# Simultaneous Determination of Quinapril and Hydrochlorothiazide in Tablets by Ratio Spectra Derivative Spectrophotometric and Chemometric Methods

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*Simultaneous determination of quinapril (QA) and hydrochlorothiazide (HCT) in tablets were accomplished by ratio spectra first order derivative spectrophotometry (graphical method) and chemometric method (numerical method). Both methods do not require any chemical separation step. In the application of two analytical methods, the absorption spectra in the working range of 4.0-20.0 μg/mL QA and 2.5-12.5 μg/mL HCT were plotted in the wavelength range of 210-350 nm. In the graphical approach, the absorption spectra of QA and its binary mixtures in the selected spectral range of 210-280 nm were divided by the standard spectrum of 10 μg/ mL HCT and their absorption spectra were obtained. In the similar way, the ratio spectra of HCT in the wavelength region of 210-350 nm were also obtained by using the standard spectrum of 12 μg/mL QA. First derivative of the ratio spectra obtained in the above steps were calculated by $\Delta\lambda$=5 nm interval for both drugs. Calibration equation functions were obtained by measuring the ratio spectra derivative amplitudes of the minima at 219.9 nm for QA and 283.2 nm for HCT in the above mentioned spectral ranges for each drug. In the numerical method, the critical wavelengths corresponding to maximum points at 213.0 nm for QA and 220.0 nm for HCT in the zero-order absorption spectra were selected to construct the least squares calibration (CLS). Both graphical and numerical methods developed in this study were completely validated and applied to the quantitative analysis of tablets containing QA and HCT. The results obtained from the developed methods were compared with each other as well as to those obtained by classical derivative spectrophotometry, which have different experimental conditions than the previous derivative method, and the difference was not observed statistically significant.*

*Keywords: quinapril, hydrochlorothiazide, ratio spectra first order derivative, chemometric method, binary mixture, commercial tablet formulation*

Quinapril (QA) is named as {(3S)-2-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenyl –propyl ] amino]-1-oxopropyl -1,2,3,4-tetrahydro-3-isoqinolincarboxylic asid]} (fig. 1). QA is a member of a class of drugs called antihypertensive drug and its combination with the diuretic drug HCT is used in" Accuzide® tablets. HCT is described chemically as (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadizýne-7-sulphonamide 1,1-dioxide ). The simultaneous use of QA and HCT in pharmaceutical formulation increases the above therapeutic effects [1,2].

Simultaneous quantitative analysis of QA and HCT in their mixtures was reported by derivative spectrophotometry [3] and TLC method [4].

The resolution of the overlapping absorption spectra is an important task in the analytical chemistry. In order to solve this problem, chemometric and classical derivative methods have been widely used in the analytical application of the UV-Visible spectrophotometry. However, chemometric calibration methods require the use of abstract mathematical calculation together with properly designed software, and the preparation of appropriate calibration samples. In some cases, the application of classical derivative spectrophotometric method to the quantitative resolution of binary mixtures may not give better results because the analytes do not have suitable or repeated zero crossing points in the derivative treatment of the spectra. Another disadvantage of the derivative method is the interference of the main peaks with the noises in the higher order derivation. To eliminate or reduce the drawbacks of this classical derivative method, ratio spectra derivative spectrophotometric method is a promising approach for the quantitative evaluation of binary mixtures. This method based on the measurement of the amplitudes corresponding to the maximum or minimum points doesn't require the utilization of the zero crossing point or the researching critical point in the derivative of the ratio spectra.

In this study, the simultaneous quantitative resolution of QA and HCT having overlapping absorption spectra in the wavelength region of 210-300 nm was performed by using the ratio spectra derivative spectrophotometry. In this application, the method doesn't require any chemical separation procedure. In addition to this, a chemometric least squares calibration method based on the measurement of the absorption values at the maximum wavelength points was applied to the quantitative simultaneous prediction of these drugs in tablet samples. This chemometric approach was used as an alternative method. The performance of both graphical and numerical methods was tested by using the synthetic mixtures of two drugs in the different concentration compositions and standard addition techniques. The methods proposed in the present study were successfully subjected to the real samples containing QA and HCT drugs. The analytical results of the applied methods were compared with those

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obtained by the derivative spectrophotometry, which has different experimental condition than the previous derivative method [3] and a good coincidence was observed between results.

