

# Validated UV Spectrophotometric Method for Quantification of Zofenopril in Pharmaceutical Formulations

LAURA SBARCEA<sup>1\*</sup>, LUCRETIA UDRESCU<sup>2</sup>, LIANA DRAGAN<sup>1</sup>, CRISTINA TRANDAFIRESCU<sup>1</sup>, MARIUS BOJITA<sup>3</sup>

<sup>1</sup> Victor Babeş" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Eftimie Murgu Square, 300041, Timișoara, Romania

<sup>2</sup> Victor Babeş" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Drug Analysis, 2 Eftimie Murgu Square, 300041, Timișoara, Romania

<sup>3</sup> "Iuliu Hațieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Drug Analysis, 6 Louis Pasteur Str., 400015, Cluj-Napoca, Romania

*A simple, sensitive, accurate, precise and economical UV spectrophotometric method has been developed and validated for the determination of zofenopril calcium in bulk and in pharmaceutical formulations. Zofenopril calcium exhibits maximum absorbance at 248 nm with apparent molar absorptivity of  $1.317 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ . The Lambert-Beer law is obeyed in the concentration range of 2-70  $\mu\text{g/mL}$ . In order to validate the method, the linearity, the limit of detection (LOD), limit of quantification (LOQ), selectivity, precision, accuracy and recovery have been examined according to ICH guidelines. The LOD and LOQ values are calculated to be 0.5052  $\mu\text{g/mL}$  and 1.5310  $\mu\text{g/mL}$  respectively. The percent recovery ranged from 98.088 to 101.988 %. No interferences from the common excipients were detected and therefore the method is considered specific. The proposed method was successfully applied for the quantitative determination of zofenopril from pharmaceutical dosage form.*

**Key words:** zofenopril calcium, UV spectrophotometry, method validation, quantification, pharmaceutical formulation

Zofenopril, (2S,4S)-1-[(2S)-3-(benzoylsulphonyl-2-methylpropanoyl)-4-phenylsulphonylpyrrolidine-2-carboxylic acid, (fig. 1) is a sulphur containing angiotensin-converting enzyme (ACE) inhibitor characterized by high lipophilicity ( $\log P = 3.5$ ) and low water solubility [1]. It exhibits selective and sustained cardiac ACE inhibition which makes it effective and well-tolerated in the treatment of cardiovascular diseases such as: essential hypertension, acute myocardial infarction, heart failure, and slows the development of atherosclerosis [1-5]. Due to the presence of sulphur in its molecule, zofenopril possesses antioxidant and cardioprotective activity, responsible for the prevention of the coronary events post myocardial infarction [2,3,5].

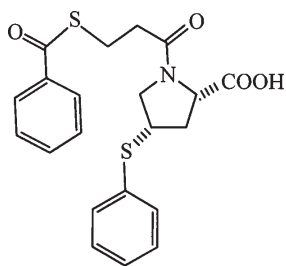


Fig. 1. Zofenopril chemical structure

Several analytical methods including radioimmunoassay [6], gas chromatography with mass detection [7] and HPLC-mass spectrometry [8-11] have been developed for the determination of zofenopril and its active metabolite zofenoprilat in human plasma. Most of these methods involve chemical derivatization and require the usage of expensive equipment and reagents. A HPLC-DAD method and a RP-LC method have been reported for the assay of zofenopril in the presence of hydrochlorothiazide in oral pharmaceutical formulation [12, 13].

Spectrophotometric methods still have practical and economical advantages over the other methods, and thus are widely used for the assay of pharmaceuticals in bulk and in dosage forms [14, 15]. In the literature there has been reported a derivative UV spectrophotometric method for simultaneous determination of zofenopril and hydrochlorothiazide in pharmaceutical dosage form [16].

In this study a simple and reproducible UV spectrophotometric method for the assay of zofenopril in bulk and in pharmaceutical formulation is developed and validated.

## Experimental part

### Apparatus

SPECTRONIC UNICAM – UV 300 UV-VISIBLE double beam spectrophotometer with 1 cm matched quartz cells has been used for all spectrophotometric measurements.

### Materials and reagents

All the chemicals used have been of analytical reagent grade. Distilled water has been used to prepare all solutions. ZOMEN tablets containing 7.5 and 30 mg zofenopril calcium have been purchased from the local market.

