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Kinetic spectrophotometric determination of flavonoids using alkaline potassium permanganate

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ABSTRACT

Two simple and sensitive kinetic methods were developed for the determination of diosmin and hesperidin in bulk and pharmaceutical preparations. The proposed methods are based on kinetic investigation of the oxidation reaction of the drugs with alkaline potassium permanganate at room temperature for a fixed time of 20 min and 15 min for the first and second methods, respectively. In the first method the absorbance of the green colored manganate ion produced by diosmin and hesperidin was measured at 610 nm. Alternatively in the second method, the decrease in the absorbance of permanganate ion after addition of diosmin was measured at 525 nm. Calibration graphs were linear over the concentration range 5.0-25.0 and 1.0-20.0 μg/mL for diosmin using the two methods, respectively and 1.10-2.77 μg/mL for hesperidin, using the first method. The results obtained were compared statistically with those obtained by a reported method and showed no significant differences regarding accuracy and precision. In addition, the activation parameters such as E_a, ΔH, ΔS and ΔG were also evaluated for the reaction and found to be 15.85 KJ mol^{-1}, 40.79 KJ mol^{-1}, 25.59 JK^{-1} mol^{-1} and -7.63 KJ mol^{-1}, respectively.

KEYWORDS

Diosmin;
Hesperidin;
Kinetic spectrophotometric determination;
Potassium permanganate;
Activation parameters.

INTRODUCTION

Flavonoids are important polyphenolic secondary metabolites that are widely distributed in medicinal plants and foods of plant origin providing much of the flavor and color to fruits and vegetables[1]. Among these flavonoids; the flavone glycoside diosmin (3′,5,7- trihydroxy- 4′-methoxyflavone 7-rutinoside) and its flavanone analog hesperidin (3′,5,7- trihydroxy-4′-methoxyflavanone 7-rhamnoglycoside), the structures are represented in Figure 1.

The two flavonoids are common in many citrus species[1,2]. Diosmin and hesperidin possess antioxidant, blood lipid lowering, anti-carcinogenic activities[3,4]. In addition, both drugs improve venous tone, enhance microcirculation, assist healing of venous ulcers and they are used for the treatment of chronic venous insufficiency, haemorrhoids and the prevention of postoperative thromboembolism[1]. In view of the increasing interest in these bioflavonoids, especially for the treatment of chronic venous insufficiency, chronic hemorrhoids and as antioxidants, several methods have been reported for the determination of diosmin and hesperidin in plant extracts, biological fluids and pharmaceuti-

cal formulations, the majority of which are chromatographic in nature[1-3,5-11]. In addition, capillary electrophoretic techniques[12-14] as well as electrochemical methods have been also used for the determination of both drugs[15-18]. However, few spectrophotometric methods for analyzing diosmin[19-21] and hesperidin[20,22,23] could be found in literature. In addition spectrofluorimetric methods has been developed for the determination of hesperidin based on the formation of fluorescent metal chelates[24,25].

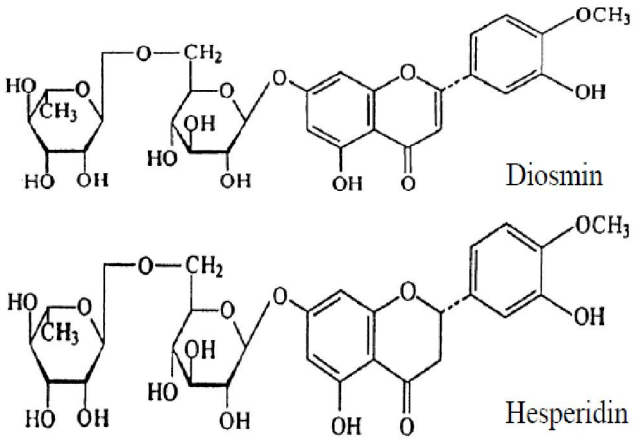

Figure 1 : Structures of diosmin and hesperidin.

Kinetic spectrophotometric methods are becoming of great interest in the pharmaceutical analysis[26-32]. The application of these methods offers some specific advantages such as improved selectivity, avoiding the interference of the colored and/or turbidity background of the samples, possibility of avoiding the interference of the other active ingredients present in the commercial products, and reduction of the analysis time when the analytical reaction requires long time for completion[33].

To the best of our knowledge, no attempt has been made for the kinetic spectrophotometric determination of diosmin and hesperidin. Therefore, the development of a kinetic spectrophotometric method for their determination was necessary. Thus, the aim of the present work was to study the reaction between the studied drugs with potassium permanganate in alkaline medium kinetically in an attempt to evaluate them in their dosage forms. The proposed method was simple and did not need sophisticated instruments or special skill, sensitive, rapid and readily adaptable to both the bulk drug and dosage forms.

EXPERIMENTAL

Apparatus

JASCO V-630 BIO spectrophotometer (S/N B206561148) equipped with kinetic accessory provided with temperature controlled cell (EHCS - 760) thermo-electric temperature. Recording range, 0-2; wavelengths 610 nm, 525 nm; factor 1; number of cell, 1; reaction time 30 min.; cycle time, 0.2 minutes.

Reagents and materials

All used chemicals were of analytical reagent grade and all solvents were of spectroscopic grade.

