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Research Article

Simultaneous Spectrophotometric Determination of Valsartan and Ezetimibe in Pharmaceuticals

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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*

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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 spectro-photometric 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

Chemicals, Canada. Valsartan and ezetimibe powders were obtained from Matrix Pvt. Ltd and Glenmark Pvt Ltd, India, respectively. The commercial formulation of valsartan (Valzar 80 mg Lot# 06039005) was obtained from Torrent Pharmaceuticals Ltd while ezetimibe (Zeteze 10 mg Lot# 1997604) was obtained from Ranbaxy Laboratories Ltd, India

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 ℃ 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.

Selection of dye

The complex formation ability of valsartan and ezetimibe were first tested with 10 different dyes in the pH range of 0 to 10. The dyes used (with their pH range in parenthesis) are: eosin (0 to 3), cresol red (0.2 to 1.8), methyl orange (2.8 to 4.6), bromophenol blue (2.8 to 4.6), methyl red (4.2 to 6.3), bromocresol green (3.6 to 5.2), bromothymol blue (6.0 to 7.6), thymol blue (1.2 to 2.8 and 7.8 to 9.5), phenolphthalein (8.2 to 10.0) and methylene blue. The analysis was repeated with three different solvents, namely, methanol, acetonitrile and acetone. BPB and BCG both showed similar results at 425 and 428 nm, respectively. Preliminary trials performed for the extraction of valsartan-dye ion pair with two immiscible solvents - dichloromethane and chloroform followed by UV analysis - showed that there was no absorbance at 425 nm for BPB, thus indicating that the complex formed is stable in methanol only.

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		BCG	
	Valsartan	Ezetimibe	Valsartan	Ezetimibe
λ_{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]	1.5×10^4	8.9×10^4	1.45×10^4	8.5×10^4
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).

Stochiometric relationship

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

Table 3: Assay results for valsartan and ezetimibe in tablet formulations

Dye	Sample drug content	Amount found (%, mean ± SD)				Calculated value of t
		***MF*[c]**	***CV*[d]**	***DF*[e]**	***CV*[d]**	
BPB[a]	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[b]	Valsartan (80 mg)	99.41± 0.48	0.48	99.44± 0.37	0.37	0.94
	Ezetimibe (10 mg)	98.16± 0.89	0.91	98.24± 0.85	0.87	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.

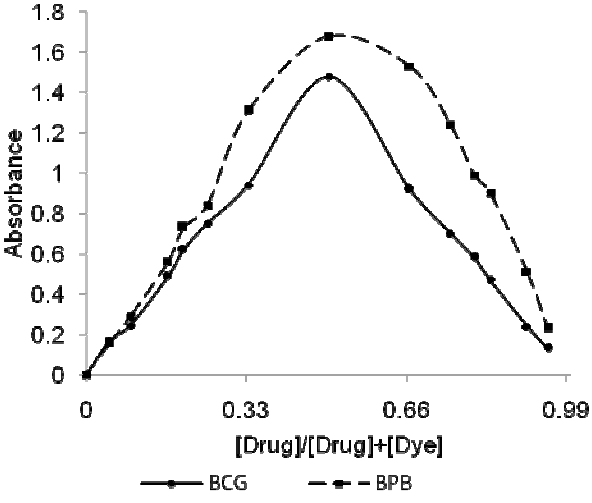

Figure 1: Job`s method of continuous variation for valsartan-dye complex

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]. T he 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.

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