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Development and validation of two spectrophotometric methods for simultaneous determination of diosmine and hesperidin in mixture and their applications

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ABSTRACT

Two spectrophotometric methods were used to determine two substances simultaneously in a binary mix which are diosmin (DSM) and hesperidin (HSP). First, we used the derivative spectrophotometry by the zero-crossing measurements based on the elaboration of the linear calibration graphs of first derivative values, which are plotted at 269 nm for HSP and 262.5 nm for DSM. Then, for the second method, we opted for the calculation of the peak absorbance ratio λ_{max} of both drug substances (DSM-HSP) in a binary mixture, and the percentage of drugs was determined. Two different ratios were selected.

The two methods presented are simple, selective, and reliable, providing satisfactory accuracy. The recoveries obtained in both cases are good and agreed well with the reported values. The results confirmed that the methods can be considered as a good alternative to other costly techniques, in particular, chromatographic techniques. These methods can be used easily and effectively for simultaneous dosing of two active ingredients mixed in pharmaceutical forms with precision. Furthermore, we can use them for monitoring the synthesis of DSM from HSP. Considering the time saved by these methods presented in the brief period of the analysis, we can use them for daily quality assurance.

INTRODUCTION

Hesperidin (HSP) (Fig. 1), a well-known flavonoid in citrus fruit, is the active component of the skin of oranges and mandarins: anti-inflammatory action of cross-linked citrus fruits by inhibiting the synthesis of eicosanoids (Crespo *et al*., 1999) and other physiological activities including hypotensive effect, lowering cholesterol rate, sepsis protective agent, and diuretic and antioxidant activities (Garg *et al*., 2001; Tanaka *et al*., 1997). It also has a protective role against intoxication due to heavy metal poisoning. It is also used to treat some cases of diabetes and gastroesophageal reflux. Before being absorbed by the intestinal mucosa, the intestinal microflora converts HSP into hesperetin.

Diosmin (DSM) (Fig. 2), also called DSM aglycone or diosmetin, is a flavonoid synthetic drug derived from HSP. It is an oral venotonic phlebropathic medicine indicated in the treatment of hemorrhoidal and venous diseases, particularly in the cases of chronic insufficiency. It has a potential effect on the case of neurodegenerative diseases including Alzheimer's disease and has demonstrated its actions on neuron cells, especially antiapoptotic and anti-inflammatory actions. It decreases venous pressure in patients with chronic venous insufficiency. It increases the frequency of the lymphatic contraction and also its intensity. It promotes the development of lymphatic drainage in a better way by increasing the number of functional lymphatic capillaries (Srilatha *et al*., 2013).

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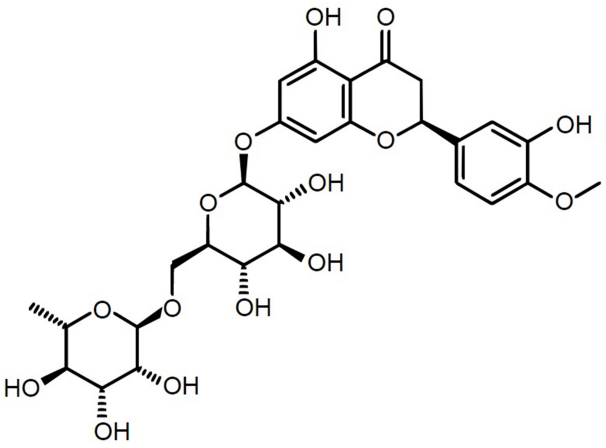

Figure 1. Structure of HSP.

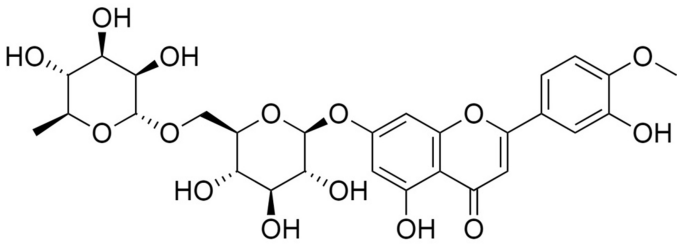

Figure 2. Structure of DSM.