## Experimental part

*Instrument*

A Shimadzu UV-160 double beam UV–Vis spectrophotometer having a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer were used for the registration of absorption spectra of samples against a black. The absorption data were transformed into ASCII files and transferred to EXCEL and ratio spectra procedure was performed. After that the transferred data vectors were processed by ratio treatments and derivative transformation method. Data treatments, regressions and statistical analysis were performed by using the Microsoft EXCEL.

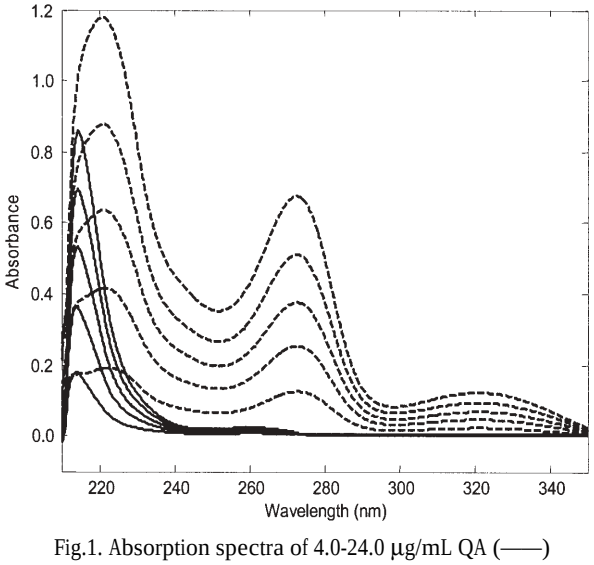


Fig.1. Absorption spectra of 4.0-24.0 μg/mL QA (——) and 2.50-12.50 μg/mL HCT (- - - -) in 0.05 M NaOH

*Commercial tablet formulation*

A commercial pharmaceutical product (Accuzide® tablet, Phizer Pharm. Ind., Istanbul, Turkey,) was studied. Its declared content was as follows: 20 mg QA, and 20 mg HCT per tablet. QA and HCT were obtained as a donation from Phizer Pharm. Ind., Istanbul (Turkey).

*Reagents and solutions*

Stock solutions of 25 mg/100 mL for QA and HCT were prepared in 0.05 N NaOH. Standard series of QA and HCT in the linear concentration range of 4.0-20.0 μg/mL and 2.5-12.5 μg/mL were obtained from the above stock solutions, respectively. An independent validation set of various binary mixtures of QA and HCT in the working concentration range was prepared by using the stock solutions. The proposed methods were subjected to the absorption spectra of the prepared solutions.

*Tablet analysis*

Ten tablets containing QA and HCT were totally weighed and powdered. The content equivalence to one tablet was dissolved in a 100 mL calibrated flask by using 0.05 N NaOH.

The content of the flask was mechanically shaken for 20 min and filtrated into a 100 mL volumetric flask through a 0.45 mm membrane filter. Appropriate dilutions were done into the range of calibration curve with 0.05 N NaOH. The absorption spectra of the resulting solutions were recorded against 0.05 N NaOH as a reference solution.