- Potassium permanganate (Merck, Darmstadt, Germany): $5x10^{-3}$M and 7.6×10^{-3} M aqueous solutions, freshly prepared.
- Sodium hydroxide (BDH, UK): 0.1M and 0.5M aqueous solutions are prepared.
- Acetonitrile (Merck, Darmstadt, Germany).
- Distilled water from "Aquatron" Automotive water Still A 4000 (bibby Sterillin Ltd., Staffordshire-UK).
- Diosmin and hesperidin were obtained as gifts from Sedico Pharmaceutical Co., 6 October City, Egypt. Their purities were certified and analyzed by a reported method[3] and were found to be 99.32% and 98.49%, respectively. They were used as provided.
- Pharmaceutical formulations of:
- Daflon 500 mg Tablets: Diosmin 450 mg, Hesperidin 50 mg B.N. 17162 (Servier Egypt industries limited, 6th October city, Giza, Egypt).
- Dioven 500 Tablets: Diosmin 500 mg B.N. 614710[A] (Amriya pharm. ind., Alexandia, Egypt),
- Veinatonic Tablets: Diosmin 450 mg, Hesperidin 50 mg B.N. 12227 (Sigma pharmaceutical industries, Egypt, S.A.E.)

Standard solutions

- Diosmin stock solution: 10 mg of diosmin were quantitatively transferred into a 100 mL volumetric flask and dissolved in 100 mL of 0.1 M NaOH.
- Hesperidin stock solution: 10 mg of hesperidin were quantitatively transferred into a 100 mL volumetric flask and dissolved in 100 mL of 0.1 M NaOH.
- Total flavanoids: 10 mg of diosmin and an aliquot equivalent to 1.1 mg of hesperidin from hesperidin

stock solution were quantitatively transferred into a 100 mL volumetric flask and the volume was completed to 100 mL with 0.1 M NaOH.

The stock solutions were protected from daylight and stored in a refrigerator (4^0C) where they are stable for up to five days.

Procedures

(a) Construction of kinetic calibration graph (first method)

Aliquot solutions containing 50-250 μg of standard diosmin and 111 - 277.7 μg of total flavanoids solutions were accurately and separately transferred into a series of 10-mL volumetric flasks. 1 mL of 0.5 M NaOH was added followed by 2 mL of 5×10^{-3} M $KMnO_4$. Mixtures were shaken well and completed to the volume with distilled water. The increase in absorbance at 610 nm was scanned during 20 minutes at room temperature against an appropriate blank prepared simultaneously.

(b) Construction of kinetic calibration graph (second method)

Aliquot solutions containing 10-200 μg of standard diosmin were accurately transferred into a series of 10-mL volumetric flasks. 1 mL of 0.5 M NaOH was added followed by 0.5 mL of 7.6×10^{-3} M $KMnO_4$. Mixtures were shaken well and completed to the volume with distilled water. The decrease in absorbance was measured during 15 minutes at room temperature at 525 nm against a similar blank prepared simultaneously.

For both methods; the reaction order was estimated by plotting log reaction rate ($\Delta A/\Delta t$) over the specified time period *versus* log concentration of the drug. The calibration graphs and the regression equations were constructed by plotting absorbance (A) at specified time *versus* concentration of the drug in μg/mL.

(c) Application to pharmaceutical formulation

(A) Dioven 500 tablets

Five tablets were pulverized well; an accurately weighed quantity of the powdered tablets equivalent to 10 mg of diosmin was transferred into a 100-mL measuring flask. The drug was dissolved in 0.1 M NaOH and the volume was completed to the mark with the same solvent. The resulting solution was sonicated for 5 min and filtered then the procedure described under calibration curve was carried out.

(B) Daflon 500 and veinatonic tablets

(1) For diosmin determination

Five tablets were pulverized well; an accurate amount of the powdered tablets equivalent to 10 mg of diosmin was transferred into a 150-mL beaker. Then 25 mL of methanol was added and the resulting mixture was sonicated for 15 min and filtered. The filtrate (containing hesperidin) was rejected. The residue (containing diosmin) was collected in the beaker and washed twice with water. Then the residue was dissolved in 0.1 M NaOH then transferred quantitatively to 100 mL volumetric flask with the aid of the same solvent. The volume was completed to the mark with 0.1 M NaOH. The resulting solution was sonicated for 5 min and filtered. Finally the procedure was completed as described under calibration curve.

(2) For total flavanoid determination

Five tablets were pulverized well; an accurate amount of the powdered tablets equivalent to 10 mg of diosmin and 1.1 mg hesperidin was weighed and transferred to a 100 mL volumetric flask. The drugs were dissolved in 0.1 M NaOH and the volume was completed to the mark with the same solvent. The resulting solution was sonicated for 5 min and filtered then the procedure described under calibration curve was carried out.

The nominal content of all dosage forms was calculated either from a previously plotted calibration graph or using the regression equation.

RESULTS AND DISCUSSION

Oxidation-reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds. $KMnO_4$ is a strong oxidant, and its oxidation for the organic compounds is pH dependent. During the course of the reaction, the valance of manganese changes and the intermediate ions have been suggested as participating oxidants. The species that are considered as potential oxidants depend on the nature of the substrate and the pH of the medium[34,35].

$KMnO_4$ has not been previously used for the spectrophotmetric determination of diosmin and hesperidin. In the present study, the reaction of alkaline $KMnO_4$ with the cited drugs was investigated, and it was found that $KMnO_4$ in alkaline medium has oxidized diosmin and hesperidin and yielded the green color of manganate radical, which absorbs maximally at 610 nm (Figure 2). As the intensity of the color increases with time, this has been used as a useful method for the determination of both drugs in bulk as well as in combined dosage forms (first method). Alternatively, owing to the consumption of $KMnO_4$ in the reaction, the absorbance of $KMnO_4$ at 525 nm decreases with time. This has been also used as a useful method for the determination of diosmin (second method).