Based on extensive literature search, several methods of analysis have been found for determination of DSM and HSP simultaneously such as spectrophotometric method (Chen *et al*., 2002; Srilatha *et al*., 2013), fluorometric method (Mir *et al*., 2013), chromatographic methods (Alpdoğan *et al*., 2002; El-Shahawi *et al*., 2006; Janeczko *et al*., 2004; Pooralhossini *et al*., 2017), electrophoresis method (El-Zinati and Abdellatif, 2015), colorimetric method, and stripping adsorptive voltammetry (Erk, 2002).

As mentioned before and according to an extensive literature search, no spectrophotometric method by derivation or absorbance ratio was mentioned for the synchronous dosage of HSP with DSM in a binary mixture; hence, the interest for developing a spectrophotometric method can be validated for the simultaneous dosage of DSM and HSP.

The main purpose of this study is to develop two simple, fast, accurate, and economical methods for simultaneous dosage of DSM and HSP in the binary mixture and present their applications to determine the quantity of these active substances in the pharmaceutical form and to make a monitoring synthesis of DSM from HSP.

MATERIALS AND METHODS

The present work was conducted in the Laboratory of Analytical Chemistry, Faculty of Medicine and Pharmacy of Rabat.

Reagents

DSM and HSP with working standards and with different synthesis standards were purchased from Dioma Laboratory, Morocco.

A commercially available drug product was procured from a local pharmacy containing 500 mg of purified micronized flavonoid fraction corresponding to 90% of DSM (450 mg) and 10% of HSP (50 mg). Acetonitrile, methanol, DMSO, and sodium hydroxide were purchased from Merck Chemicals. Water of all dilutions was collected by using purification system of water.

Apparatus

A double-beam UV–visible spectrophotometer (PerkinElmer Lambda 12) with a wavelength accuracy of 0.3 nm and a spectral bandwidth of 1 nm was used.

Methods

Spectra of DSM and HSP with various solvents mentioned previously were recorded. 0.2 N NaOH was the best solvent of dissolution for the two substances which present the best absorbances.

The zeroth-order UV absorption spectrum of each solution was established against 0.2 N NaOH as a blank, and the wavelengths of DSM and HSP were selected.

Two different methods were developed.

First derivative method (Method A)

Method A is the derivative spectrophotometry by measurements in the zero-crossing, i.e., at λ_{max} of product 1, the absorbance of product 2 is 0. The first derivative absorption spectra of UV spectrum for each solution against NaOH as a blank were recorded, and the curves were established.

The calibration curve was obtained by plotting the maxima of the first derivative at λ_{max} corresponding to zero-crossing of HSP versus the corresponding concentrations. The same procedure was used for the determination of the DSM content in mixtures using the second wavelength.

Ratio absorbance peak (Method B)

Method B is used to calculate the ratio of the absorbance peak of DSM and HSP in a binary mixture.

Different mixtures of DSM and HSP were scanned, and the absorbances were noted. After that, the calibration curves are plotted to calculate the concentration of two substances in a mixture. The percentage of DSM and HSP was determined. Two ratios were chosen, and the percentage of DSM was calculated by two different ways:

For ratio 1: % DSM = A_{DSM}/A_{HSP}

For ratio 2: % DSM = $A_{HSP} - A_{DSM}/A_{HSP}$

where A_{DSM} is the absorbance of DSM (at 268 nm) and A_{HSP} is the absorbance of HSP (at 285 nm).

The method validation

The studied methods were validated precisely according to the ICH Q2 guidelines with respect to the following parameters: linearity, precision, and accuracy (International Conference Harmonization guideline ICH Q2 (R1), 2015).

The method application

Analysis of tablet formulation

Six 500-mg tablets of commercially available drug were analyzed, and each tablet was prepared by dissolving in 500 ml of

NaOH (0.2 N) after suitable dilution. The sample contains HSP and DSM diluted to 5 and 50 µg/ml^{-1}, respectively. The absorbances were recorded, respectively, in the corresponding wavelengths for both methods A and B.

