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Simultaneous spectrophotometric determination of rutin, quercetin and ascorbic acid in drugs using a Kalman Filter approach

H.N.A. Hassan [a,*], B.N. Barsoum [b], I.H.I. Habib [a]

[a] *Microanalytical Research Laboratory, National Research Centre, Dokki, Cairo, Egypt*
[b] *Chemistry Department, Faculty of Science, Cairo University, Cairo, Egypt*

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Abstract

A UV-spectrophotometric analysis of severely overlapped spectra yielded by anti-capillary fragility pharmaceutical materials, viz. rutin, quercetin and ascorbic acid, in authentic mixtures and in tablets or soft elastic capsules was proposed. The method was based on Kalman Filter calibration of either orthogonal experimental design, i.e. standard solutions containing only one component at a time, or non-orthogonal experimental design, i.e. standard solutions containing more than one component. This calibration was followed by quantitative determinations of two- and three-component mixtures in synthetic mixtures or in oral dosages within the concentration range 2–10 μg ml^{-1}. A statistical analysis of the results was reported.

Keywords: UV-spectrophotometry; Vitamins P and C; Kalman Filter

1. Introduction

Rutin and quercetin, which chemically classified as flavonols, have a direct construction action on the capillary bed and decrease the permeability and fragility of the blood vessels [1]. Some workers have claimed better results from the use of rutin in combination with ascorbic acid than from rutin alone. It has been suggested that these substances could be classed as vitamins, particularly of the 'Vitamin P'. Quercetin is present as a natural co-existence with rutin in plants. It may also be present as a hydrolytic product of rutin in pharmaceuticals, containing rutin and ascorbic acid as tablets and soft gelatine capsules, when they are not well stored [2].

Various methods for the determination of rutin in drugs and extractants have been reviewed [3]. Few methods for determining the active ingredients in binary mixtures were reported. Binary mixture of rutin and ascorbic acid in drugs was determined UV-spectrophotometrically in combination with dual-wavelength technique [4] or orthogonal function method [5]. Both were based on limited number of wavelengths. The two cited

* Corresponding author. Fax: +20-2-3370931.
E-mail address: hassanhna@yahoo.com (H.N.A. Hassan)

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components were chemically determined by flow-injection spectrophotometric analysis [6], high-performance liquid chromatography (HPLC) [7], or recently by reversed-phase HPLC [8]. Rutin and ascorbic acid have been also estimated but by two steps [9,10], the first was by direct spectrophotometric determination of rutin in alcoholic solution and the second involved dual-wavelength spectrophotometric determination of ascorbic acid after treatment with HCl [9] or with NaCl [10] to separate rutin. Another form of binary mixtures to be analyzed was rutin and quercetin, in the presence of ascorbic acid, which have been estimated simultaneously either using first derivative spectrophotometry [2], or from the ratio of the absorbances after reaction with powdered magnesium and HCl [11,12].

The aim of the present work is the application of computer program based on Kalman Filter multivariate calibration and prediction to analyze the full-spectra of binary and ternary mixtures of rutin, quercetin and ascorbic acid without chemical separation.

2. Experimental

2.1. Apparatus

A SHIMADZU UV-240 recording spectrophotometer with 2 nm slit width and 1 cm quartz cell were used. A PC IBM or compatible computer was used for the calibration and prediction processes, the numerical data or ASCII file was inline entered into CASAP program [13] which includes the Kalman Filter program.

Table 1
The composition of non-orthogonal calibration samples in μg ml^{-1}

Sample no.	1	2	3	4	5	6	7	8
Ascorbic acid	10	2	10	10	10	2	2	2
Rutin	5	5	2	5	2	2	5	2
Quercetin	5	5	5	2	2	5	2	2

2.2. Reagents

Methanol (Merck), Rutin (Fluka), Quercetin (Fluka) and Ascorbic acid (Analar) were used. Ruta-C tablets (El-Kahira, Egypt) and Rutin C capsules (Pharco Pharmaceuticals, Egypt) labelled to contain 60 and 50 mg for Rutin and 160 and 100 mg for Ascorbic acid per tablet and capsule, respectively. The fillers used in tablets were lactose, magnesium stearate, sodium benzoate, maize starch, sucrose and talc, whereas that in soft elastic capsules were soybean oil, partially hydrogenated vegetable oil, soylecithin and ethyl vanillin.

