Research Article

Simultaneous Spectrophotometric Determination of Valsartan and Ezetimibe in Pharmaceuticals

Sridevi Ramachandran¹,²*, Badal Kumar Mandal¹ and Sameer G Navalgund²

¹School of Advance Sciences, Vellore Institute of Technology University, Vellore Tamil Nadu-632041, ²Analytical Research Department, International Specialty Products (India) Private Limited, 2nd and 3rd Floor Bhupal Towers Somajiguda, Hyderabad Andhra Pradesh - 500082, India.

Abstract

Purpose: To develop a direct, simple and extraction-free spectrophotometric method for the simultaneous estimation of valsartan and ezetimibe in pharmaceuticals.

Methods: A spectrophotometric method for the determination of valsartan and ezetimibe was developed using acidic dyes, namely, bromophenol blue (BPB) and bromocresol green (BCG). The method was based on selective ion-pair formation between valsartan and the acidic dye. The yellow coloured ion-pair induces a bathochromic shift in the spectrum with maximum absorbance at 425 and 428 nm for BPB and BCG, respectively. The developed method was validated as per ICH guidelines.

Results: With BPB, the ion-pair formed obeyed Beer’s law in the ranges 5 - 40 and 1 - 50 µg/mL for valsartan and ezetimibe, respectively. The assay data for valsartan and ezetimibe were, 99.39 ± 0.53 and 98.17 ± 0.91 %, respectively, for the commercial formulation, and 99.41 ± 0.48 and 98.16± 0.89 %, respectively, for the developed formulation. The method was validated and the correlation coefficient for valsartan and ezetimibe were 0.995 and 0.999, respectively. Recovery was in the range 99.3 - 100.3 %. Conclusion: The proposed method is reproducible, accurate, robust and suitable for the simultaneous quantitative analysis of the studied drugs in bulk and dosage formulation.

Keywords: Valsartan, Ezetimibe, Bromophenol blue, Bromocresol green, Spectrophotometric method

Received: 9 March 2011

Revised accepted: 23 October, 2011

*Corresponding author: Email: sri_devir@yahoo.com; Tel: +91-040-44747000
INTRODUCTION

Valsartan (3-methyl-2- [pentanoyl-[ [4-[2-(2H-
tetrazol-5-yl) phenyl] phenyl] methyl]amino]-
butanoic acid) is an angiotensin II receptor
antagonist acting on the AT1 subtype. It is
indicated for the treatment of high blood
pressure, congestive heart failure (CHF), and
post-myocardial infarction (MI). Ezetimibe
((3R, 4S)-1-(4-fluorophenyl)-3-((3S)-3-(4-
fluorophenyl)-3-hydroxypropyl)-4-(4-hydroxy-
phenyl)-2-azetidinone) is an anti-
hyperlipidemic medication used to lower
cholesterol levels. It acts by decreasing
cholesterol absorption in the intestine.

A literature survey on valsartan indicates that
several methods are available including those
based on high performance liquid
chromatography (HPLC) [1,2] and chiral
HPLC [3]. Valsartan and hydrochlorothiazide
have been determined in tablets
simultaneously by HPLC and first derivative
UV Spectrophotometry [4]; tandem mass
spectrometry has been used for the
determination of nebivolol and valsartan in
biological fluids [5], while valsartan and
amilodipine besylate in capsules have been
analyzed using reverse phase HPLC [6].
Ezetimibe has been determined in biological
fluids using HPLC [7] and in pharmaceutical
dosage forms [8]. Stress degradation studies
have been carried out on ezetimibe using
HPLC [9] while simvastatin and ezetimibe
have been simultaneously quantified in a
drug product using HPLC [10]. Although
studies on insulin-sensitizing agents
(metformin and rosiglitazone) in combination
with ezetimibe and valsartan for the treatment
of fatty liver disease have been reported [11],
to the best of our knowledge, no method for
the simultaneous analysis of valsartan and
ezetimibe in a combined dosage form has
been reported.

Ion-pair extraction technique is a well
recognized spectrophotometric method. An
ion-pair is usually formed between the drug
molecule (a basic compound) and an anionic
dye such as bromophenol blue (BPB) and
bromocresol green (BCG). The drug-dye
complex formed is either extracted using a
suitable solvent or measured directly using a
spectrophotometer [12]. The extraction
technique is often fraught with complications
such as incomplete extraction and formation
of emulsion between the hydrocarbon solvent
and the solution containing the basic
compound. Spectrophotometric procedures
are popular for their sensitivity and simplicity;
thus, ion-pair extractive spectrophotometry
has received considerable attention for the
quantitative determination of many
pharmaceutical compounds [12].