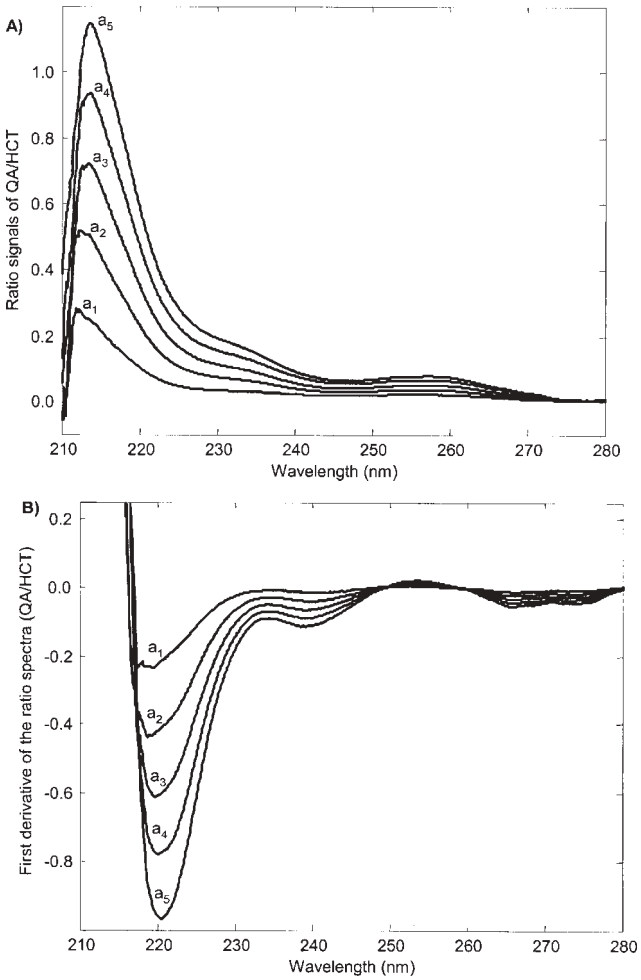


Fig. 2. Ratio spectra (A) and their first derivative spectra (B) of $a_1$) 4.0 μg/mL, $a_2$) 8.0 μg/mL, $a_3$) 12.0 μg/mL, $a_4$) 16.0 μg/mL and $a_5$) 20.0μg/mL QA, (when 10.0μg/mL HCT was used as a divisor)

## Results and discussion

Since the absorption spectra of QA and HCT overlapped in the wavelength region of 210-350 nm as shown in figure 1, the simultaneous determination of these drugs in binary mixtures is not possible from the direct use of the zero order absorption spectra. For this analytical problem, classical derivative spectrophotometric method was already applied to the same mixture containing QA and HCT drugs [2]. As mentioned in the introduction section, for the elimination or reduction of the disadvantages of classical derivative approach, ratio spectra derivative spectrophotometric method was proposed for the simultaneous determination of QA and HCT in their binary mixtures and commercial tablet formulation. For a comparison, classical least squares (CLS) calibration as an alternative approach was applied to the resolution of the above analytical problem. The application of both graphical and numerical methods will be explained below.

*Ratio spectra derivative spectrophotometry*

In the application of this method, the procedure was carried out by using two mathematical steps. These steps for each drug are the division and derivation treatments of the absorption spectra.

Absorption spectra of the standard solutions of QA and its samples with HCT in the spectral rage of 210-350 nm were recorded and divided by the standard spectra of 10 μg/ mL HCT ($\beta_{HCT}C^{o}_{HCT}$) and their ratio spectra were obtained as indicated in figure 2A. The first derivative of the ratio spectra of QA was obtained with the interval of

$\Delta\lambda$= 5 nm and scaling factor = 10 as indicated in figure 2B. The concentration of QA in the binary mixture was proportional to the amplitude of the minimum at 219.9 nm with respect to the following equation:

$$\frac{d}{d\lambda}\left(\frac{A_{mix}}{\beta_{HCT}\, C^{o}_{HCT}}\right) = \frac{d}{d\lambda}\left(\frac{\alpha_{QA}}{\beta_{HCT}}\right)\frac{C_{QA}}{C^{o}_{HCT}} \quad (1)$$

The amplitudes measured, $\frac{d}{d\lambda}\left(\frac{A_{mix}}{\beta_{HCT}\, C^{o}_{HCT}}\right)$ were drawn as a graph, versus concentrations of QA and a straight line was obtained. By using the calibration graph, QA was determined in samples containing QA and HCT.