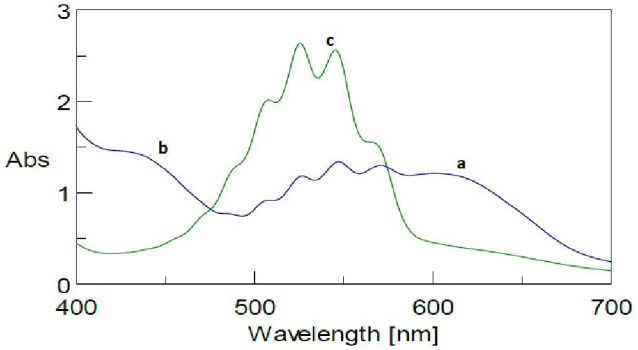

Figure 2 : Absorption spectra of diosmin after reaction with $KMnO_4$ / NaOH system. (a) The produced manganate ions after the reaction of $KMnO_4$ with diosmin (25 µg/mL); (b) Oxidation product of diosmin; (c) $KMnO_4$ (7.6×10^{-3} M).

It is worthy to note that in the Egyptian market there are not any dosage forms for hesperidin as a single component, in addition, it is present as a minor component in the combined dosage forms with diosmin, in a ratio of 1: 9 (hesperidin : diosmin). Hence regarding hesperidin, our aim was not to construct a calibration curve for the drug in bulk samples using the proposed methods and apply these methods to dosage forms. Because, obviously the linear concentration range for hesperidin will be very close to that of diosmin which would be an un-useful concentration range above the actual ratio present in the dosage forms. So, our main goal was to be able to analyze hesperidin quantitatively as a minor ingredient with keeping its linear concentration range nine times below that of diosmin. This was achieved by applying the oxidation procedure (of first method) on diosmin alone then on the total flavanoid mixture keeping the ratio of 1: 9 (hesperidin : diosmin) and obtaining the different kinetic spectra for diosmin and total flavanoids. Since both drugs are oxidized similarly with the production of manganate ion, thus, it was possible to subtract the kinetic spectra of diosmin from the corresponding ones of the total flavanoid mixture to obtain spectra representative of hesperidin. These spectra were used for constructing the calibration curve of hesperidin.

The following sections describe the optimization of different factors affecting the reaction, kinetics, and the use of the optimized conditions in the development of the assay procedures.

Study of experimental parameters

(a) Effect of diluting solvent

For both methods, the effect of diluting solvent was tried and the most suitable and economic one is distilled water.

(b) Effect of $KMnO_4$ concentration

In the first method, the reaction rate and maximum absorbance increased with increasing $KMnO_4$ concentration. It was found that 2 mL of 5×10^{-3} M $KMnO_4$ was adequate for the maximum absorbance, both for diosmin and total flavanoids (Figure 3). Higher concentrations of $KMnO_4$ yielded lower absorbance values probably due to decomposition of the products (Figure 2). While in the second method, the reaction rate and maximum absorbance reduction increased with increasing $KMnO_4$ concentration. It was found that 0.5 mL of 7.6×10^{-3} M $KMnO_4$ was adequate for the maximum absorbance reduction for diosmin (Figure 4).

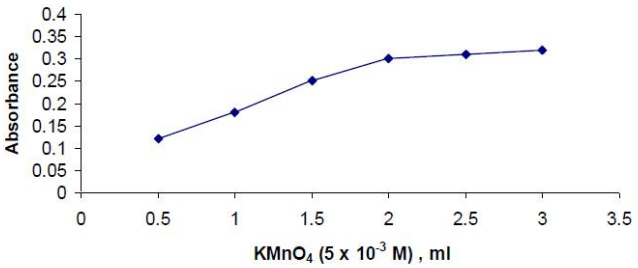

Figure 3 : Effect of $KMnO_4$ on the absorbance intensity at 610 nm.

(c) Effect of NaOH concentration

It was found that increasing the volume of 0.5 M NaOH would increase the absorbance of the reaction

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products up to 1 mL for the first method (Figure 5). In the second method increasing the volume of 0.5 M NaOH would increase the reduction in the absorbance of $KMnO_4$ up to 1 mL.

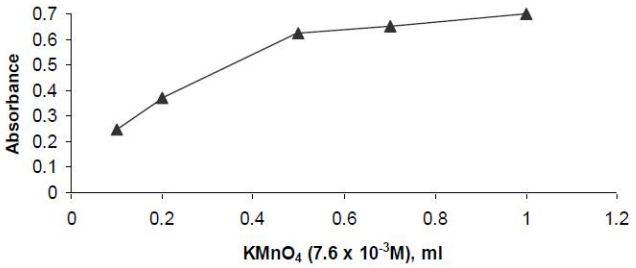

Figure 4 : Effect of $KMnO_4$ on the absorbance intensity at 525 nm.

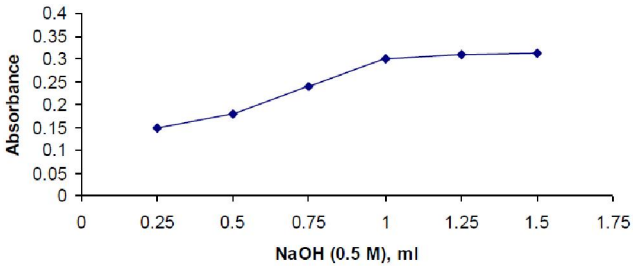

Figure 5 : Effect of NaOH on the absorbance intensity at 610 nm.

