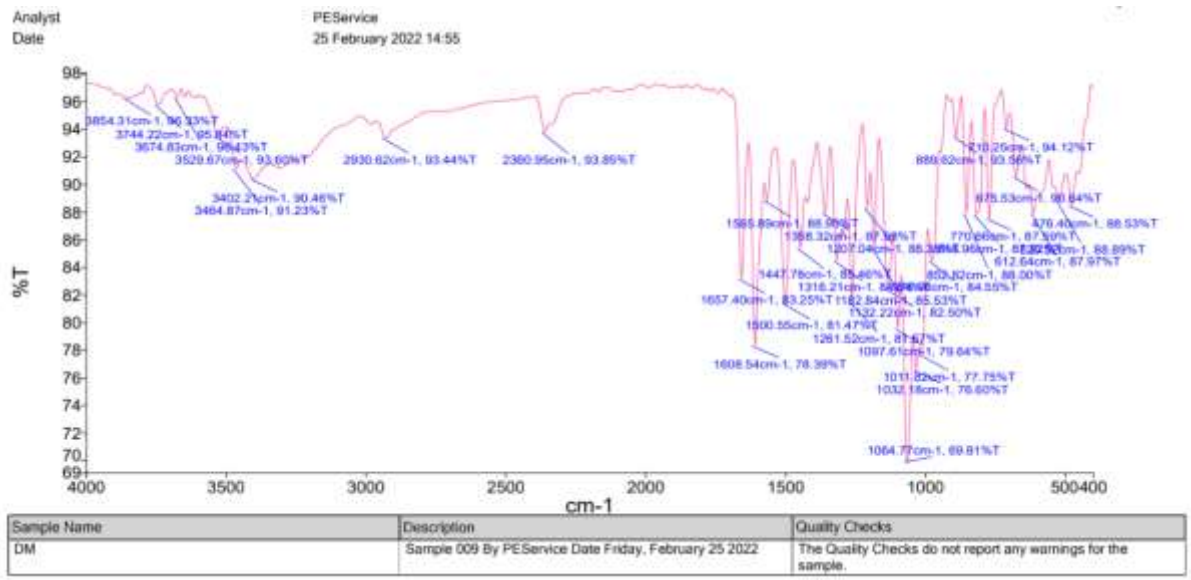

Figure 7. FTIR Result of DM. Fourier Transform Infrared Spectroscopy (FTIR) **identifies chemical bonds in a molecule by producing an infrared absorption spectrum**. The spectra have a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. Hereafter analysis of the graph, we found a peak in 1565 due to C=C aromatic peak, a peak in 1657 due to C=O, a peak in 1064 due to C-O stretching, C-H Bending peak at 1065, a C=C Aromatic peak at 1515, a C=O peak at 1644,

2361 peak due to carbon dioxide, 2915 peak due to C-H stretching, and 3675 peak due OH group. After analysis, we found that our compound has the same components in its structure.