The aim of the present study is to develop a
method for the simultaneous spectrophotometric assay of valsartan and ezetimibe
by ion-pair formation using the dyes, bromophenol blue (BPB) and bromocresol
green (BCG).

EXPERIMENTAL

Apparatus and reagents

A uv/visible spectrophotometer (Agilent 8453,
China) with spectral bandwidth of 2 nm,
wavelength accuracy of ± 0.5 nm and 1 cm
quartz cells was used for all absorbance
measurements. Spectra were automatically
obtained from the system software
ChemStation. A Perkin Elmer UV/vis
spectrophotometer (Lambda 12) was used for
intermediate precision. Vortex cyclomixer
was used for obtaining solution of uniform
concentration. The formulations were
compressed with the automated Cadmach
(Model# CMD4) Compression machine using
the software, AIM, version 3.6 and presented
below. Labnet Hermle Z200A centrifuge
device was used for centrifugation of solution
during the study.

Methanol of chromatographic grade,
bromophenol blue and bromocresol green
(both 0.04 % solution) were procured from E.
Merck. Reference standard of valsartan (Lot
# TRC-200306011) and ezetimibe (5-YM-
118-1) were obtained from Toronto Research
Preparation of combined tablet formulation

Ezetimibe (50 %), lactose (20 %) and Plasdone K29/32 (3 %) were pre-mixed with water for 1 min in Pro-C-Ept granulator at an impeller speed of 1000 rpm. The granulator speed was increased to 2300-2500 rpm 2 min later following the addition of water. The mass was sieved through #18-mesh sieve and dried at 60 °C in an oven. The dried granules were sized through #30-mesh sieve, blended valsartan (38 %), Eudragit RLPO (36 %), microcrystalline cellulose (14 %), hydroxypropyl methylcellulose (10 %), magnesium stearate (1 %) and fumed silica (1 %) in a V-cone blender (8 rpm) to form a homogeneous mix and compressed using 11 mm round shaped standard punch set was used on a 16-station rotary tablet press (Cadmach, model CMD4, India). Advanced Instrumentation Monitor (AIM) software (Metropolitan Computing Corporation, USA) was used with the tablet press to determine the compression force required to give tablets of approximately equal hardness in the study.

Optimisation of solvent

Optimisation of conditions is necessary for rapid and quantitative formation of colored ion-pair complexes with maximum stability and sensitivity. Maximal absorbance was observed in methanol solution and this solvent was found to be suitable for both BPB and BCG while chloroform and dichloromethane were not.

Optimisation of dye concentration

Four different standard solutions of valsartan containing 10, 20, 30 and 70 µg/mL were prepared by diluting valsartan stock solution (500 µg/mL) with methanol in 50 mL standard volumetric flasks. To each concentration of valsartan, varying concentrations of dye were prepared from the stock solution (400 µg/mL) to constitute the final dye concentration of 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96 and 104 µg/mL, and the volumes made up with methanol. The solutions were shaken well in a vortex shaker for uniform concentration and scanned spectrophotometrically. The optimised dye concentration was found to be 72 µg/mL, based on the validation data obtained and this concentration was applied in all subsequent analyses.

Standard and sample solutions

Twenty tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder equivalent to 80 mg of valsartan (Valzaar 80 mg) and 10 mg of ezetimibe (Zeteze 10 mg) was transferred to
a 200 mL standard volumetric flask; the solution was made up to the mark using methanol and centrifuged for 20 minute at 2500 rpm. The solution was suitably diluted to contain 24 µg/mL valsartan and 3 µg/mL ezetimibe. A dye concentration of 72 µg/mL was added to the final solution and made up to the mark with methanol. The combined formulation of valsartan (80 mg) and ezetimibe (10 mg) was formulated in-house and the solutions for the analysis were prepared as per the procedures described above.

A stock standard solution of 400 µg/mL valsartan and 50 µg/ mL ezetimibe was prepared and suitably diluted with methanol to obtain final concentrations within Beer Lambert’s range, i.e., 24 µg/mL valsartan and 3 µg/mL ezetimibe, in a 50 mL volumetric flask. A dye (BPB) concentration of 72 µg/mL was added to the final solution and made up to the mark with methanol. The solutions were vortexed to obtain a uniform concentration. Both standard and sample solutions were scanned using Agilent UV spectrophotometer.