Monitoring of the synthesis of DSM

The monitoring of the synthesis of DSM was realized by method B. Different samples were taken from DSM synthesis. These samples were prepared by NaOH solution (0.2 N). After the dilution, the absorbances were scanned at λ_{max1} and λ_{max2}, respectively. Three determinations were conducted at each level of the amount.

RESULTS AND DISCUSSION

For DSM and HSP determination in a mixture, zeroth-order absorption spectra of both drugs were recorded. It was found that DSM could be determined at λ_{max1} = 268 nm and HSP at λ_{max2} = 285 nm without interferences (Fig. 3).

First derivative method (Method A)

The first derivative (by zero-crossing measurements) gave two lambda values specific to each substance: 262.5 and 269 nm for DSM and HSP, respectively (Fig. 4). The calibration curve of the first derivative was established (Fig. 5a and b).

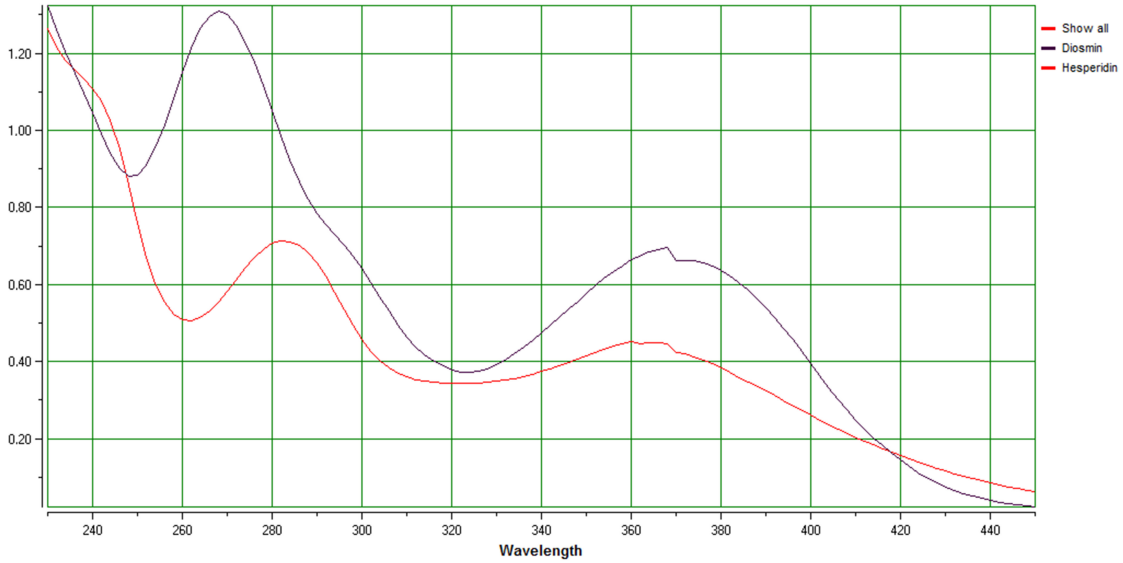

Figure 3. The spectra of HSP and DSM in NaOH.