### Standard drug solution

Pharmaceutical grade of zofenopril calcium salt (ZOF) was kindly provided by Berlin-Chemie Menarini (Berlin, Germany). A stock standard solution of 200  $\mu\text{g/mL}$  ZOF was prepared by dissolving 20 mg of pure drug in distilled water and diluting to 100 mL in a calibrated flask with distilled water. The standard ZOF solution of 100  $\mu\text{g/mL}$  was prepared from the stock solution, by appropriate water dilution.

\* email: laura\_sbarcea@yahoo.com

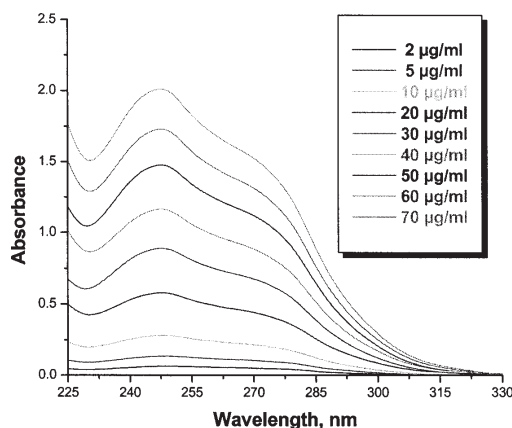


Fig. 2. UV absorption spectra of ZOF 2-70 µg/mL in distilled water

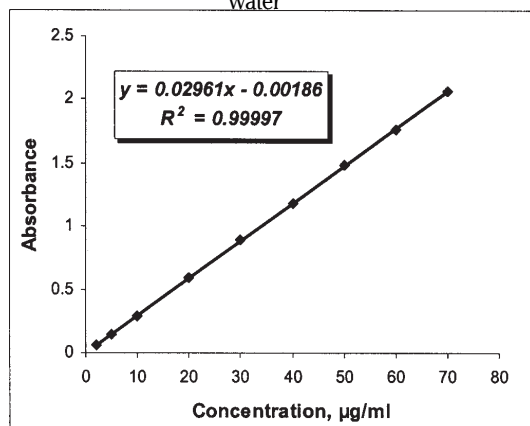


Fig.3. Calibration curve of ZOF

#### Proposed procedure

Different aliquots of 100 µg/mL ZOF solution were accurately measured and transferred into a series of 10 mL volumetric flasks and the volume has been made up to the mark with distilled water to obtained final concentration of 2, 5, 10, 20, 30, 40, 50, 60, 70 µg/mL ZOF. The absorbance has been measured at 248 nm against distilled water as a blank. The calibration graph has been performed by plotting the measured absorbance values versus concentration values.

#### Procedure for pharmaceutical formulation

Twenty tablets have been accurately weighed and powdered. A quantity of powder containing 10 mg of ZOF has been transferred into a 100 mL volumetric flask with 60 mL distilled water. The mixture has been shaken for 20 min, diluted to volume with distilled water and then filtered using 0.2 µm Nylon filter membrane. The filtrate has been subsequently subject to analysis using the above described procedure.

#### Results and discussions

The absorption spectra of ZOF present maximum at 248 nm, which was the wavelength used (fig. 2)

#### Method validation

The proposed method has been validated according to the present International Conference of Harmonization (ICH) guidelines [17].

#### Linearity and sensitivity

A linear correlation between the absorbance and the concentration of ZOF has been obtained in the range of 2-70 µg/mL ZOF (fig. 3). The calibration graph is described by the equation:

$$Y = a + bC$$

where Y = absorbance, a = intercept, b = slope and C = concentration in µg/mL, which have been obtained by the least squares method. The intercept, slope and correlation coefficient are presented in table 1. The sensitivity parameters such as molar absorptivity and Sandell sensitivity are also presented in table 1.

#### Limit of detection and quantification

The ICH guidelines were followed in order to determine the LOD and LOQ. Accordingly, the method based on the standard deviation has been applied, so that three and ten times the standard deviation values of the response and the slope of the calibration curve has been used to calculate the LOD and LOQ. The computed values were found to be 0.5052 and 1.5310 µg/mL, respectively.