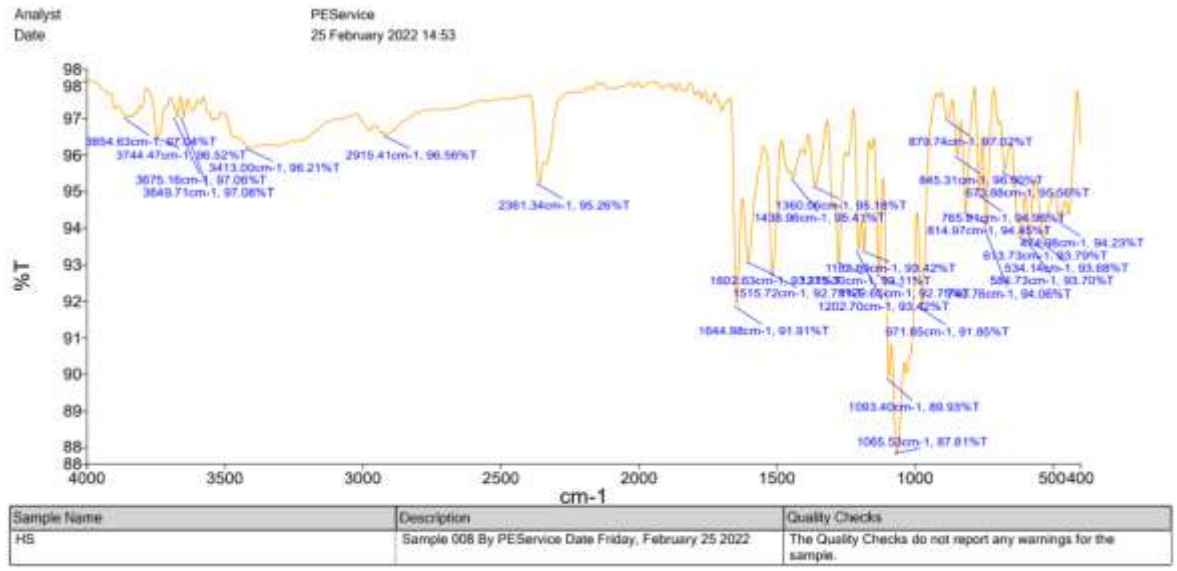

Figure 8. FTIR result of HS. After analysis of the graph, we found different peaks, i.e., C-H Bending peak at 1065, C=C Aromatic peak at 1515, C=O peak at 1644, 2361 peak due to carbon dioxide, 2915 peak due to C-H stretching, 3675 peak is due OH group. However, after analysis, we found that our compound has the same components in its structure.

NMR:

It is a technique for assessing the chemical structure of a substance by viewing and quantifying the interconnection of nuclear spins in an intense magnetic field. It investigates the material's physical, chemical, and biological characteristics (Figures 9 & 10.). Pharmacologists employ it to detect the identity and composition of molecules. In addition, MRI, a multidimensional NMR imaging technology, is used by doctors for medical diagnostics.

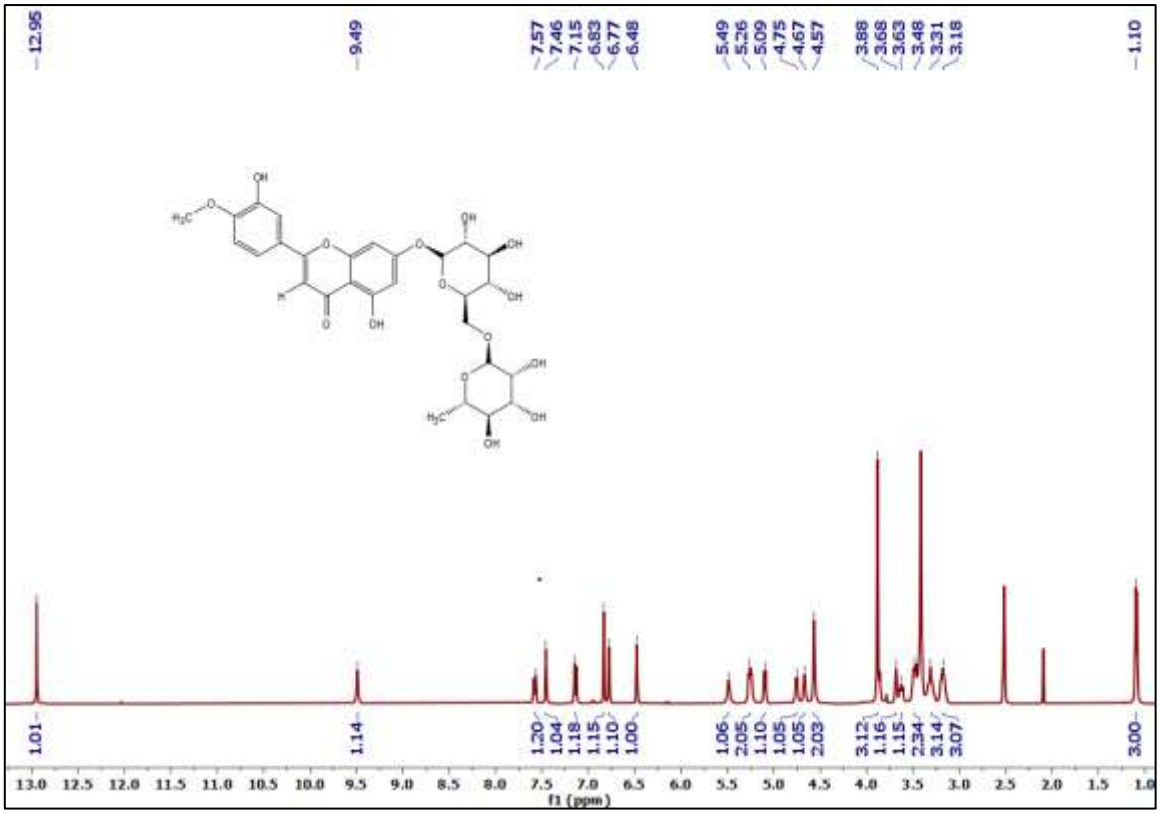

Figure 9. shows the NMR result of DM.