(d) Effect of temperature

The effect of temperature on the reaction rate was studied, it was found that, permanganate was reduced to manganate radical at room temperature (25°C) while at higher temperatures, manganese dioxide was produced. Therefore, room temperature was selected as the optimum temperature.

(e) Effect of time

The effect of time on the reaction between $KMnO_4$ and the studied drugs was studied. The absorbance of the reaction mixture changed with time so quantification was made at fixed times of 20 minutes in the first method and at 15 minutes in the second one (Figure 6 & 7).

Evaluation of kinetic parameters

As mentioned above, the reaction between $KMnO_4$ and the studied drugs never reach completion and a decision was made to apply a kinetic method for their determination. Consequently, the order of the reaction and reaction rate constants were determined at 610 and 525 nm. The rate of the reaction was found to be dependent on diosmin and hesperidin concentration. The rates were followed at room temperature with various concentrations in the range of 5.0-25.0 µg/mL and 1.0-20.0 µg/mL for diosmin using the first and second methods, respectively, and 1.10-2.77 µg/mL for hesperidin using the first method keeping $KMnO_4$ and NaOH concentrations constant at the recommended levels mentioned above. The reaction rate obeys the following equation:

$$\text{Rate of the reaction} = \Delta A/\Delta t = K'[\text{drug}]^n \quad (1)$$

Where K´ is the pseudo-order rate constant and n is the order of the reaction. The rate of the reaction may be estimated by the variable time method measurement[36], where A is the absorbance and t is the time in seconds. Taking logarithms of rates and drug concentrations (TABLE 1), the previous equation is transformed into:

$$\log(\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log[\text{drug}] \quad (2)$$

Plot of log reaction rate versus log drug concentration at 610 nm and 525 nm gave the regression equation, correlation coefficient, pseudo-order rate constant and order of the reaction which are indicated in TABLE 1. These results indicate that the reaction is pseudo first order reaction in the drug concentration.

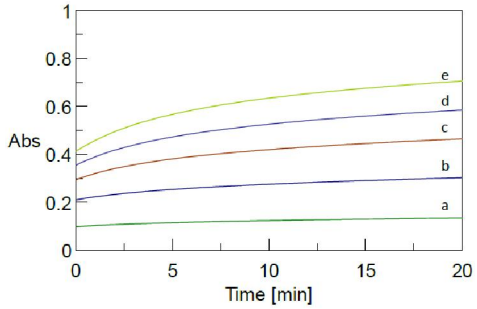

Figure 6 : Absorption versus time graphs for the reaction between diosmin and $KMnO_4$ at 610 nm. a) 5 µg/mL b) 10 µg/mL c) 15 µg/mL d) 20 µg/mL e) 25 µg/mL.

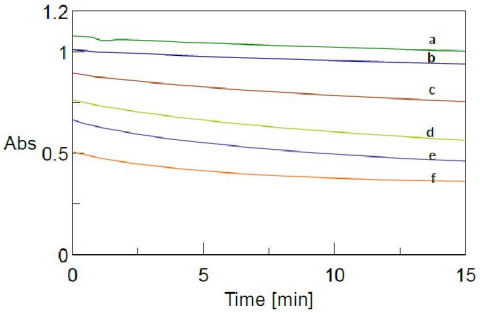

Figure 7 : Absorption versus time graphs for the reaction between diosmin and $KMnO_4$ at 525 nm. a) Blank b) 1 µg/mL c) 5 µg/mL d) 10 µg/mL e) 15 µg/mL f) 20 µg/mL.

TABLE 1 : Logarithms of rate for different concentrations of the drugs at room temperature.

Drug	Log ΔA/Δt	Log [drug]	Regression equation	Correlation coefficient	Rate constant (S^{-1})	Order of reaction (n)
At 610 nm Diosmin	-4.509	-5.084	Log rate= 0.6591+ 1.0167 log C	0.9999	4.561	1.0167
	-4.202	-4.783				
	-4.029	-4.607				
	-3.897	-4.481				
	-3.795	-4.384				
Hesperidin	-4.611	-5.744	Log rate= 0.9952+ 0.9761 log C	0.9999	9.890	0.9761
	-4.321	-5.443				
	-4.221	-5.346				
At 525 nm Diosmin	-4.996	-5.785	Log rate= 0.5505+ 0.9600 log C	0.9999	3.552	0.9600
	-4.552	-5.308				
	-4.336	-5.085				
	-4.048	-4.785				
	-3.870	-4.607				
	-3.746	-4.481				

Selection of the best kinetic method

Several kinetic techniques were adopted for the selection of the best method. Rate constant, fixed absorbance and fixed time methods[36] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, i.e. the slope of the calibration graph and the correlation coefficient (r).

(a) Rate constant method

Graphs of log absorbance versus time for drugs concentration in the range of $1.64 \times 10^{-5} - 3.30 \times 10^{-5}$ M and $8.22 \times 10^{-6} - 3.30 \times 10^{-5}$M for diosmin using the first method and second method, respectively and $1.80 \times 10^{-6} - 4.50 \times 10^{-6}$M for hesperidin using the first method were plotted and all appeared to be rectilinear. Pseudo-first order rate constants (K′) corresponding to different drug concentrations (C) were calculated from the slopes multiplied by –2.303 and are presented in TABLE 2.