In the same way, to determine HCT, the absorption spectra of the solution of HCT and its mixtures with QA were divided by the standard spectra of QA 12 μg/ mL as a divisor ($\alpha_{QA} C^{o}_{QA}$) . From the resulting ratio spectra shown in figure 3A, the first derivative of the ratio spectra was calculated with the interval of $\Delta\lambda$= 5 nm as indicated in Figure 3B. The concentration of HCT in the binary mixture was proportional to the amplitude of the maximum at 283.2 nm as given in the following equation:

$$\frac{d}{d\lambda}\left(\frac{A_{mix}}{\alpha_{QA}\, C^{o}_{QA}}\right) = \frac{d}{d\lambda}\left(\frac{\beta_{HCT}}{\alpha_{QA}}\right)\frac{C_{HCT}}{C^{o}_{QA}} \quad (2)$$

As explained above, a straight line was also obtained by using the amplitudes measured, $\frac{d}{d\lambda}\left(\frac{A_{mix}}{\alpha_{QA}\, C^{o}_{QA}}\right)$ for HCT. By means of the calibration graph, the content of HCT was determined in samples.

Calibration equations and their statistical results were given in table 1. The liner regression results given in the table were found suitable for the application of the method to the synthetic and real samples.

In the validation of this method, various mixtures of QA and HCT were prepared and tested between 4.0-20.0 μg/ mL for QA and 2.50-12.50 μg/mL HCT in their binary mixtures. Mean recoveries and the relative standard deviations of the method were found as 99.0 and 1.85 for QA, 99.3 and 1.93 for HCT in the synthetic mixtures prepared by adding known amounts of QA and HCT (table 2).

*Chemometric method*

In the chemometric method, CLS calibration as an alternative method in the present study was applied to the simultaneous prediction of QA and HCT in their samples. This method contains the application of the linear equation system to the Lambert-Beer law.

The absorbance values of a mixture of two drugs (QA, and HCT) were measured at a two-wavelength set ($\lambda_i$ =213 and 220 nm) corresponding to the maximum wavelengths for both drugs, the following equations were written for a two-component analysis:

$$\lambda_i = 213 \text{ nm}, \quad A_{mix1} = k_{QA,1}\, C_{QA} + k_{HCT,1}\, C_{HCT}$$
$$\lambda_i = 220 \text{ nm}, \quad A_{mix1} = k_{QA,2}\, C_{QA} + k_{HCT,2}\, C_{HCT} \quad (3)$$

where $A_{mix1}$ and $A_{mix2}$ represent the absorbances of the mixture of QA and HCT analytes at the two-wavelength set, $k_{QA,1}$, $k_{HCT,1}$ and $k_{QA,2}$ $k_{HCT,2}$ are the absorptivities obtained from the individual drugs at 213 and 220 nm.

Equation (3) can be formulated in matrix notation as:

$$\begin{bmatrix} A_{mix1} \\ A_{mix2} \end{bmatrix} = \begin{bmatrix} k_{QA,1} & k_{HCT,1} \\ k_{QA,2} & k_{HCT,2} \end{bmatrix} * \begin{bmatrix} C_{AQ} \\ C_{HCT} \end{bmatrix} \quad (4)$$

This procedure is the mathematical basis of the CLS method for two-component analysis. As explained here, the proposed calibration model was applied easily to the simultaneous quantitative analysis of the two-component mixtures.

*Derivative spectrophotometry*

In the case of the classical derivative method, the second derivative of the absorption spectra of QA and HCT under the same experimental condition with the above ratio spectra derivative method was calculated by using the interval of $\Delta\lambda$=4 nm and scaling factor = 20 as shown in figure 4.

