**Reviewer’s Comments**

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**SIMULTANEOUS DETERMINATION VALSARTAN and HYDROCHLOROTHIAZIDE by FIRST ORDER DERIVATIVE- ZERO CROSSING UV-VISIBLE SPECTROPHOTOMETRIC METHOD**

**ABSTRACT**

**Background**: Antihypertensive drugs are widely used to treat high blood pressure in patients. The aim of this study is to prepare various mixtures of valsartan and hydrochlorothiazide, to create a matrix effect in the drug, and to determine the determination of these active substances in drug samples after optimum conditions by first-order derivative spectroscopy method.

**Method**:In the first-degree derivative spectrophotometry method applied, valsartan and hydrochlorothiazide were dissolved in methanol and 100 mgL-1 solutions were prepared, and then the mixtures of these solutions were determined in spectrophotometry. After mixing, tablet (drug) sample was prepared and absorbance values were recorded and the first derivative spectrophotometric method was applied to the values obtained.

**Results**:As a result of the spectrophotometric determination of various mixtures of valsartan and hydrochlorothiazide used in the study, it was determined that the first-order derivative spectrophotometric method applied gave very consistent results.

**Conclusion**:The first-order derivative spectrophotometry method used in the study can be easily applied in routine analyzes in drug research laboratories.

**Keywords**: Valsartan, Hydrochlorothiazide, First Order Derivative Spectrophotometric Method.

**INTRODUCTION**

Today, a wide variety of drugs are used for various purposes and new ones are added to them every day. The active parts of the drugs are the active ingredients of the drugs. Accurate determination of the amounts of active pharmaceutical ingredients during both manufacturing and market controls is very important in terms of use. Medicines with missing active ingredients do not show the required benefit and when the side effects of the drugs are taken into account, those with too much active ingredient cause many harmful side effects. Since drug active ingredients are found in different matrices in different drugs, it is also important scientifically to determine them correctly.

It is observed from the studies that classical analytical methods and chemometric calibrations are used with increasing intensity in the analysis of pharmaceutical preparations, since they give successful results in the analysis of mixtures. Pharmaceutical preparations consist of a fixed matrix of excipients and one, two or more active samples containing the compound. Simultaneous quantitative analysis of combined pharmaceutical preparations without any separation or using a pre-separation process is extremely important in analytical chemistry.

It is possible to carry out simultaneous quantitative determination of drug preparations in combination with Derivative spectrophotometer without any separation process. In addition, since graphical detail will also increase with increasing selectivity with the method of taking derivatives, analyzes performed on preparations containing a single active ingredient of the drug are performed more easily.

With the use of diuretic mixtures, it is aimed to increase the effectiveness by obtaining synergistic effects. Combinations of thiazides and diuretics are often used in complex mixtures. The advantages of complex drugs include low dosages and increase the activity of hypertension.

Valsartan (VAL) is a new antihypertensive drug belonging to the family of angiotensin II receptor antagonists. It is used in the treatment of hypertension and heart failure to reduce cardiovascular mortality in patients with left ventricular dysfunction following myocardial infarction.Hydrochlorothiazide (HCT)[6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide -1, 1-dioxide,] is a diuretic effective on the distal convoluted tubule. Its molecular weight is 297.7 g/mol and its closed molecular formula is C7H8ClN3O4S2. Due to their synergistic anti-hypertensive effects, valsartan and hydrochlorothiazide are commercially available as a combined dosage form.The chemical explicit formulas of VAL and HCT studied in this study are shown in Figure 1.

Various methods for the determination of the amount of VAL1-5 or HCT6-11 individually or in combination with other antihypertensive drugs in various matrices have been found in the literature search. Although the individual appointments of both drugs are not preferred due to the high cost and low efficiency of the studies to be conducted, they have been encountered at least a little in the literature research conducted again.Methods such as HPLC methods12-15, spectrofluorimetric method16 and capillary electrophoresis17 for VAL analysis, and methods such as HPLC18-21, capillary zone electrophoresis22, spectrophotometry23-25 for HCT analysis have been found in the literature.

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Valsartan Hydrochlorothiazide

**Figure 1** Drug formulas studied

In this study, it was tried to determine the active drug substances by derivative spectrophotometer in a drug formulation where VAL and HCT, which are very difficult to determine by classical UV methods, due to their absorbance in very close places.