Regression of (C) versus K′ gave equations:

At 610 nm

$K' = 1.2 \times 10^{-3} + 28.15C$ r = 0.9958 (for diosmin)

$K' = 7.0 \times 10^{-4} + 120.53C$ r = 0.9986 (for hesperidin)

At 525 nm

$K' = 5.0 \times 10^{-4} + 13.40\ C$ r = 0.9957 (for diosmin)

Where C is the molar concentration of the drug.

TABLE 2 : Application of the rate constant method in the quantification of the studied drug with $KMnO_4$.

Drug	[drug]	K′/S^{-1}	
		At 610 nm	At 525 nm
Diosmin	8.22×10^{-6}		-4.00×10^{-4}
	1.64×10^{-5}	-7.21×10^{-4}	-2.70×10^{-4}
	2.47×10^{-5}	-4.50×10^{-4}	-1.55×10^{-4}
	3.30×10^{-5}	-2.50×10^{-4}	-6.91×10^{-5}
Hesperidin	1.80×10^{-6}	-4.60×10^{-4}	
	3.60×10^{-6}	-2.30×10^{-4}	
	4.50×10^{-6}	-1.38×10^{-4}	

(b) Fixed absorbance method

Reaction rates were recorded for different concentrations of the drugs in the range of $2.47 \times 10^{-5} - 4.11 \times 10^{-5}$M and $1.64 \times 10^{-5} - 1.97 \times 10^{-5}$ M for diosmin using the first and second method, respectively, and $1.80 \times 10^{-6} - 4.50 \times 10^{-6}$M for hesperidin using the first method. Preselected values of the absorbance (0.45) and (0.35) for diosmin using the first and second method, respectively and (0.07) for hesperidin in the first method were fixed and the time was measured in seconds. The reciprocal of times (1/t) versus the initial concentrations of drug (TABLE 3) were plotted and the following equations of the calibration graphs were obtained:

At 610 nm

$1/t = -1.71 \times 10^{-2} + 718.05C$, r = 0.9977 (for diosmin)

$1/t = -6.2 \times 10^{-3} + 4004.6C$, r = 0.9992 (for hesperidin)

At 525 nm

$1/t = -1.12 \times 10^{-1} + 6920.20C$, r = 0.9973 (for diosmin)

Where C is the molar concentration of the drug.

(c) Fixed time method

At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of the absorbance versus initial concentrations of diosmin and hesperidin at fixed times of 20 min. in the first method and 15 min. in the second method, were established with the regression equations and correlation coefficients assembled in TABLE 4. It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) was chosen as the most suitable time interval for the measurement.

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As a conclusion, the fixed time method was chosen for quantification because it gave the best correlation coefficient in a reasonable time.

TABLE 3: Application of the fixed absorbance method in the quantification of the studied drugs with $KMnO_4$.

Drug	[drug]	1/t (sec.$^{-1}$)	
		At 610 nm	At 525 nm
Diosmin	2.47×10^{-5}	9.01×10^{-4}	
	3.30×10^{-5}	6.17×10^{-3}	
	4.11×10^{-5}	1.28×10^{-2}	
	1.64×10^{-5}		1.11×10^{-3}
	1.80×10^{-5}		1.39×10^{-2}
	1.97×10^{-5}		2.38×10^{-2}
Hesperidin	1.80×10^{-6}	1.1×10^{-3}	
	3.60×10^{-6}	8.0×10^{-3}	
	4.50×10^{-6}	1.2×10^{-2}	

TABLE 4: Application of the fixed time method in the quantification of the studied drugs with $KMnO_4$.

Drug	Time (min.)	Regression equation	Correlation Coefficient
At 610 nm Diosmin	5	A= 0.0070 + 0.0226C	0.9982
	10	A= -0.0024 + 0.0263C	0.9993
	15	A= -0.0070 + 0.0286C	0.9995
	20	A= -0.0050 + 0.0298C	0.9998
Hesperidin	5	A= 0.0456 + 0.0119C	0.9972
	10	A= 0.0513 + 0.0132C	0.9994
	15	A= 0.0564 + 0.0134C	0.9998
	20	A= 0.0562 + 0.0144C	0.9999
At 525 nm Diosmin	5	A= 0.0436 + 0.0298C	0.9990
	10	A= 0.0495 + 0.0303C	0.9995
	15	A= 0.0587 + 0.0309C	0.9999

Method validation

(a) Linearity and range

After optimizing the reaction conditions, the fixed time method was applied to the kinetic determination of pure diosmin and hesperidin. The calibration graphs for the determination of the drugs were constructed and were found to be rectilinear within the concentration ranges and with the regression equations cited in TABLE 5.

(b) Accuracy and precision

The accuracy of the proposed method was evaluated by analyzing five levels of standard solutions of the studied drugs, each three times. The results obtained by the proposed method were favorably compared with those of a reported one[3]. Statistical analysis[37] obtained by the proposed and reported methods using student´s t-test and variance ratio F-test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (TABLE 6).

TABLE 5 : Performance data for diosmin and hesperidin by the proposed methods.