#### Selectivity

The proposed method was tested in order to assess its selectivity using the artificial mixture analysis. It has been confirmed that the measured absorbance was only produced by the analyte. A synthetic mixture was prepared, containing zofenopril (20 mg) as zofenopril calcium salt (21.96 mg), talc (100 mg), starch (150 mg), lactose (180 mg) and magnesium stearate (50 mg). The extract was yielded according to the procedure that was described for tablets and subsequently analyzed using the procedure that was earlier described. The replicate analysis (n = 5) for a concentration level of 30 µg/mL ZOF has yielded the % ZOF recovery at  $100.30 \pm 1.19$ , and thus revealed that the inactive ingredients did not interfere with ZOF determination.

#### Precision and accuracy

Intra-day and inter-day precision values have been calculated by replicate analysis (n = 5) of calibration standard, at three different concentration levels, during the same day, and then during five consecutive days. The RSD (%) values of intra-day and inter-day measurements have indicated good precision (table 2).

Accuracy, defined as the closeness between the reference and the found values, has been evaluated as percentage relative error between the measured and theoretical concentration of ZOF. The results are presented in table 2, and show good accuracy for this method.

#### Application to pharmaceutical formulation

The proposed method has been applied to the quantification of ZOF, in tablets of available commercial

Table 1  
REGRESSION PARAMETERS AND SENSITIVITY VALUE

| Parameter   | Value               |
|---|---------------------|
| $\lambda_{\max}$ / nm   | 248                 |
| Linearity range, µg/mL  | 2-70                |
| Molar absorptivity ( $\epsilon$ ), $\text{lmol}^{-1}\text{cm}^{-1}$   | $1.317 \times 10^4$ |
| Sandell sensitivity, $\mu\text{g}/\text{cm}^2/0.001 \text{ abs unit}$ | 0.034               |
| Slope   | 0.02961             |
| Intercept   | 0.00186             |
| Limit of detection (LOD) µg/mL  | 0.5052              |
| Limit of quantification (LOQ) µg/mL                                   | 1.5310              |
| Standard deviation of slope ( $S_b$ )                                 | 0.000036            |
| Standard deviation of intercept ( $S_a$ )                             | 0.001395            |
| Correlation coefficient (r)   | 0.999985            |

| ZOF taken, $\mu\text{g/ml}$ | Intra-day accuracy and precision |             |        |        | Inter-day accuracy and precision |             |        |        |
|-----------------------------|----------------------------------|-------------|--------|--------|----------------------------------|-------------|--------|--------|
|                             | ZOF found, $\mu\text{g/ml}$      | Recovery, % | RE, %  | RSD, % | ZOF found, $\mu\text{g/ml}$      | Recovery, % | RE, %  | RSD, % |
| 20                          | 20.01                            | 100.05      | 0.05   | 1.74   | 20.32                            | 101.60      | 1.60   | 1.19   |
| 40                          | 39.69                            | 99.23       | - 0.77 | 0.96   | 40.06                            | 100.15      | 0.15   | 1.21   |
| 60                          | 59.94                            | 99.90       | - 0.10 | 1.76   | 59.09                            | 98.48       | - 1.52 | 1.23   |

RE stands for Relative error and RSD for Relative standard deviation.

| Tablet brand name  | Labeled amount, mg/tablet | Amount found <sup>a</sup> , mg/tablet | Label claim $\pm$ SD, % |
|--------------------|---------------------------|---------------------------------------|-------------------------|
| Zomen <sup>b</sup> | 7.5                       | 7.445                                 | 99.260 $\pm$ 0.263      |
| Zomen              | 30                        | 29.904                                | 99.680 $\pm$ 0.323      |

<sup>a</sup> Mean value of six determinations; <sup>b</sup> Berlin-Chemie AG Menarini Group, Germany

| Tablet studied | ZOF in tablet, $\mu\text{g/ml}$ | Pure ZOF added, $\mu\text{g/ml}$ | Total found, $\mu\text{g/ml}$ | Pure ZOF recovered <sup>a</sup> $\pm$ SD, % |
|----------------|---------------------------------|----------------------------------|-------------------------------|---|
| Zomen 7.5      | 19.852                          | 10                               | 29.854                        | 100.025 $\pm$ 0.683                         |
|                | 19.852                          | 20                               | 39.467                        | 98.088 $\pm$ 0.603                          |
|                | 19.852                          | 30                               | 49.092                        | 98.181 $\pm$ 1.248                          |
| Zomen 30       | 19.936                          | 10                               | 30.135                        | 101.988 $\pm$ 0.377                         |
|                | 19.936                          | 20                               | 40.088                        | 100.758 $\pm$ 0.829                         |
|                | 19.936                          | 30                               | 49.840                        | 99.680 $\pm$ 1.112                          |

<sup>a</sup> Mean value of three measurements

brand ZOMEN tablets and the results are presented in table 3.