**Method validation**

**Linearity and range**

Calibration curve was constructed with various concentrations of Valsartan and Ezetimibe in the range of 5- 40 µg/mL and 1- 50 µg/ mL respectively. The linearity graph was plotted with absorbance against concentration.

**Stability of the complexes**

The absorbance of nine different concentrations (in the range of 5 - 50 µg/mL) of valsartan-dye was measured periodically after 24, 60, 72 and 120 h.

**Intermediate precision**

The intermediate precision study was performed by scanning the solution of valsartan-dye complexes using an alternative spectrophotometer (Perkin Elmer double beam spectrophotometer).

**Recovery study**

Recovery experiments were conducted to determine the accuracy of the proposed method. In order to detect possible interaction with excipients, the recovery of the sample solution was studied by spiking a known quantity of the drug in the range 20 - 30 µg/mL and 2 - 4 µg/mL for valsartan and ezetimibe respectively with placebo (the placebo was obtained by mixing all the content in the tablet excluding the drug).

**Job’s Method**

The stochiometry of the ion pair was determined by Job’s method using equimolar solution. In this method the complex is made to form at different mole fraction and the absorbance is measured.

**Statistical analysis**

The results obtained were subjected to statistical analysis using two tailed student’s t-test using MS Excel 2007. Differences were considered significant at $p < 0.05$.

**RESULTS**

The developed method was optimised and validated as per international conference on harmonisation (ICH) guidelines [13]. The stochiometric relationship of the complex formed was estimated using Job’s method. The method was applied to obtain the assay of the formulation.

**Optical characteristics**

The factors affecting color development, reproducibility and adherence to Beer’s law are presented in Table 1.
### Table 1: Optical characteristics of valsartan and ezetimibe (n = 5)

| Parameter                        | BPB | Valsartan | Ezetimibe | BCG | Valsartan | Ezetimibe |
|----------------------------------|-----|-----------|-----------|-----|-----------|-----------|
| $\lambda_{\text{max}}$ (nm)     | 423 | 250       | 428       | 250 |           |           |
| Stability (h)                    | 120 | 120       | 120       | 120 |           |           |
| Beer’s law range (µg/mL)        | 5-40| 1-50      | 5-35      | 1-50|           |           |
| Molar absorptivity $^a$          | $1.5 \times 10^4$ | $8.9 \times 10^4$ | $1.45 \times 10^4$ | $8.5 \times 10^4$ |

#### Regression data

- Slope (a): 0.033, 0.036, 0.032, 0.037
- Intercept (b): 0.006, 0.012, 0.042, 0.008
- Correlation coefficient (r): 0.9950, 0.9990, 0.9930, 0.9990
- RSD (%) $^a$: 1.12, 0.43, 1.30, 0.40

$^a$ε L mol$^{-1}$ cm$^{-1}$; BPB = bromophenol blue; BCG = bromocresol green

### Table 2: Recovery data for combined dosage form of valsartan and ezetimibe using bromophenol blue (mean ± SD, n = 5)

| Sample     | Dye Used | µg/mL | Recovery (%) | CV$^a$ |
|------------|----------|-------|--------------|--------|
|            |          | Taken | Found        |        |
| Valsartan  | BPB      | 20    | 20.02 ± 0.04 | 100.11 ± 0.25 | 0.25   |
|            |          | 25    | 25.06 ± 0.09 | 100.26 ± 0.35 | 0.35   |
|            |          | 30    | 30.02 ± 0.08 | 100.06 ± 0.28 | 0.28   |
| Ezetimibe  |          | 2     | 1.99 ± 0.01  | 99.73 ± 0.42 | 0.42   |
|            |          | 3     | 2.99 ± 0.01  | 99.53 ± 0.38 | 0.38   |
|            |          | 4     | 4.01 ± 0.02  | 100.16 ± 0.45 | 0.45   |
| Valsartan  | BCG      | 20    | 19.98 ± 0.03 | 100.04 ± 0.17 | 0.16   |
|            |          | 25    | 24.96 ± 0.05 | 99.83 ± 0.20 | 0.20   |
|            |          | 30    | 29.97 ± 0.06 | 99.89 ± 0.19 | 0.19   |
| Ezetimibe  |          | 2     | 1.99 ± 0.01  | 99.29 ± 0.40 | 0.4    |
|            |          | 3     | 2.98 ± 0.01  | 99.35 ± 0.30 | 0.3    |
|            |          | 4     | 3.99 ± 0.02  | 99.86 ± 0.48 | 0.48   |