Parameter	At 610 nm		At 525 nm
	Diosmin	Hesperidin	Diosmin
Concentration range (µg/ml)	5-25	1.1-2.77	1-20
Regression equation	$-5.00\times10^{-3}+2.98\times10^{-2}C$	$5.62\times10^{-2}+1.44\times10^{-2}C$	$5.87\times10^{-2}+3.09\times10^{-2}C$
Slope	2.98×10^{-2}	1.44×10^{-2}	3.09×10^{-2}
Intercept	-5.00×10^{-3}	5.62×10^{-2}	5.87×10^{-2}
Correlation Coefficient	0.9998	0.9999	0.9999
$S_{y/x}$	4.83×10^{-3}	1.54×10^{-4}	4.02×10^{-3}
SE of slope	3.06×10^{-4}	1.28×10^{-4}	2.43×10^{-4}
SE of intercept	5.07×10^{-3}	2.74×10^{-4}	2.73×10^{-3}
LOD (µg/ml)	0.53	0.34	0.43
LOQ (µg/ml)	1.62	1.03	1.43

$S_{y/x}$ = Standard deviation of residuals, LOD = Limit of detection, LOQ = Limit of quantification

TABLE 6 : Accuracy and precision data obtained by the proposed method and a reported one[3] for the analysis of the studied drugs in pure form.

Item	Diosmin			Hesperidin	
	Proposed At 610 nm	Proposed At 525nm	Reported[3]	Proposed At 610 nm	Reported[3]
Mean ± SD	100.24 ± 0.86	99.91 ± 0.63	99.32 ± 0.67	99.09 ± 0.75	98.49 ±0.71
%RSD	0.86	0.63	0.67	0.76	0.72
Variance	0.74	0.40	0.45	0.56	0.51
t- test	1.77(2.36)	1.38(2.36)		1.22(2.36)	
F- test	1.65(9.12)	1.12(6.59)		1.11(9.12)	
Intraday precision	100.96 ± 0.95	100.35 ± 0.69		102.06 ± 0.84	
%RSD	0.94	0.69		0.84	
Variance	0.90	0.48		0.74	
Interday precision	99.85 ± 0.79	99.54 ± 0.61		97.81 ± 1.07	
%RSD	0.79	0.61		1.07	
Variance	0.62	0.37		1.1	

S.D = Standard deviation, % RSD = Percentage relative standard deviation, Tabulated t-test and F-ratio at P = 0.05 and n = 5 for the proposed method and n = 4 for the reported method. Intra-day and interday relative standard deviation of the average of three concentrations

TABLE 7 : Statistical comparison for the results obtained by the proposed method and the reported one[3] for the analysis of diosmin and hesperidin in their dosage forms.

Item	Dioven 500 tablets			Daflon 500 mg tablets			Veinatonic tablets		
Diosmin	Proposed At 610 nm	Proposed At 525 nm	Reported[3]	Proposed At 610 nm	Proposed At 525 nm	Reported[3]	Proposed At 610 nm	Proposed At 525 nm	Reported[3]
Mean±SD	98.39±0.72	98.16±0.45	97.52±0.70	99.01±0.83	98.41±0.89	97.99±0.86	98.01±0.83	97.97±0.48	98.43±0.75
Variance	0.52	0.20	0.49	0.68	0.79	0.75	0.69	0.23	0.56
t- test	1.82(2.36)	1.68(2.36)		1.80(2.36)	0.71(2.36)		0.78(2.36)	1.12(2.36)	
F- test	1.07(9.12)	2.41(6.59)		1.09(6.59)	1.06(9.12)		1.23(9.12)	2.41(6.59)	
Hesperidin				**Daflon 500 mg tablets**			**Veinatonic tablets**		
Mean±SD				98.10±0.70		97.54±0.54	98.79±0.76		98.31±0.66
Variance				0.49		0.29	0.57		0.44
t- test				1.30(2.36)			1.01(2.36)		
F- test				1.65(9.12)			1.31(9.12)		

Tabulated t-test and F-ratio at P = 0.05 and n = 5 for the proposed method and n = 4 for the reported method.

In addition, the intraday precision was evaluated through replicate analysis of the standard solutions of the drugs. While the interday precision was performed through replicate analysis of the standard solutions of the drugs on three successive days. The percentage recoveries as well as the percentage relative standard deviations were calculated as abridged in TABLE 6.

(c) Limit of detection (LOD) and limit of quantitation (LOQ)

The Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to ICH Q2B recommendations[38], the results are shown in TABLE 5.

(d) Robustness of the method

The robustness of the method adopted was demonstrated by the consistency of the absorbance values with the deliberately minor changes in the experimental parameters.

Application of the proposed method

The proposed method was successfully applied to the determination of diosmin and hesperidin in tablets of different brands. The concentrations of the drugs were calculated referring to the corresponding regression equation. Commonly used tablet excipients did not interfere in the analysis as indicated by the percentages found. The results obtained are abridged in TABLE 7 and were in accordance with that obtained from the reference method[3].

Mechanism of the reaction

The data used in the optimization of $KMnO_4$ concentration and the data of the calibration graphs were used to calculate the stoichiometry of the reaction adopting the limiting logarithmic method[39]. The ratio of the reaction between diosmin and $KMnO_4$ in alkaline medium was calculated by dividing the slope of $KMnO_4$ curve over the slope of the drug curve (Figures 8A & 8B). It was found that, the ratio was 0.90 : 0.92 for diosmin pointing out to a ratio of 1 : 1 ($KMnO_4$ to drug). Based on the obtained molar reactivity, the reaction pathway is proposed to proceed as in Scheme 1.