Calibration graphs were obtained by measuring the second derivative amplitudes at 233.3 nm for QA and 275.6 nm for HCT. Linear regression analysis and its statistical results were presented in table 1. Calibration equations were used for the quantitative analysis of QA and HCT in samples.

*Method validation*

The developed method was validated according to the literature (5 -7).

The linearity of the calibration graphs obtained by application of the ratio spectra derivative and second derivative methods in the concentration range of 4.0-20.0 μg/mL for QA and 2.50-12.50 μg/mL HCT in the binary mixture was verified by high correlation coefficients (r) as shown in table 1.

**Table 1**
LINEAR REGRESSION ANALYSIS AND ITS STATISTICS

| Method | Ratio spectra first derivative | | Classical second derivative | |
|---|---|---|---|---|
| | QA | HCT | QA | HCT |
| λ (nm) | 220.0 | 283.2 | 233.3 | 275.6 |
| r | 0.9998 | 0.9973 | 0.9997 | 0.9982 |
| m | 0.0047 | 8.6100 | 0.0437 | 0.1630 |
| n | -0.0029 | 2.9250 | -0.0504 | -0.0616 |
| S(r) | 0.0007 | 2.8792 | 0.0081 | 0.0442 |
| S(m) | 0.0001 | 0.3642 | 0.0006 | 0.0056 |
| S(n) | 0.0007 | 3.0197 | 0.0085 | 0.0464 |
| LOD | 0.34 | 0.70 | 0.33 | 0.85 |
| LOQ | 1.13 | 2.33 | 1.10 | 2.82 |

Various mixtures containing QA and HCT drugs were used to test the precision of the proposed methods and second derivative spectrophotometry. The relative standard deviations were found between 97.2% and 102.5% and these values demonstrate satisfactory reproductibility of the methods as presented in table 2. A good coincidence of the obtained experimental results was observed for the validation of the method. No degradation product, interference and systematical errors were observed during the analysis procedure.

Another recovery study of the methods was performed by using the standard addition technique and its calculation results, namely the mean % recovery, standard deviation (SD) and relative standard deviation (RSD) were presented in Table3. These results were obtained on the average of six replicate for each drug at two different concentration levels and a good agreement was observed for the standard addition assay results by application of these methods. We observed that the effect of excipients in tablets was not observed on the quantitative analysis of drugs.

The limit of detection (LOD; signal-to –noise ration of 3:1) and the limit of quantitation (LOQ;signal-to-noise ratio of 10:1) were calculated and presented in table 1.