$^a$CV- Coefficient of variance (in %)

The correlation coefficient of the calibration curves of valsartan and ezetimibe were 0.995 (at 425 nm) and 0.999 (at 250 nm), respectively in the concentration range 5 - 40 and 1 - 50 µg/mL, respectively. The valsartan - complex formed were stable for up to 120 h at ambient temperature. The intermediate precision analysis was performed using an UV spectrophotometer from another manufacturer (Perkin Elmer). The concentration of valsartan – BPB dye complexes were linear in the range 5 - 40 µg/mL with a correlation coefficient of 0.9934 and 0.9954 using both Perkin Elmer and Agilent spectrophotometers, respectively. The accuracy (% recovery) was performed and it was found to be in the range 99.3 - 100.3 % (Table 2).

#### Stoechiometric relationship

The composition of ion-pair was determined by Job’s method [14] using equimolar

---

Trop J Pharm Res, December 2011;10 (6):813
Table 3: Assay results for valsartan and ezetimibe in tablet formulations

| Dye      | Sample drug content | Amount found (%) | Calculated value of t |
|----------|---------------------|------------------|-----------------------|
|          |                     | MF ± SD          | CV                    | DF ± SD | CV |
| BPB      | Valsartan (80 mg)   | 99.39 ± 0.53     | 0.54                  | 99.41 ± 0.40 | 0.40 | 0.97 |
|          | Ezetimibe (10 mg)   | 98.17 ± 0.91     | 0.92                  | 98.15 ± 0.89 | 0.92 | 0.98 |
| BCG      | Valsartan (80 mg)   | 99.41 ± 0.48     | 0.48                  | 99.44 ± 0.37 | 0.37 | 0.94 |
|          | Ezetimibe (10 mg)   | 98.16 ± 0.89     | 0.89                  | 98.24 ± 0.85 | 0.85 | 0.89 |

*a* Bromophenol blue; *b* bromocresol green; CV = coefficient of variation (in %); SD = standard deviation; *c* commercial formulation; DF = formulation prepared in-house

Valsartan solution at concentrations of 239 and 229 µM for BPB and BCG, respectively. The plot reached maximum value at a mole fraction of 0.5, indicating complex formation in the ratio of 1:1 (valsartan: dye) as Fig 1 shows.

![Graph showing absorbance against mole fraction of drug and dye](image)

**Figure 1:** Job’s method of continuous variation for valsartan-dye complex

**Application of the developed method to pharmaceutical formulations**

The developed method was applied to analyse a commercial product and a tablet formulation developed in-house, both of which contained valsartan and ezetimibe. The UV spectrum was recorded and their drug contents were calculated by comparing the absorbance of the sample at 425 nm (using BPB dye) and 250 nm against a standard sample. Drug contents of both the standard and samples were calculated for a concentration of 24 µg/mL Valsartan and 3µg/mL Ezetimibe (Table 3).

**DISCUSSION**

Anionic dyes such as BPB and BCG form ion-association complexes selectively with one of the drug molecules, i.e., valsartan, forming a yellow colored complex. This might be due to the electron-donating groups (tetrazole and amino butanoic acid) present in the valsartan structure [15]. Valsartan forms ion-pair complex selectively with the dye, as indicated by the formation of a yellow coloured complex. The other drug, ezetimibe, does not interact with the dye due to the presence of fluorine [16]. Fluorine, being highly electronegative, does not make available any free electron in the molecule to interact with the acidic dye and hence it does not show a characteristic color change. Valsartan dye complex behaves as a single unit held together by electrostatic force of attraction [14]. The absorbance of valsartan shifted from 250 nm to 425 nm (bathochromic shift) after the complex formation with the dye solution.

The method developed was validated according to ICH guidelines, being found to adhere to Beer’s law in the concentration range of 5 - 40 µg/mL and 1 - 50 µg/ mL for valsartan and ezetimibe, respectively (when BPB was used as the dye).