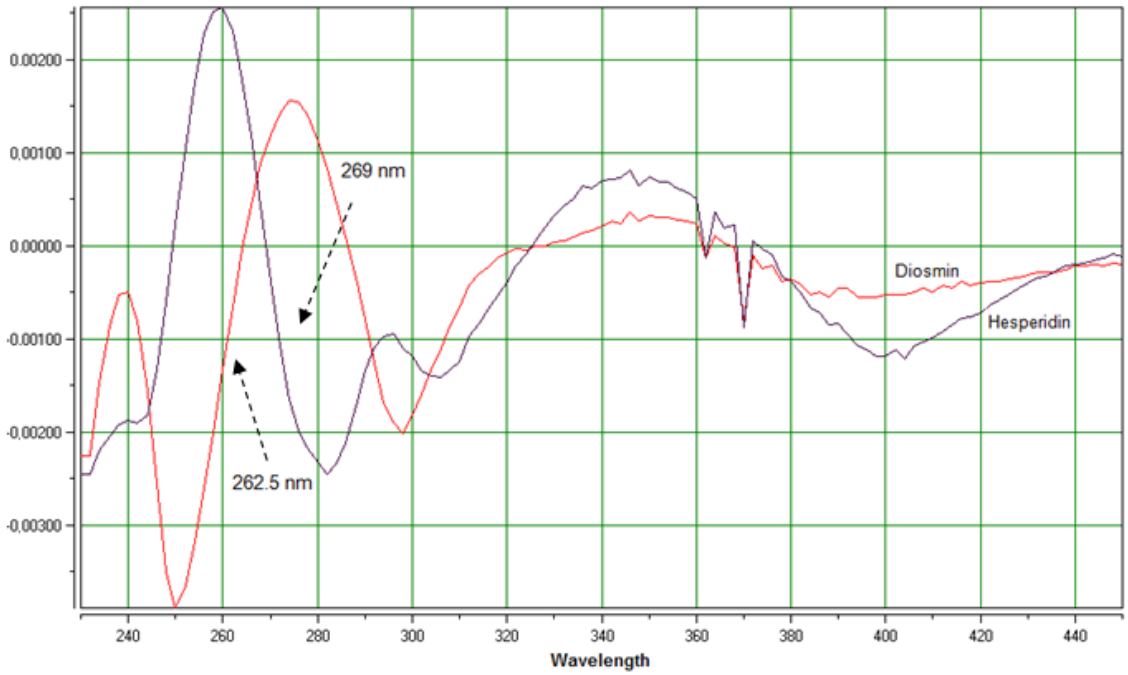

Figure 4. First derivative spectra of HSP and DSM.

Ratio absorbance method (Method B)

The mixtures with ratios ranging from 0% to 100% were measured for DSM and HSP at 285 and 268 nm, respectively, for (A_{DSM}/A_{HSP} or A_{HSP}-$_{DSM}/A_{HSP}$). Two absorbances were noted at λ_{max1} of DSM and λ_{max2} of HSP. The ratios were calculated, and the calibration lines were established (Fig. 5a and b).

Linearity was established by the least-squares linear regression analysis of calibration curve, and the calibration curves were linear in the range of 0%–100% [0%, 25% (1/4), 50% (1/2), 75% (3/4), and 100%], respectively, for absorbance ratios and absorbance difference ratios.

The method validation

Linearity

The linearity was established by the linear regression analysis of calibration curves for both the methods. For method A, the calibration curves were plotted by the absorbances as a function of the concentrations. The concentration range was 16–84 µg/ml^{-1} for DSM and 3–25 µg/ml^{-1} for HSP.

For method B, the calibration curves were plotted by the absorbance ratio of 1 or 2, and the range was from 0% to 100%. Table 1 shows all the parameters of linearity.

Precision

An appropriate statistical evaluation has been established to test the repeatability of the studied method. The concentrations of two substances were analyzed three times a day at an interval of a few days. The standard deviation (SD) and the relative SD (RSD) were calculated for both the methods (Table 2).

Accuracy

Recovery was carried out by the method of assaying a sample of drug tested at a known standard amount. For method A, the DSM and HSP samples corresponding to claims 80%, 100%, and 120% of the standard were added. For method B, mixtures [HSP%–DSM%] corresponding to [100%–0%], [80%–20%], and [60%–40%] were prepared and measured. For each sample, three determinations were made (Table 3).

The method application

Analysis of tablet formulation

For the determination of the amount of DMN and HSP in tablet formulation, the absorbances were noted, respectively, in the wavelengths of 262.5/269 nm for method A and 268/285 nm for method B. Table 4 shows the results of the determination of DMN and HSP in the tablets: quantity found (method A), percentages found (method B), and the percentages of claims on the label and RSD of the two methods.

These results showed a good agreement between the results found and the quantities labeled by the manufacturer. There is no difference between the methods A and B, and both the methods present the satisfactory results.

These methods are faster and simpler than several techniques, and hence, there was the interest in using these methods which can be considered as a useful alternative to the chromatography techniques [high-performance liquid chromatography (HPLC)] used routinely for the quality control of finished products, allowing not only qualitative information but also quantitative information to be obtained simultaneously and quickly with inexpensive instrumentation and reagents. These methods offer a clear improvement compared to conventional absorption spectrophotometry, especially in the characterization of a compound (Erk, 2000; Haripriya *et al*., 2013).