5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-((((2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (DM).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (s, 1H), 9.49 (s, 1H), 7.57 (s, 1H), 7.46 (s, 1H), 7.15 (s, 1H), 6.83 (s, 1H), 6.77 (s, 1H), 6.48 (s, 1H), 5.49 (s, 1H), 5.26 (s, 2H), 5.09 (s, 1H), 4.75 (s, 1H), 4.67 (s, 1H), 4.57 (s, 2H), 3.88 (s, 3H), 3.68 (s, 1H), 3.63 (s, 1H), 3.48 (s, 2H), 3.31 (s, 3H), 3.18 (s, 3H), 1.10 (s, 3H);^{13}C NMR (100 MHz, DMSO-d_6) δ 182.42, 164.65, 163.40, 161.67, 157.41, 151.77, 147.22, 123.33, 119.42, 113.58, 112.68, 105.90, 104.28, 100.99, 100.05, 95.25, 76.72, 76.05, 73.56, 72.50, 71.19, 70.78, 70.02, 68.80, 66.51, 56.24, 18.28.

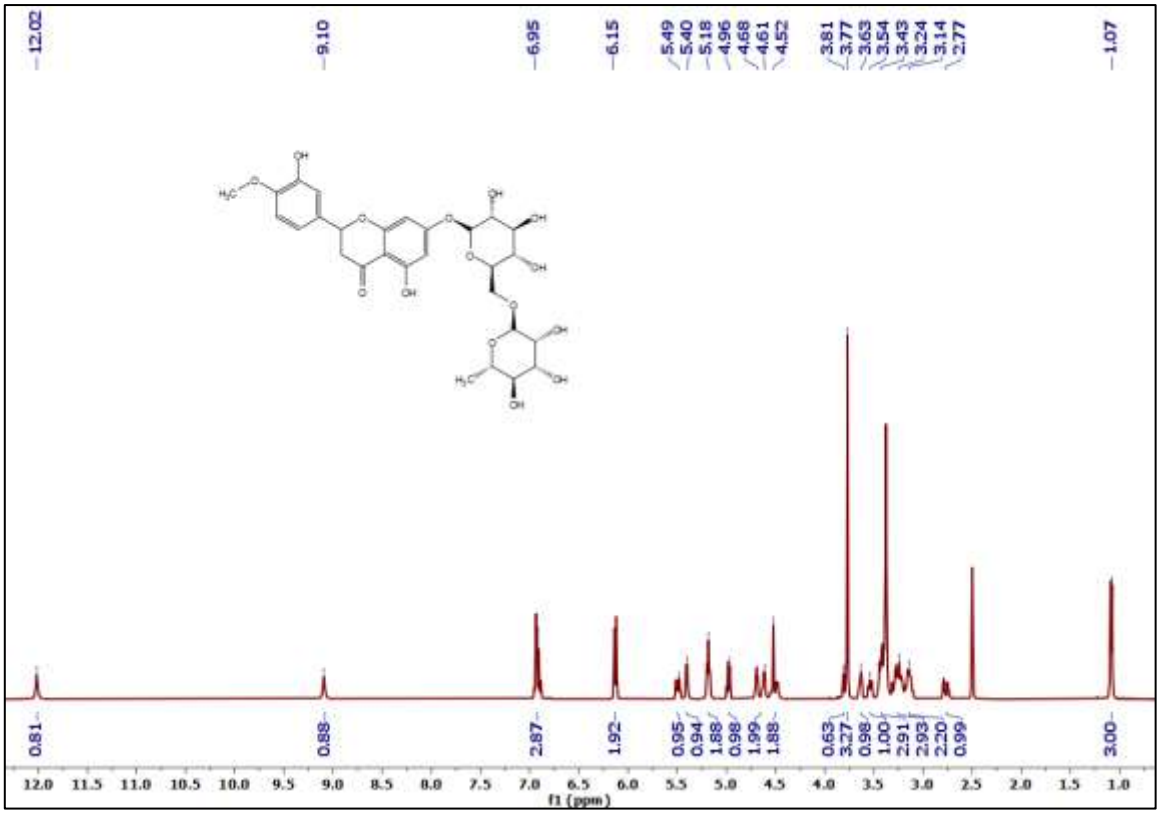

Figure 10 shows the NMR of HS.