**CONCLUSION**

Both valsartan and ezetimibe were successfully determined in commercial tablets containing the drugs separately as...
well as in combined formulation. The ion-pair complex formation takes place instantaneously and the color formation is stable. Excipients used in the pharmaceutical formulation did not interfere in the analysis. Based on the results obtained, the proposed method is accurate, precise, reproducible, economical and can be employed for routine analysis of valsartan and ezetimibe in a dosage formulation containing both drugs.

ACKNOWLEDGMENT

Ms R Sridevi acknowledges the help of VIT University, Vellore - 632014, India for making facilities available to carry out this research. She also acknowledges the support of International Specialty Products (P) Ltd, Hyderabad - 500082, India, who also provided access to some of their facilities in the course of this research work.

REFERENCES

1. Koseki N, Kawashita H, Hara Hisanori, Niina M, Tanaka M, Kawai Ryosei, Nagae Y, Masuda N. Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry. J. Pharm. Biomed. Anal, 2007; 43: 1769-1774.

2. Iriarte G, Gonzalez O, Ferreiros N, Maguregui MI, Alonso RM, Jimenez RM. Validation of a fast liquid chromatography-UV method for the analysis of drugs used in combined cardiovascular therapy in human plasma. J. Chromatogr. B, 2009; 877: 3045-3053.

3. Francotte E, Davatz A, Richert P. Development and validation of chiral high-performance liquid chromatographic methods for the quantitation of valsartan and of the tosylate of valinebenzyl ester. J. Chromatogr. B, 1996; 686: 73-83.

4. Satana E, Altinay S, Goger NG, Ozkan SA, Senturk Z. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. J. Pharm. Biomed. Anal, 2001; 25: 1009-1013.

5. Senthilvelan P, Gowda P, Mandal KV, Sam Solomon WD, Pal TK. Simultaneous determination of fixed dose combination of nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to pharmacokinetic study. J. Chromatogr. B, 2007; 858: 143-150.

6. Chitlange SS, Bagi K, Sakarkar DM. Stability Indicating RP-HPLC method for Simultaneous Estimation of Valsartan and Amilodipine in Capsule Formulation. Asian J. Research Chem, 2008; 1: 15-18

7. Li S, Liu G, Jia J, Li X, Yu C. Liquid chromatography- negative ion electrospray tandem mass spectrometry method for the quantification of Ezetimibe in human plasma. J. Pharm. Biomed. Anal, 2006; 40: 987-992.

8. Sistla R, Tata VSSK, Kashyap YV, Chandrasekar D, Diwan PV. Development and validation of a reversed-phase HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms. J. Pharm. Biomed. Anal, 2005; 39: 517-522.

9. Singh S, Singh B, Bahuguna R, Wadhw L, Saxena R. Stress degradation studies on Ezetimibe and development of a validated stability-indicating HPLC assay. J. Pharm. Biomed. Anal, 2006; 41: 1037-1040.

10. Hefnawy M, Mohammed AO, Julkhuf S. Rapid and sensitive simultaneous determination of Ezetimibe and Simvastatin from their combination drug products by monolithic silica high-performance liquid chromatographic column. J. Pharm. Biomed. Anal, 2009; 50: 527-534.

11. Asy N, Grozovski M, Bersudsky I, Szvalb S, Hussein O. Effect of insulin-sensitizing agents in combination with Ezetimibe, and Valsartan in rats with non-alcoholic fatty liver disease. World Gastroenterology, 2006; 12(27): 4369-4376.

12. Abidine H, Belal F, Zoman N. Simple spectrophotometric determination of cinnarazine in its dosage forms. Farmaco, 2002; 57:267-271.

13. ICH Guidelines, “Validation of Analytical Procedures: Text and Methodology” [Accessed 1 March 1995, Vol. 60, p. 11260 [cited 2011 Jan 10]. Available from: http://www.ich.org

14. Ashour S, Al-Khail. R. Simple extractive colorimetric determination of levofloxacin by acid-dye complexation methods in pharmaceutical preparations. Farmaco, 2005; 60: 771-775.

15. El Sherif ZA, Mohamed AO, Walash MI, Tarras FM. Spectrophotometric determination of loperamide hydrochloride by acid-dye and charge-transfer complexation methods in the presence of its degradation products. J. Pharm. Biomed. Anal, 2000; 22: 13-23.

16. Carroll TX, Thomas TD, Bergersen H, Borve KJ, Saethere LJ. Fluorine as a pi donor. Carbon 1s photoelectron spectroscopy and proton affinities of fluorobenzenes. J. Org. Chem. 2006; 71(5): 1961-1968.