**Table 2**

RECOVERY RESULTS OBTAINED BY APPLYING THE PROPOSED ANALYTICAL METHODS TO THE SYNTHETIC MIXTURES

| Mix No. | Added (µg/mL) | | Found (µg/mL) | | | | | | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CLS | | Ratio spectra first derivative | | Classical second derivative | | CLS | | Ratio spectra first derivative | | Classical second derivative | |
| | QA | HCT | QA | HCT | QA | HCT | QA | HCT | QA | HCT | QA | HCT | QA | HCT |
| 1 | 4.0 | 5.0 | 3.94 | 5.02 | 3.91 | 5.01 | 4.01 | 5.17 | 98.6 | 100.4 | 97.7 | 100.1 | 100.3 | 103.5 |
| 2 | 12.0 | 5.0 | 11.38 | 5.02 | 11.57 | 5.00 | 12.32 | 5.15 | 94.8 | 100.4 | 96.4 | 100.0 | 102.6 | 103.0 |
| 3 | 20.0 | 5.0 | 19.23 | 5.04 | 20.32 | 5.01 | 20.48 | 5.24 | 96.2 | 100.8 | 101.6 | 100.2 | 102.4 | 104.8 |
| 4 | 4.0 | 10.0 | 3.83 | 9.63 | 3.91 | 9.70 | 4.11 | 10.44 | 95.7 | 96.3 | 97.8 | 97.0 | 102.9 | 104.4 |
| 5 | 12.0 | 10.0 | 11.34 | 9.82 | 11.69 | 9.78 | 12.38 | 10.08 | 94.5 | 98.2 | 97.4 | 97.8 | 103.1 | 100.8 |
| 6 | 20.0 | 10.0 | 19.29 | 9.84 | 19.80 | 9.77 | 20.59 | 10.17 | 96.4 | 98.4 | 99.0 | 97.7 | 103.0 | 101.7 |
| 7 | 8.0 | 2.5 | 7.63 | 2.61 | 7.90 | 2.54 | 8.23 | 2.53 | 95.3 | 104.2 | 98.7 | 101.4 | 102.8 | 101.2 |
| 8 | 8.0 | 7.5 | 7.60 | 7.48 | 7.86 | 7.38 | 8.00 | 7.53 | 95.0 | 99.7 | 98.2 | 98.4 | 100.0 | 100.4 |
| 9 | 8.0 | 12.5 | 7.49 | 12.33 | 7.83 | 12.65 | 8.29 | 13.05 | 93.7 | 98.6 | 97.9 | 101.2 | 103.6 | 104.4 |
| 10 | 15.0 | 2.5 | 15.45 | 2.61 | 15.27 | 2.56 | 14.86 | 2.55 | 103.0 | 104.4 | 101.8 | 102.2 | 99.1 | 102.1 |
| 11 | 15.0 | 7.5 | 15.25 | 7.50 | 15.04 | 7.45 | 15.36 | 7.69 | 101.7 | 100.0 | 100.2 | 99.4 | 102.4 | 102.5 |
| 12 | 15.0 | 12.5 | 15.19 | 11.97 | 15.23 | 12.01 | 15.36 | 12.71 | 101.3 | 95.7 | 101.5 | 96.1 | 102.4 | 101.7 |
| | | | | | | | | Mean | 97.2 | 99.8 | 99 | 99.3 | 102.1 | 102.5 |
| | | | | | | | | RSD | 3.24 | 2.65 | 1.85 | 1.93 | 1.41 | 1.46 |

RSD = Relative standard deviation

**Table 3**

RECOVERY RESULTS FOR STANDARD ADDITION TECHNIQUE

| | CLS | | Ratio spectra first derivative | | Classical second derivative | |
|---|---|---|---|---|---|---|
| | QU | HCT | QU | HCT | QU | HCT |
| | 103.5 | 99.6 | 101.6 | 103.0 | 103.2 | 103.4 |
| | 101.6 | 99.3 | 101.4 | 102.7 | 101.6 | 103.4 |
| | 100.8 | 98.9 | 102.7 | 103.1 | 104.0 | 102.8 |
| | 102.5 | 98.8 | 99.8 | 98.9 | 99.4 | 100.9 |
| | 101.5 | 99.2 | 103.7 | 98.8 | 99.2 | 99.9 |
| | 100.6 | 99.5 | 94.2 | 99.5 | 97.5 | 101.2 |
| Mean | 101.7 | 99.2 | 100.6 | 101.0 | 100.8 | 101.9 |
| RSD | 1.06 | 0.31 | 3.37 | 2.13 | 2.51 | 1.44 |