2.3. Procedure

Appropriate amounts of rutin, quercetin and ascorbic acid were dissolved in methanol so that after further dilution with the same solvent, the final concentrations for measuring the absorbances were in the range 2–10 μg ml^{-1} for each. Two experimental designs were used. One was based on orthogonal design where only three standard solutions of pure components were prepared, i.e. 10 μg ml^{-1} for each, and the other was based on non-orthogonal design which was constructed as given in Table 1. The absorption measurements of each standard solution were recorded at room temperature (25°C) against a blank of methanol at 5 nm wavelength intervals over the range 450–200 nm (51 data points were used in calculations).

To assay tablets, ca. 0.5 g finely powdered of ten tablets was transferred into a 50-ml measuring flask and completed to the mark with methanol. Further dilution with methanol was achieved to get a reasonable concentration range, 2–10 μg ml^{-1}, and the procedure was completed as described before.

To assay soft elastic capsules, a capsule was placed in a 50-ml beaker containing 10 ml methanol and heated over water bath until all the contents in the capsule, using a glass rod, was emerged into methanol. The beaker was well shaken and left to cool, then the solution was transferred into a 50-ml measuring flask and the procedure was completed as described before. The

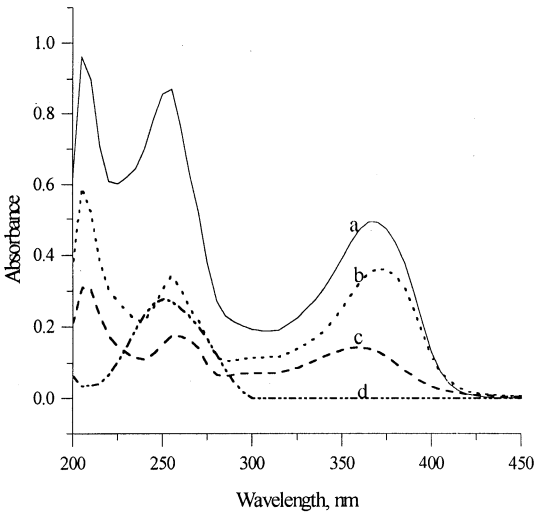

Fig. 1. Overlapped UV-visible spectrum (a) including spectra of (b) 5 μg ml^{-1} quercetin, (c) 5 μg ml^{-1} rutin and (d) 10 μg ml^{-1} ascorbic acid.

oily paste remained, after dissolution procedure, was insoluble in methanol and had no effect on the determination.

3. Results and discussion

The absorption spectra of rutin, quercetin and ascorbic acid over the wavelength range 450–200 nm (Fig. 1), shows markedly overlapping each other that high errors would be expected if the conventional univariate regression method was used. The calibration and then prediction of multicomponent systems are of the most important research areas in chemometrics. One of the simplest methods investigated in this field was Kalman Filter. The theoretical background of recursive Kalman filter KF calibration and prediction algorithms can be found elsewhere [14–16]. To start the recursion, the initial guesses for the state variable $k(0)$, the diagonal elements in covariance matrix $P(0)$ and the measurement noise scalar $R(0)$ were assigned as 0, 100 and 1×10^{-5}, respectively