Monitoring the DSM synthesis

The ratio absorbance method can be used for the control of the process for the synthesis of DMN from HSP without interferences with other substances such as excipients or products used for synthesis.

The absorbances were noted at the wavelength fixed by this study (268–285 nm), and at each step, three determinations were made (Table 5).

The different spectra established for all the standards taken during the synthesis show a change in the shape of the spectrum from HSP starting product to the synthesis of DMN (Fig. 7), which consolidates the results obtained in Table 5.

The verification of the result of monitoring was realized by another reliable method which is the HPLC. The different mixtures

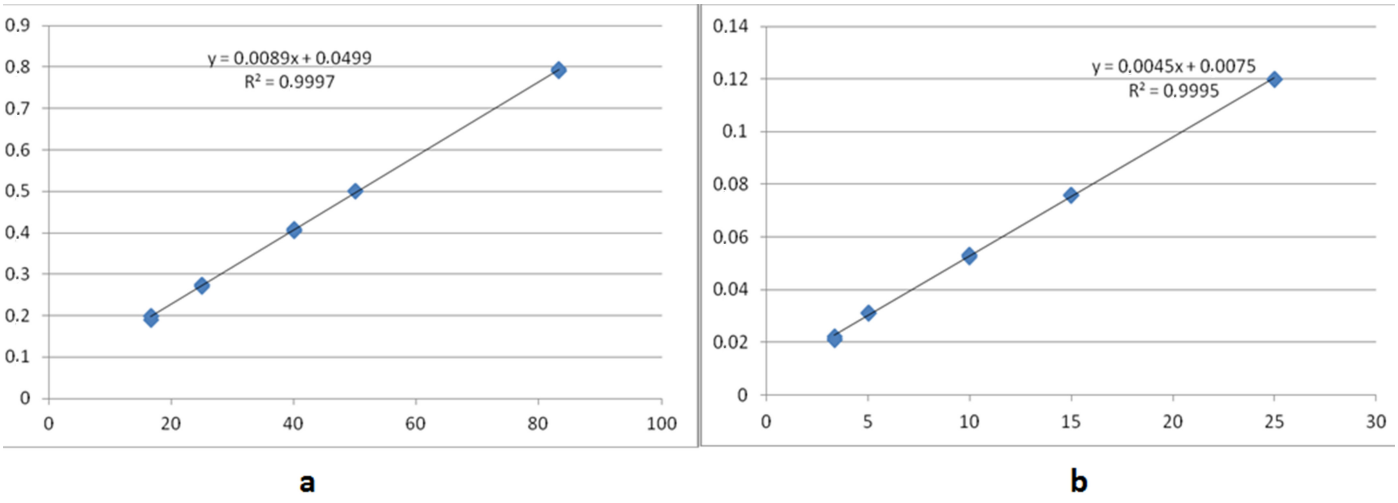

Figure 5. Calibration curves of DSM after derivation at 262.5 nm (a) and HSP after derivation at 269 nm (b).

Table 1. Parameters of linearity of methods A and B.

Parameters	Method A		Method B	
	DSM	HSP	Ratio 1	Ratio 2
Working lambda	262.5 nm	269 nm	268 nm DMN 285 nm HSP	268 nm DMN 285 nm HSP
Beer's Lambert limit µg/ml^{-1}	16–84	3–25	0%–100%	0%–100%
Correlation coefficient (R^2)	0.9997	0.9995	0.997	0.9979
Slope	0.0089	0.0045	0.6894	−0.6732
Intercept	0.0499	0.0075	0.7479	0.2492

Table 2. Result of precision of methods A and B.

Method		Drug	Percentage or concentration	RSD%
Method A		DMN	50 µg/ml	0.91885984
		HSP	10 µg/ml	0.54638827
Method B	Ratio 1	DMN	50%	0.44
		HSP	100	0.86
			75%	0.05
			100	0.22
	Ratio 2	DMN	50%	0.1
		HSP	100	0.42
			75%	0.07
			100	0.27

Table 3. Accuracy values of methods A and B.