The values of the active substance recovery from pharmaceutical formulation ZOMEN tablets is between 98.997 and 99.523% for 7.5 mg/tablet, respectively between 99.357 and 100.003% for 30 mg/tablet, thus confirming the repeatability and the reproductibility of the proposed method.

#### Recovery analysis

The validity and the accuracy of the proposed method have been further demonstrated by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ZOF at three concentration levels (50, 100 and 150 % of that present in tablet powder), and the total amount has been found using the proposed method. The percentage values for ZOF added recovery are ranging between 98.088 and 101.988, with a standard deviation of 0.377 – 1.248%, which reveals a good recovery (table 4)

#### Conclusions

In this paper, a simple, rapid, sensitive, accurate and precise UV spectrophotometric method has been developed and validated for the quantification of zofenopril calcium in bulk and in pharmaceutical formulation. It has been found that common excipients present in the pharmaceutical formulation did not interfere with the proposed method and can be used for the routine analysis of ZOF in bulk and in marketed formulation.

#### References

- SUBISSI, A., EVANGELISTA, S., GIACHETTI, A., Cardiovascular Drug Reviews, 17, 2, 1999, p. 115.
- EVANGELISTA, S., MANZINI, S., Journal of international medical research, 33, 1, 2005, p. 42.

**Table 2**  
EVALUATION OF INTRA-DAY AND INTER-DAY ACCURACY AND PRECISION

**Table 3**  
ASSAY OF ZOF IN  
PHARMACEUTICAL FORMULATION BY  
THE PROPOSED METHOD

**Table 4**  
RECOVERY ANALYSIS RESULTS, OBTAINED  
WITH THE STANDARD-ADDITION METHOD

- MALACCO, E., OMBONI, S., Current Therapeutic Research, 69, 3, 2008, p. 232.
- BORGHI, C., BACCHELLI, S., DEGLI, D., AMBROSIONI, E., Expert Opin Pharmacother, 5, 2004, p. 1965.
- COMINACINI, L., FRATTA PASINI, A., GARBIN, U., EVANGELISTA, S., CREA, A., TAGLIACOZZI, D., NAVA, C., DAVOLI, A., LOCASCIO, V., AJH, 15, 2002, p. 891.
- KELLY, J.G., O'MALLEY, K., Clin. Pharmacokinet., 19, 1990, p. 177.
- JEMAL, M., IVASHKIV, E., TEITZ, D., COHEN, A.I., J. Chromatogr., 428, 1988, p. 81.
- WU, F., GAO, F., DING, L., MAO, X.M., MA, P.C., J. China Pharm. Univ., 40, 2009, p. 353.
- GAO, F., DING, L., MA, P., WU, F., Chromatographia, 71, 2010, p. 1007.
- DAL BO, L., MAZZUCHELLI, P., MARZO, A., J. Chromatogr. B., 749, 2000, p. 287.
- JIANG, Y., YAN, F., DI, B., FENG, F., YOU, L., HUANG, L., LU, J., J. Pharm. Biomed. Anal., 55, 3, 2011, p. 527.
- CARLUCCI, G., DI FEDERICO, L., IULIANI, P., J. Sep. Sci., 33, 2010, p. 1717.
- SERAP, A.S., J. Chromatogr. Sci., 49, 2011, p. 259.
- SEGI, T.U., Rev. Chim. (Bucharest), 62, no. 3, 2011, p. 261
- MĂNDRESCU, M., ȘPAC, A.F., DORNEANU, V., Rev. Chim. (Bucharest), 60, no. 2, 2009, p. 160
- CARLUCCI, G., DI FEDERICO, L., IULIANI, P., Analytical Letters, 43, 2010, p. 2609.
- \*\*\*, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Complementary Guideline on methodology dated 6 November 1996 incorporated in November 2005, London

Manuscript received: 5.07.2011