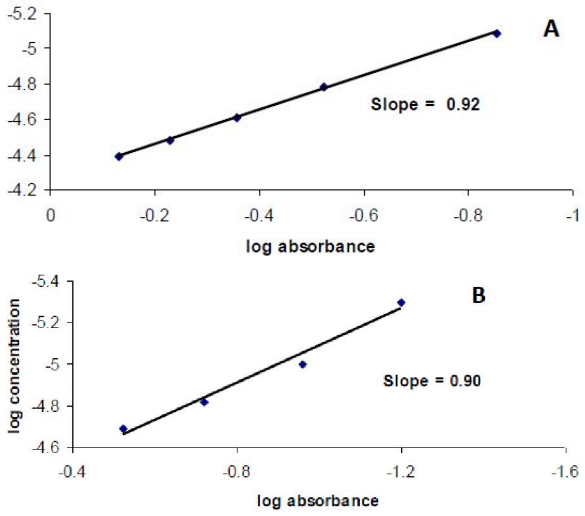

Figure 8 : Stoichiometry of the reaction between diosmin and $KMnO_4$ adopting limiting logarithmic method[39]. (A)Variable concentrations of diosmin at constant $KMnO_4$ concentration; (B) Variable concentrations of $KMnO_4$ at constant diosmin concentration.

$$\text{Diosmin} + MnO_4^- + OH^- \longrightarrow \text{REACTION PRODUCTS} + MnO_4^{2-} + H_2O$$

Scheme 1 : The proposed pathway for the reaction between diosmin and potassium permanganate in alkaline medium.

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Activation parameters

For the evaluation of apparent activation parameter[40], the reaction was studied at 298, 303, and 308 K at diosmin = 1.65 x 10^{-5} M, [$KMnO_4$] = 5x10^{-3} M and [NaOH] = 0.5 M at 610 nm. The Arrhenius plot of ln k versus 1/T was found to be linear with a correlation coefficient of 0.9997 (Figure 9). The Eyring plot of ln k/T versus 1/T was linear with a correlation coefficient of 0.9999 (Figure 10). The value of Ea was evaluated from the slope (-Ea/R) of Arrhenius plot and found to be 15.85 KJ mol^{-1}. The value of ΔH and ΔS were evaluated from the slope (-ΔH/R) and intercept [ln (k_b/h) + ΔS/R] of Eyring plot and found to be 40.79 KJ mol^{-1} and 25.59 JK^{-1} mol^{-1}, respectively. The value of Gibbs free energy (ΔG) of activation of the reaction product was found to be -7.63 KJ mol^{-1}. This value indicated that the proposed reaction is a favored reaction (spontaneous) can occurr without external supply of energy.

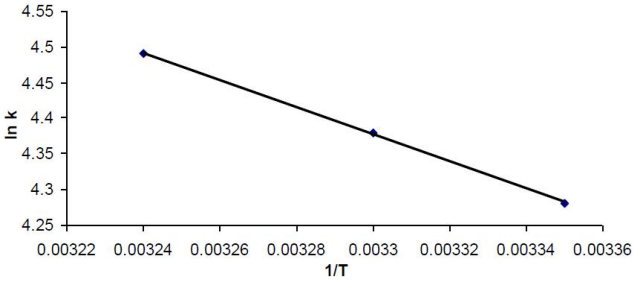

Figure 9 : Arrhenius plot of ln k versus 1/T at 298.0, 303.0, and 308.0 K for determination of activation energy at 610 nm.

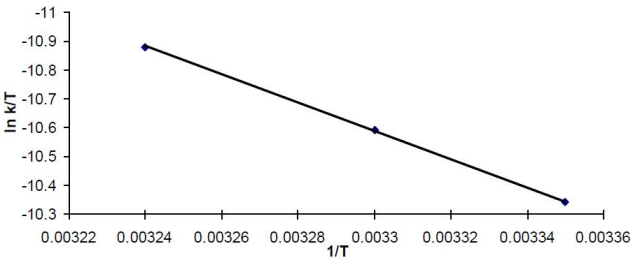

Figure 10 : Eyring plot of ln k/T versus 1/T at 298.0, 303.0, and 308.0 K for determination of ΔH and ΔS at 610 nm.

CONCLUSION

The proposed kinetic methods are appreciable with a view that the oxidation of drugs can be exploited for the routine quality control analysis of diosmin and hesperidin in their pharmaceutical formulations. The proposed methods are sensitive with a simple calibration system that does not require any laborious clean up procedure prior to analysis. Moreover the present technique has the advantage of using inexpensive and easily available reagents and therefore can be frequently used in the laboratories of research, hospitals and pharmaceutical industries. The only limitation for these method, if used in other pharmaceutical preparations containing antioxidant or any other oxidisable matter which will cause interference and this can be solved by using suitable solvent extraction.