5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-((((2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)chroman-4-one (HS).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 9.10 (s, 1H), 6.95 (s, 3H), 6.15 (s, 2H), 5.49 (s, 1H), 5.40 (s, 1H), 5.18 (s, 2H), 4.96 (s, 1H), 4.64 (d, J = 29.7 Hz, 2H), 4.52 (s, 2H), 3.81 (s, 1H), 3.77 (s, 3H), 3.63 (s, 1H), 3.54 (s, 1H), 3.43 (s, 3H), 3.24 (s, 3H), 3.14 (s, 2H), 2.77 (s, 1H), 1.07 (s, 3H);^{13}C NMR (100 MHz, DMSO-d_6) δ 197.49, 165.59, 163.46, 163.00, 131.34, 118.42, 114.61, 103.77, 101.06, 99.84, 96.84, 96.00, 78.84, 76.73, 75.97, 73.44, 72.53, 71.16, 70.73, 70.05, 68.78, 56.13, 18.30

DSC:

DM and HS were determined using DSC apparatus with thermal expansion. Standardisation was formerly conducted using a calibrating reagent. At 1.90 mg, the specimen was weighed and stored in an aluminium testing crucible, capped, and then warmed to 300$^{\circ C}$ in N_2 atmosphere at 10$^{\circ C}$ minute-1. This method is employed to determine the melting range of a material. The result is of Diosmin and hesperidin shown in Figures 11 &12.

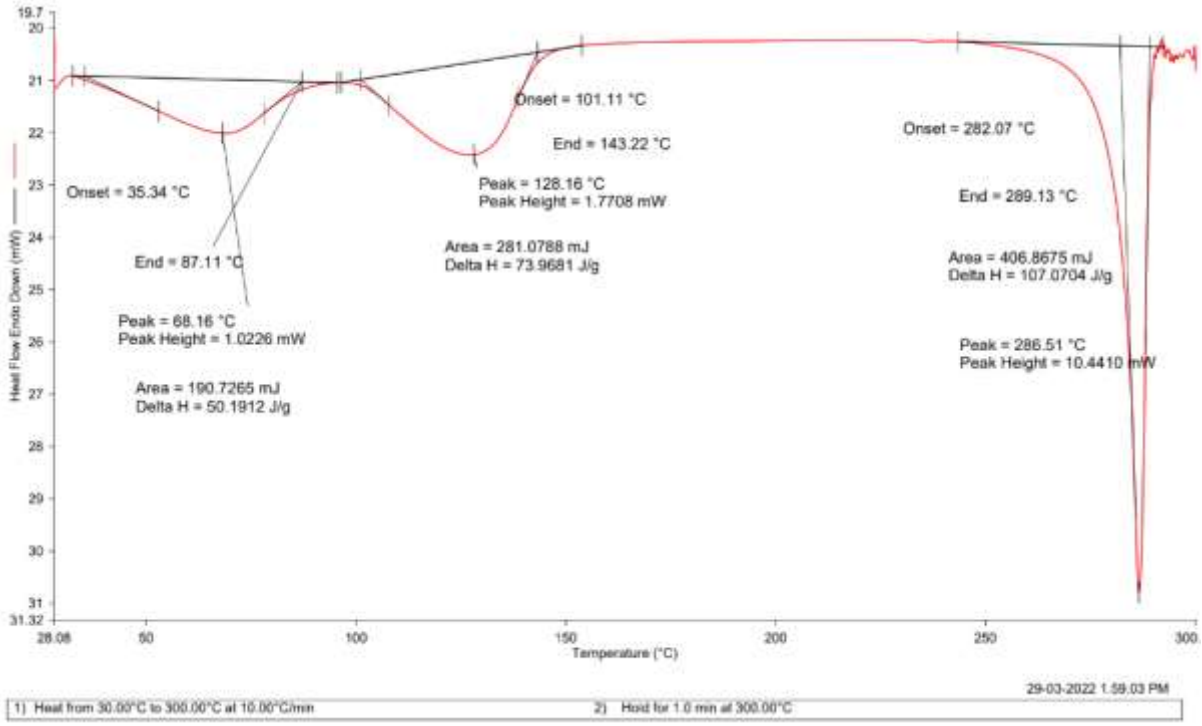

Figure 11. showing the DSC Graph of DM. The DSC curve shows an exothermic peak around 128°C, indicating an exothermic reaction caused by crystallisation. The endothermic peak observed at around 286°C refers to an endothermic reaction by "melting".