Method	Mixture	Recovery			RSD		
		80%	100%	120%	80%	100%	120%
Method A	DSM	99.1	100.2	99.8	0.63	0.15	0.15
	HSP	100	101	102.5	0.1	0.70	1.74
Method B	DSM	0%	20%	40%	0%	20%	40%
	HSP	100%	80%	60%	100%	80%	60%
	Ratio 1	99.9	102	103	0.07	1.44	2.08
	Ratio 2	100.9	101	104	0.63	0.7	2.7

Table 4. Analysis of tablet formulation by method A and method B.

Method		Drug	Percentage labeled or amount labeled	Percentage found or percentage found	% label claim	% RSD
Method A		DSM	450	459	102	0.93
		HSP	50	49.1	98.2	0.85
Method B	Ratio 1	DSM	90	91.2	101.33	0.87
		HSP	10	98	98	
	Ratio 2	DSM	90	92.8	103.11	0.8
		HSP	10	7.2	72	

Table 5. Analysis of different stages of the DMN synthesis by ratio absorbance method and % RSD values.

Methods	Step of synthesis of DSM	Percentage labeled of DSM	Percentage found	% Label claim	% RSD
Ratio 1 A_{DSM}/A_{HSP}	Before synthesis	0	0	0	0
	After 1 hours 30	10	10.27	102.7	1.7
	After 2 hours 30	35	36.3	103.7	1.04
	In the middle of the synthesis	40	40.4	101	1.35
	After 3 hours 30	70	69.5	99.3	1.34
	After 4 hours 30	80	79.99	99.9	0.79
	In the end of synthesis	100	100.7	100.7	0.82
Ratio 2 $A_{HSP} - A_{DSM}/A_{HSP}$	Before synthesis	0	0	0	0
	After 1 hours 30	10	10.04	100.4	1.8
	After 2 hours 30	35	36.1	103.1	1
	In the middle of the synthesis	40	40.7	101.7	1.3
	After 3 hours 30	70	69.8	99.7	1.35
	After 4 hours 30	80	80.9	101.1	0.8
	In the end of synthesis	100	101.7	101.7	0.83

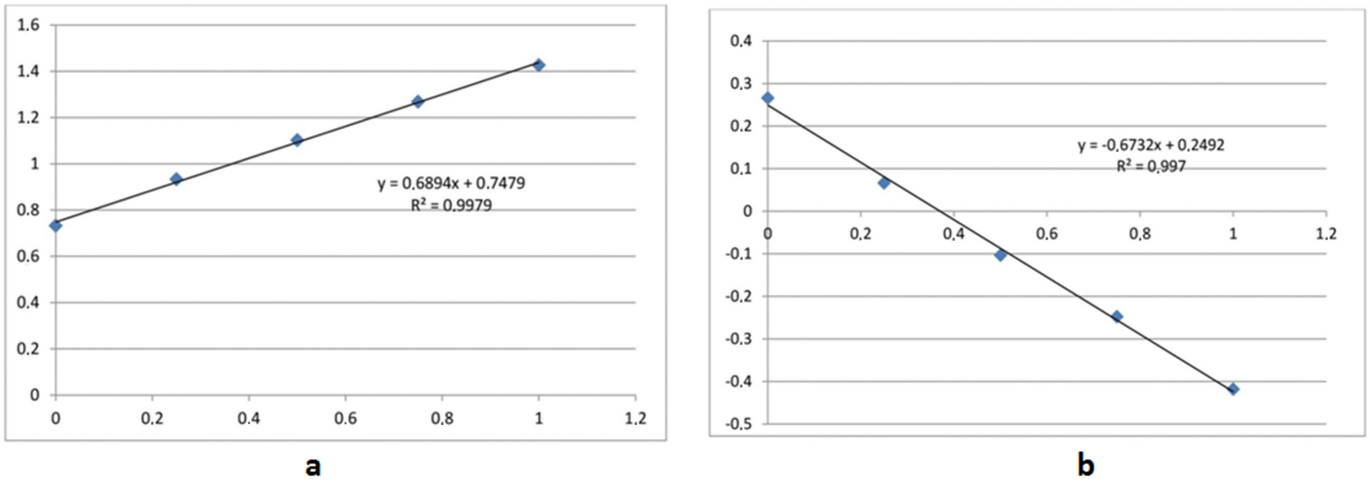

Figure 6. Calibration lines of the ratios of absorbances (a) and ratios of the difference of absorbances (b) according to percentage in different mixtures.