REFERENCES

[1] F.I.Kanaze, C.Gabrieli, E.Kokkalou, M.Georgarakis, I.Niopas; J.Pharm.Biomed.Anal., **33**, 243 **(2003)**.
[2] T.H.Tsai, M.C.Liu; J.Chromatogr.B, **806**, 161 **(2004)**.
[3] A.M.El-Shafae, M.M.El-Domiaty; J.Pharm. Biomed.Anal., **26**, 539 **(2001)**.
[4] F.I.Kanaze, E.Kokkalou, M.Georgarakis, I.Niopas; J.Pharm.Biomed.Anal., **36**, 175 **(2004)**.
[5] M.A.Campanero, M.Escolar, G.Perez, E.Garcia-Quetglas, B.Sadaba, J.R.Azanza; J.Pharm.Biomed. Anal., **51**, 875 **(2010)**.
[6] B.Abad-Garcia, L.A.Berrueta, D.M.Lopez-Marquez, I.Crespo-Ferrer, B.Gallo, F.Vicente; J.Chromatogr.A, **1154**, 87 **(2007)**.
[7] X.Li, H.Xiao, X.Liang, D.Shi, J.Liu; J.Pharm. Biomed.Anal., **34**, 159 **(2004)**.
[8] C.Caristi, E.Bellocco, V.Panzera, G.Toscano, R.Vadala, U.Leuzzi; J.Agric.Food Chem., **51**, 3528 **(2003)**.
[9] D.J.Guedon, B.P.Pasquier; J.Agric.Food Chem., **42**, 679 **(1994)**.
[10] Z.Janeczko, U.Hubicka, I.Podolak, J.Krzek; Chem.Anal.(Warsaw,-Pol), **49**, 309 **(2004)**.
[11] Z.Janeczko, U.Hubicka, J.Krzek, I.Podolak; J.Planar Chromatogr.Mod.TLC., **16**, 377 **(2003)**.
[12] S.Sawalha, D.Arraez-Roman, A.Segura-Carretero, A.Fernandez-Gutierrez; Food Chem., **116**, 567 **(2009)**.
[13] T.Wu, Y.Guan, J.Ye; Food Chem., **100**, 1573 **(2007)**.
[14] Z.Aturki, M.Sinibaldi; J.Sep.Sci., **26**, 844 **(2003)**.
[15] M.J.Sims, Q.Li, R.T.Kachoosangi, G.G.Wildgoose, R.G.Compton; Electrochim.Acta., **54**, 5030 **(2009)**.
[16] M.S.El-Shahawi, A.S.Bashammakh, T.El-mogy;

Anal.Sci., **22**, 1351 **(2006)**.
[17] N.Pejic, S.Blagojevic, S.Anic, V.Vukojevic, L.Kolar-Anic; Anal.Bioanal.Chem., **381**, 775 **(2005)**.
[18] D.Sun, F.Wang, K.Wu, J.Chen, Y.Zhou; Microchim. Acta, **167**, 35 **(2009)**.
[19] Z.Moldovan, A.A.Bunaciu, M.A.Al-Omar, H.Y.Aboul-Enein; The Open Chemical and Biomedical Methods Journal, **3**, 123 **(2010)**.
[20] A.El-Bayoumi; Anal.Lett., **32**, 383 **(1999)**.
[21] S.R.Mehra; East Pharm., **33**, 179 **(1990)**.
[22] D.Malesev, Z.Radovic, V.Kuntic, M.Kosanic; Anal.Lett., **30**, 917 **(1997)**.
[23] Z.Radovic, D.Malesev, M.Jelikic-Stankov; Pharmazie, **51**, 604 **(1996)**.
[24] T.Kaito, T.Yoshida, K.Sagara; Yakuzaigaku (through analytical abstract), **39**, 210 **(1979)**.
[25] T.Perez-Ruiz, C.Martinez-Lozano, V.Tomas, J.Fenoll; Fresenius J.Anal.Chem., **364**, 279 **(1999)**.
[26] R.M.Youssef, E.F.Khamis, A.A.Gazy, H.Mahgoub, M.A.El-Sayed; Chin.Pharm.J., **58**, 85 **(2006)**.
[27] M.I.Walash, M.K.Sharaf-El-Din, M.S.Metwally, M.R.Shabana; J.Chen.Chem.Soc., **52**, 71 **(2005)**.
[28] B.S.Virupaxappa, K.H.Shivaprasad; Int.J.Chem.Res., **2**, 21 **(2011)**.
[29] N.El-Enany, F.Belal, M.Rizk; Sci.Pharm., **74**, 99 **(2006)**.
[30] F.A.Ibrahim, F.A.Ali, S.M.Ahmed, M.M.Tolba; Int.J.Biomed.Sci., **3**, 20 **(2007)**.
[31] A.M.El-Brashy, Z.A.Sheribah, M.K.Sharaf-El-Din, R.M.El-Gamal; Int.J.Biomed.Sci., **3**, 65 **(2007)**.
[32] M.I.Walash, A.M.El-Brashy, M.S.Metwally, A.A.Abdelal; Bull.Korean Chem.Soc., **25**, 517 **(2004)**.
[33] I.A.Darwish, M.A.Sultan, H.A.Al-Arfaj; Int.J.Res.Pharm.Sci., **1**, 43 **(2010)**.
[34] H.F.Askal; Bull.Pharm.Sci.Assiut Univ., **20**, 75 **(1997)**.
[35] N.Rahman, N.A-Khan, S.N.Azmi; Pharmazie, **59**, 112 **(2004)**.
[36] K.B.Yatsimirskii; Kinetic methods of analysis, Pergamon Press, London, **(1966)**.
[37] J.C.Miller, J.N.Miller; Statistics for analytical chemistry, 5th Edition, Wiley, New York, **(2005)**.
[38] ICH Harmonized tripartite guideline. Validation of analytical procedures: Text and methodology, Q2(R1), Current Step 4version, Parent guidelines on Methodology Dated November6, 1996, Incorporated in November, **(2005)**.
[39] J.Rose; Advanced physicochemical experiments, Pitman, London, **(1964)**.
[40] A.Martin; Physical pharmacy, Lippincott Williams & Wilkins, U.S.A, **(1983)**.