In multicomponent spectrophotometric analysis of overlapping spectra, the acceptable accuracy and precision results can be obtained by application of error propagation approach [17]. In this approach, a small error in response can be magnified by two factors in the worse case to produce a greater relative concentration error, error amplification or propagation. The first factor was the condition number of the calibration matrix (cond(K)), which is a measure for the selectivity of an analyte in a multicomponent system [17–22]. The second factor was the condition number of experimental design (cond(C)), which represents the numerical accuracy of standard solutions prepared that can be lost among large differences in concentrations and varying sensitivities of the analytes to the sensors [17]. Ideally, cond(K) and cond(C) should be unity for no overlapping spectra and for solutions containing single components, respectively. Two different experiments were designed, one with orthogonal solutions, i.e. standard solutions consisting of only one component at a time, and the other with multiple or non-orthogonal solutions, i.e. standard solutions containing more than one component mixed together so that prediction error due to the interaction between two or more components in the mixture is minimized. Jochum et al. [17] showed that the most accurate results can be achieved when cond(C) is unity. This was achieved practically by application of orthogonal calibration design. The scaled C^t C matrix is therefore a diagonal matrix with condition number one. Table 1 shows non-orthogonal calibration mixtures on the basis of full factorial box design L^n [18] at two concentration levels, $L = 2$, for analyzing three-component system, $n = 3$, within the calibrated concentration range, i.e. 2 and 10 μg ml^{-1} for ascorbic acid, 2 and 5 μg ml^{-1} for both rutin and quercetin. Thus there are eight different standard mixtures as outlined in Table 1. The condition number of concentration matrix in this case was 5.673, which is numerically so far from the unity that high uncertainty in the concentration prediction is expected.

On the other hand, the larger the cond(K) becomes, the larger the concentration errors will be. It has been shown that a minimum in cond(K) can be used as a criterion for determining the optimal set of sensors [17–22], which includes the most selective combination of wavelengths. This

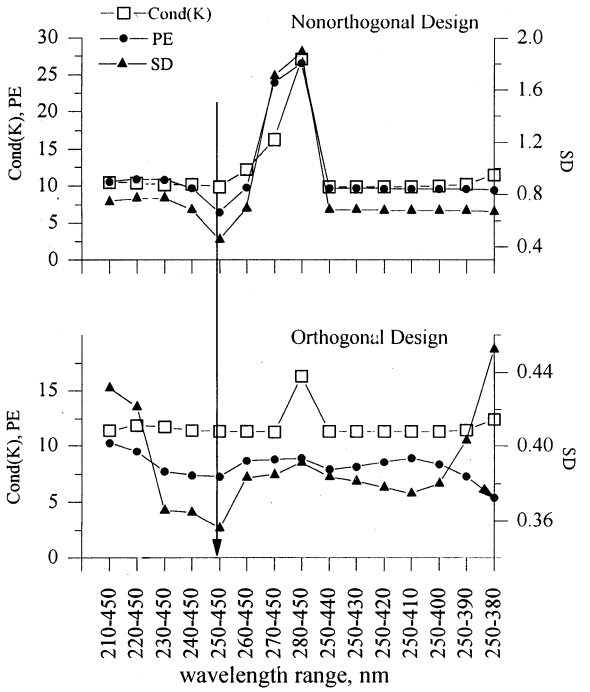

Fig. 2. Variation of wavelength range with the condition number, prediction error and standard deviation for each experimental design.

Table 2
Results obtained by Kalman Filter based on orthogonal and non-orthogonal calibration designs

Sample[a]	Taken (μg ml^{-1})	Recovery %[b]	
		Orthogonal design	Non-orthogonal design
C	10.08	96.2 ± 2.9	105.8 ± 3.0
R	2.00	101.3 ± 1.4	102.5 ± 1.2
C	2.02	93.9 ± 3.7	95.0 ± 4.1
R	5.02	102.3 ± 1.0	102.6 ± 1.6
R	5.02	102.8 ± 1.5	99.2 ± 1.2
Q	4.98	95.4 ± 4.1	104.8 ± 5.3
C	5.04	100.3 ± 0.3	97.3 ± 1.4
Q	4.98	97.5 ± 0.8	95.2 ± 3.4
C	10.08	98.3 ± 1.1	104.2 ± 1.3
R	5.02	97.1 ± 1.6	98.1 ± 2.5
Q	4.98	103.3 ± 0.2	102.4 ± 1.0

[a] C, R and Q are abbreviations of ascorbic acid, rutin and quercetin, respectively.