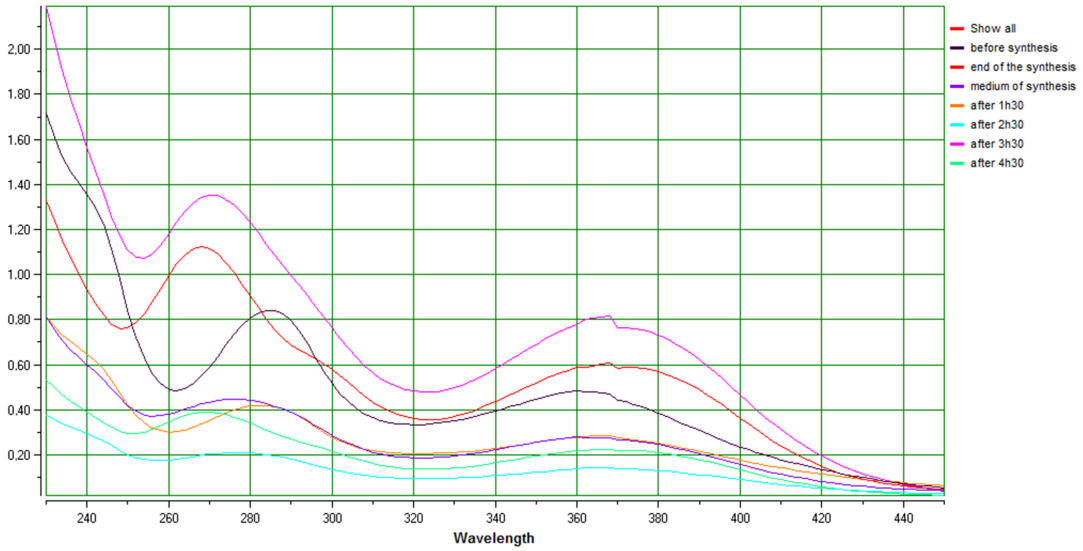

Figure 7. Overlain spectrum of different stages of the DMN synthesis.