**Table 4**

EXPERIMENTAL RESULTS OBTAINED BY APPLYING THE PROPOSED ANALYTICAL METHODS TO THE PHARMACEUTICAL TABLETS

| No. | CLS | | Ratio spectra first derivative | | Classical second derivative | |
|---|---|---|---|---|---|---|
| | QU | HCT | QU | HCT | QU | HCT |
| 1 | 20.69 | 12.85 | 20.16 | 12.80 | 19.66 | 12.64 |
| 2 | 20.40 | 12.38 | 20.36 | 12.66 | 21.00 | 12.72 |
| 3 | 20.75 | 12.30 | 20.88 | 12.72 | 19.18 | 12.84 |
| 4 | 20.75 | 12.54 | 19.86 | 12.28 | 19.41 | 12.47 |
| 5 | 20.78 | 12.39 | 20.81 | 12.26 | 19.89 | 12.47 |
| 6 | 20.02 | 12.41 | 19.95 | 12.17 | 19.66 | 12.49 |
| 7 | 20.96 | 12.61 | 20.47 | 12.69 | 20.52 | 12.46 |
| 8 | 19.61 | 12.15 | 19.51 | 12.41 | 20.46 | 12.34 |
| 9 | 19.82 | 12.24 | 19.72 | 12.58 | 20.55 | 12.42 |
| 10 | 19.71 | 12.69 | 19.80 | 12.25 | 20.34 | 12.71 |
| Mean: | 20.35 | 12.46 | 19.87 | 12.48 | 20.07 | 12.56 |
| SD | 0.51 | 0.22 | 0.47 | 0.23 | 0.59 | 0.16 |
| RSD | 2.51 | 1.73 | 2.36 | 1.88 | 2.93 | 1.28 |
| SE | 0.21 | 0.09 | 0.19 | 0.10 | 0.24 | 0.07 |
| CL(p=0.05) | 0.41 | 0.17 | 0.37 | 0.19 | 0.47 | 0.13 |

SD = Standard deviation, SE = standard error, CL = confidence limit

Label claim = 20 mg QA and 12.5 mg per tablet

Results show that the calibration models gave satisfactory results in the case of overlapping spectra of QA and HCT according to the recovery study.

*Analysis of commercial pharmaceutical tablets*

The CLS, ratio spectra derivative and second derivative methods were successfully applied to the quantitative determination of QA and HCT in tablets. The obtained

**Table 5**
STATISTICAL RESULTS OBTAINED BY APPLICATION OF ANOVA TEST TO THE PHARMACEUTICAL TABLETS

| QA | | | | | |
|---|---|---|---|---|---|
| Source of Variation | SS | df | MS | F-calculated | F-critical |
| Between Groups | 0.4185 | 2 | 0.2093 | 0.76 | 3.35 |
| Within Groups | 7.4173 | 27 | 0.2747 | | |
| Total | 7.8358 | 29 | | | |
| HCT | | | | | |
| Source of Variation | SS | df | MS | F-calculated | F-critical |
| Between Groups | 0.0538 | 2 | 0.0269 | 0.64 | 3.35 |
| Within Groups | 1.1382 | 27 | 0.0422 | | |
| Total | 1.1921 | 29 | | | |

SS = Sum of squares, df = degree of freedom, MS = Mean of squares

experimental results for the commercial preparation were presented in table 4. Statistical values: mean value, SD (standard deviation), RSD (relative standard deviation), SE (statistical value), CL (P=0.05) (confidential limit) are summarized in table 4. As it can be seen a good coincidence was noticed between the experimental results and the claimed label in the application of all the methods to the tablet formulation.

For a statistical comparison, one-way ANOVA test was applied to the results obtained by application to the proposed methods, CLS, second derivative and ratio spectra first derivative methods to the commercial pharmaceutical tablets. In this statistical test F- values were calculated by using the analysis of variance and calculated F-values and tabulated F-values were compared with each other. The results of this test were summarized in table 5. The results with %95 of confidential limit indicate that there is no significant difference between three methods in respect to tabulated values (critical).

## Conclusions

The simultaneous determination of QA and HCT in their mixtures and commercial tablets was achieved by the graphical and numerical methods proposed in this study. As it is known, the quantitative resolution of the mixture containing more than one compound is one of difficult problems of the analytical chemistry. For this reason, three different analytical approaches from second derivative spectrophotometric method reported in [2] were proposed and successfully applied to the quantitative evaluation of QA and HCT drugs in samples without using any chemical separation treatment. In addition to this, these analytical methods for QA and HCT are accurate, simple, reproducible and yet very cheap in analytical practice.

We conclude that the proposed analytical methods can be applied successfully to the routine quality control of the commercial pharmaceutical preparation containing QA and HCT in tablets.

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