[b] Confidence limits of triplicate determinations at 95% confidence level.

selection of optimal wavelength range can be confirmed also by two further criteria, namely a prediction error (PE) [23] and a standard deviation (S.D.) [24].

Results obtained for various wavelength ranges in orthogonal and non-orthogonal calibration designs are shown in Fig. 2. It illustrated that the optimum wavelength range that provides a low condition number of the matrix K and the minimum of both S.D. and PE was 250–450 nm for all calibration designs.

Thus, the prediction results for the determination of ascorbic acid, rutin and quercetin in synthetic mixtures and in pharmaceutical preparations over this selected wavelength range 250–450 nm (41 data points) and based on orthogonal and non-orthogonal calibration designs are given in Tables 2 and 3, respectively.

In order to evaluate whether there are significant differences between the concentrations found for each component and each calibration design, the variance ratio test, F-test, was used to compare the relative standard error of prediction SEP [25] at the 95% confidence level. The values of SEP are shown in Table 4. The results showed no significant differences, i.e. equal reproducibility or precision, with any of the calibration methods for all component analyzed. This indicated that there is no mutual interaction between two or more components in the mixture. Since the orthogonal design gave the lowest SEP value, it has the computation advantage over the non-orthogonal design. Besides the orthogonal design does not need a number of standard calibrations as high as that needed for full factorial design.

The determination of quercetin in Ruta-C tablets and Rutin C capsules, using the proposed method, resulted in zero% of quercetin. To prove the validity and applicability of the Kalman Filter method to resolute severely overlapped spectra of rutin, quercetin and ascorbic acid in the presence of other ingredients encountered in tablets or capsules, different concentrations of quercetin, 10, 50 and 100 mg, were added to the stock solutions of Ruta-C tablets and Rutin C capsules, respectively. Table 5 shows the superiority of Kalman Filter over the traditional chemical methods.

Table 3
Comparison between the proposed and official methods

Sample[a]	Label (mg)	Recovery%[b]		
		Proposed method		Official method
		Orthogonal	Non-orthogonal	
Ruta C tablet				
C	160	103.4 ± 3.2	103.4 ± 3.4	110.0 ± 4.5[c]
R	60	98.58 ± 1.4	102.6 ± 2.6	102.8 ± 3.4[d]
Rutin C capsule				
C	100	102.8 ± 2.8	101.4 ± 1.4	110.0 ± 3.3[c]
R	50	96.8 ± 3.2	96.4 ± 3.7	101.3 ± 2.7[e]

[a] C and R are abbreviations of ascorbic acid and rutin, respectively.
[b] Confidence limits of triplicate determinations at 95% confidence level.
[c] Determined by iodimetric method [26]
[d] Determined by UV-spectrophotometric method [27]
[e] Determined by colorimetric method [28].

Table 4
Relative SEP of each component and each design

	Degrees of freedom	$F_{critical}$ at $\alpha = 0.05$	Orthogonal design	Non-orthogonal design	F_{calc}
Ascorbic acid	11	2.82	3.05	4.85	2.53
Rutin	11	2.82	2.65	2.94	1.23
Quercetin	8	3.18	3.62	4.11	1.29

Table 5
Determination of quercetin added to the drugs

Sample	Q added (mg)	Recovery%[a] by using Orthogonal design[b]		
		C	R	Q
Ruta C tablet 160 C+60 R	10	103.2 ± 3.2	102.3 ± 1.2	98.2 ± 1.5
	50	102.4 ± 1.2	99.1 ± 2.4	104.4 ± 2.4
	100	104.5 ± 1.6	97.8 ± 1.0	100.5 ± 0.4
Rutin C capsule 100 C+50 R	10	102.8 ± 2.8	98.9 ± 2.4	103.4 ± 1.8
	50	102.6 ± 0.9	101.4 ± 1.7	97.8 ± 2.4
	100	103.0 ± 1.3	96.6 ± 2.3	98.7 ± 2.0

[a] Confidence limits of triplicate determinations at 95% confidence level.
[b] C, R and Q are abbreviations of ascorbic acid, rutin and quercetin, respectively.

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