**MATERIALS AND METHODS**

**Apparatus**

Measurements were made with a computer-controlled UV 1700 PHARMASPEC SHIMADZU spectrophotometer equipped with a 1 cm long cell. The spectra were read in the computer environment with the help of the up-to-date software of the device, and the data were evaluated with Excel and turned into graphics. Two transparent quartz cuvettes were used for absorbance measurements in the device.

**Chemical and reagents**

VAL and HCT and dosage forms (Diostar-Plus®) were supplied by Pharma International (Jordan). Methanol (Merck) was chromatographic grade. All other chemicals were used in analytical purity. All solutions were analyzed on the same day as they were prepared.

**Solutions used**

In the experimental phase of the study, stock solutions of drug active ingredients valsartan and hydrochlorothiazide were prepared as 100 ppm for UV-Vis spectrophotometric measurements and dilution was carried out to the range to be studied.

**Analysis of tablet**

Ten tablets containing VAL and HCT as active ingredients were weighed into powder and weighed. Then, dilution was performed by transferring it to the measured balloon. It was sonicated with methanol for at least 30 minutes and then made up to volume with the same solvent. Subsequently, it was centrifuged and further dilution was made using methanol for the derivatization procedure.

**RESULTS AND DISCUSSION**

In the determination studies carried out by creating a calibration graph, the calibration graphs drawn for both active components were used. Figure 2 and Figure 3 show the normal UV spectra of valsartan and hydrochlorothiazide and their first derivative spectra plotted in excel.

**Figure2**Using methanol as a solvent, valsartan a) 5.2 ppm b) 10.4 ppm c) 15.6 ppm d) 20.8 ppm and e) 26.0 ppm and hydrochlorothiazide k) 7.5 ppm l) 10.0 ppm m) 12.5 ppm n) 15.0 ppm and o) 17.5 ppm Ultraviolet visible region spectra of solutions

**Figure 3** By using methanol as a solvent, valsartan a) 5.2 ppm b) 10.4 ppm c) 15.6 ppm d) 20.8 ppm and e) 26.0 ppm and hydrochlorothiazide f) 7.5 ppm g) 10.0 ppm h) 12.5 ppm k) 15.0 ppm and l) 17.5 ppm solutions first derivative spectra

The point to be considered in the determination of the active substances in the mixture by derivative spectrophotometric method is the points where the absorbance of hydrochlorothiazide is zero in the derivative spectrum while determining the valsartan, and the points where the derivative absorbance of valsartan is zero should be selected when determining the hydrochlorothiazide. Thus, when two active substances are together, their determination can be made with accuracy.

As can be seen from the derivative spectra in Figure 3, these values are 271 nm for valsartan and 243 nm for hydrochlorothiazide. Working graphs drawn using these derivative absorbance values are given in Figures 4 and 5.

**Figure 4**First derivative calibration plot of Valsartan at 271 nm in methanol solvent

**Figure 5**First derivative calibration plot of Hydrochlorothiazide at 243 nm in methanol solvent

The results obtained when the method was applied to the commercial tablet sample containing both substances together are shown in Table 1.

**Table 1** Application of the first derivative method to tablets containing hydrochlorothiazide and valsartan together and statistical evaluation of the results

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number of experiments | The amount of HCT in the tablet (mg/tablet) | Found (mg/tablet) | The amount of VAL in the tablet (mg/tablet) | Found(mg/tablet) |
| 1 | 25 | 24,96 | 160 | 160,00 |
| 2 | 25 | 24,80 | 160 | 160,27 |
| 3 | 25 | 25,06 | 160 | 160,83 |
| 4 | 25 | 24,83 | 160 | 159,16 |
| 5 | 25 | 24,74 | 160 | 158,88 |
| 6 | 25 | 25,68 | 160 | 164,44 |
| Average value 25,01 Average value 160,60Standarddeviation0,34 Standarddeviation 2,01Confidence interval25,01± 0,35 Confidence interval 160,60± 2,10 |

**LIMITATIONS OF THE STUDY**

**CONCLUSION**

In this study, first-order derivative spectrophotometry method is proposed for simultaneous determination of VAL and HCT in binary mixtures. This method has been successfully applied to pharmaceutical products. It was observed that the determination of overlapping drug mixtures gave accurate results with the proposed method. The proposed first-order derivative spectrophotometry method has been found to be applicable for routine analysis of drug mixtures without any pre-chemical separation and without the need for time of pharmaceutical formulation.

**CONFLICT OF INTEREST**

There is no conflict of interest regarding the work done.

**Author’s Contribution**

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