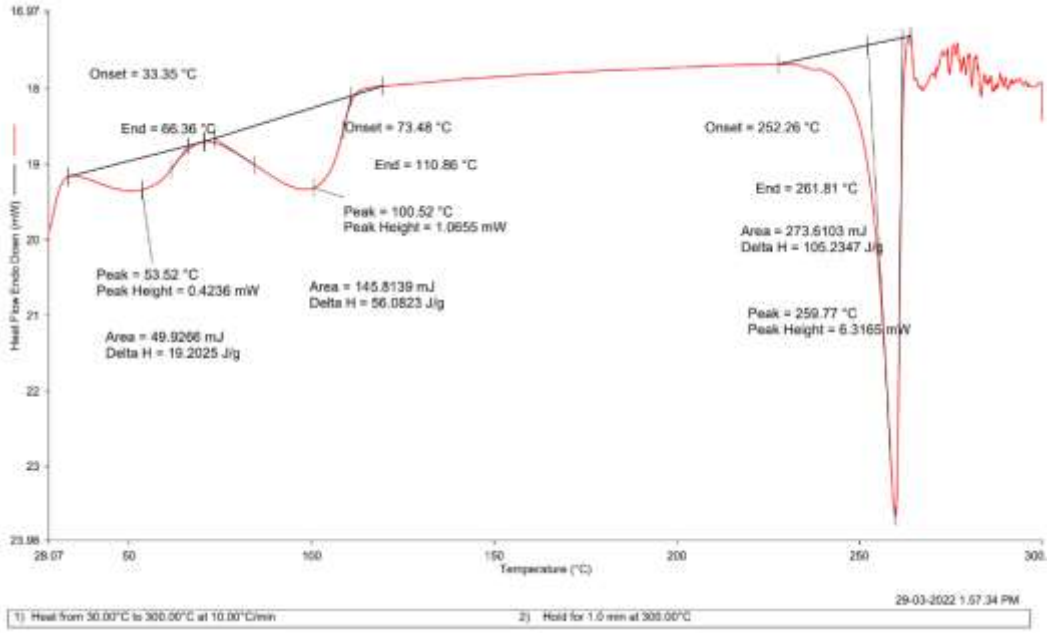

Figure 12. showing the DSC Graph of HS. The DSC curve shows an exothermic peak around 100°C, indicating an exothermic reaction caused by crystallisation. The endothermic peak observed at around 259°C refers to an endothermic reaction by "melting".

XRD:

This method is employed to identify the shape or type of the specimens, such as crystalline or amorphous. For example, if the chart has a sharp peak, the sample is crystallised; the model is amorphous if the graph has a flat mount. The result is of Diosmin, and hesperidin, shown in Figures 13 & 14.

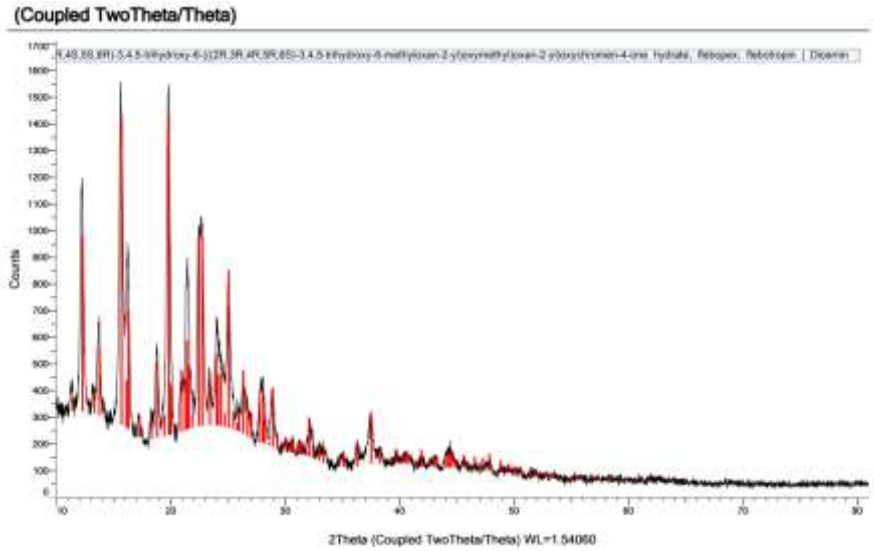

Figure 13. XRD result of DM. An XRD graph (Intensity vs two thetas) is used to find the nature of the material. For sharp peaks, the material may be taken as crystalline. For a broader peak, it may be polycrystalline, while in case of no peak but with some noisy pattern, the materials are said to have amorphous nature. In our case, Sharp peaks are there; then it shows that the nature of the sample is crystalline.