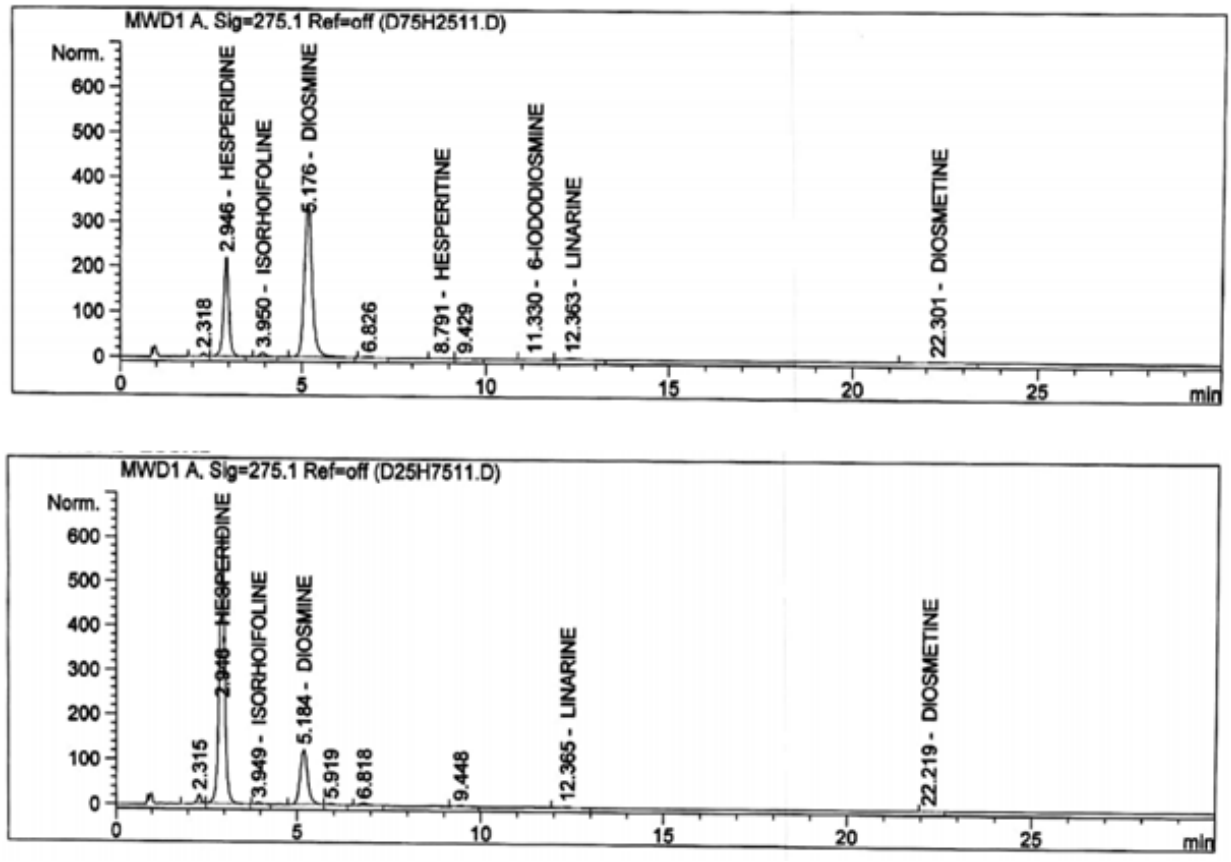

Figure 8. Chromatograms of DSM/HSP (1/4:25/75 and 3/4:75/25).

studied before are analyzed by HPLC, and the chromatograms confirm the results obtained by spectrophotometry. The example of chromatograms is shown in Figure 8. The interference effect with excess reagents and byproducts on spectrophotometric analysis is very negligible in the early stages of monitoring so that the byproducts are detected separately from the DSM and HSP peaks on a scale trace.

The various methods are reported in the prior art for the synthesis of DMN from HSP. There is a method that consists of the monobromination of acetylated flavanones in a chloroform solution by liquid bromide with ultraviolet radiation. The product obtained is a flavone derivative after the loss of hydrogen bromide and the deacetylation with an alcoholic alkali. Finally, the conversion of HSP to DMN is 37% (Kuntić *et al*., 2012).

There is also the bromination of acetylated HSP, N-bromosuccinimide in chloroform, and the benzoyl peroxide was used as a catalyst. The yield of DMN was 44% (Pavun *et al*., 2012).

The other methods describe the conversion of HSP, neohesperidin, and naringin to DSM, neodiosmin, and rhoifoline, respectively, by dehydrogenation with iodine in pyridine (El-Shafae and El-Domiaty, 2001) after separation and purification of DSM by macroporous resins and reported a 95% pure DSM (Campanero *et al*., 2010).

There is also a method describing the preparation of DSM by heating HSP, iodine, inorganic alkaline reagent, and reaction solvent in a mixture at 80°C–100°C (Mazzaferro *et al*., 2012). This method makes it possible to effectively avoid pyridine, which overcomes the defects of safety and product, and this is of the synthesis process that we will study in our study.

CONCLUSION

The methods presented are simple, selective, and reliable providing very good accuracy, with good specificity and good sensitivity. The results obtained are very good, and the agreement with reported protocol confirmed that the validated method can be considered as a good alternative to other costly techniques, in particular, chromatographic techniques. They could, therefore, be used easily and effectively for simultaneous dosage and determination of two active ingredients mixed in pharmaceutical form with precision. Given the time saved by these methods presented by the brief duration of the analysis. These methods can be used for both the quality control of pharmaceutical final forms and for monitoring the synthesis of DSM from HSP without interference by excipients.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in the publication of this research.

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None.

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