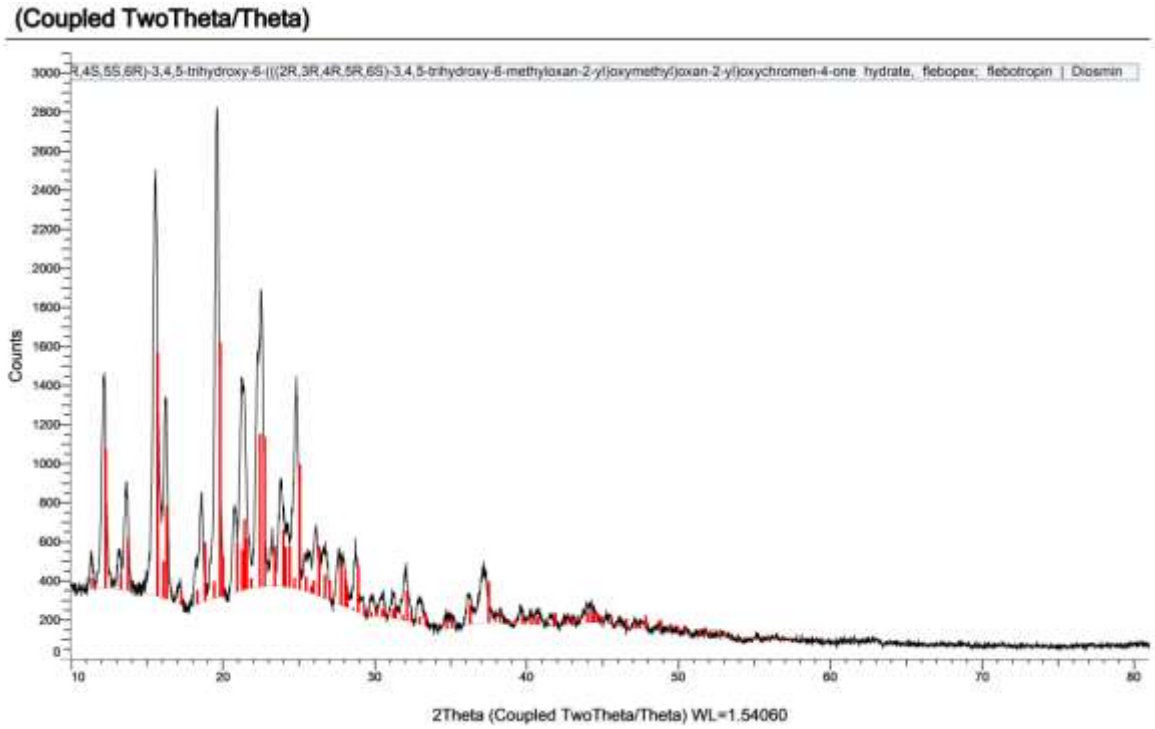

Figure 14. XRD result of HS. An XRD graph (Intensity vs two thetas) is used to find the nature of the material. For sharp peaks, the material may be taken as crystalline. For a broader peak, it may be polycrystalline, while in case of no peak but with some noisy pattern, the materials are said to have amorphous nature. In our case, Sharp peaks are there; then it shows that the nature of the sample is crystalline.

Conclusion:

This approach is regarded as straightforward, dependable, and efficient, delivering high accuracy and precision with a reduced limit of detection, more specific measurement, and responsiveness. Furthermore, the good recoveries obtained in all cases and the dependable contract with the reported procedure demonstrated that the proposed approach might be applied efficiently for the perseverance of Hesperidin and Diosmin orally administered with acceptable accuracy.

In addition, the shorter duration of Hesperidin and Diosmin assessment signifies the designed studies appropriate for routine analysis in pharmaceutical formulations or nano-formulation.

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