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Stability indicating ultraviolet spectroscopic method for the estimation of ezetimibe and carvedilol

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In this study, new and rapid stability indicating ultraviolet spectroscopic methods were developed and validated for the estimation of ezetimibe and carvedilol in pure form and in their respective formulations. Since both the drugs are poorly water soluble, 20% v/v acetonitrile in triple distilled water was selected as the solvent system for both the drugs. This ensured adequate drug solubility and maximum assay sensitivity. The linearity range for ezetimibe and carvedilol at their respective wavelength of detection of 232 nm and 238 nm was obtained as 2–50 μg/ml and 2–20 μg/ml respectively. The linear regression equations obtained by least square regression method, were $Y = 0.0443 \cdot X + 0.0106$ for ezetimibe and $Y = 0.1080 \cdot X + 0.034$ for carvedilol, where Y is the absorbance and X is the concentration (in μg/ml) of pure drug solution. The detection and quantitation limit as per the error propagation theory were found to be 0.4 μg/ml and 1.3 μg/ml respectively for ezetimibe and 0.7 μg/ml and 2.1 μg/ml respectively for carvedilol. The methods were employed with high degree of precision and accuracy for the estimation of total drug content in two commercial tablet formulations of each of the two drugs. It was concluded that both the developed methods are accurate, sensitive, precise, and reproducible. They can be applied directly for the estimation of drug content in pharmaceutical formulations.

1. Introduction

Ezetimibe, (1-(4-fluorophenyl)-3(*R*)-[3-(4-fluorophenyl)-3(*S*)-hydroxypropyl]-4(*S*)-(4-hydroxyphenyl)-2-azetidinone), initially developed as an acylcoenzyme-A cholesterol acyltransferase inhibitor, is the first drug of a new class of lipid-lowering compounds that selectively inhibit the intestinal absorption of cholesterol and related plant sterols (van Heek et al. 1997; Catapano 2001; Clader 2004). It is orally active, with a unique mechanism of action that differs from other classes of cholesterol-reducing compounds e.g., HMG-CoA reductase inhibitors (statins), bile acid sequestrants (resins), fibric acid derivatives, and plant stanols. It is rapidly absorbed and is extensively metabolized to an active phenolic glucuronide. The metabolized form is actively secreted into the lumen through entero-hepatic recirculation. Its action is localized at the brush border of the small intestine where it inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver (van Heek and Davis 2002).

Carvedilol, ±1-(carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]amino]-2-propanol) is a third-generation, neurohormonal antagonist with multiple activities. It blocks both β_1- and β_2-adrenergic receptors and also enhances vasodilatation via α_1-adrenergic blockade (Dulin and Abraham 2004). It is also reported to act as calcium channel blocker at high concentrations (Dulin and Abraham 2004). Carvedilol lacks sympathomimetic activity. In addition to these well-known properties, carvedilol has a number of ancillary activities including antioxidant, anti-inflammatory, and antiapoptotic actions (Dulin and Abraham 2004). Together, they contribute to the clinical efficacy of carvedilol in a broad spectrum of patient types and may also confer a range of cardioprotective benefits.

A detailed review of the literature revealed only a few HPLC methods for the estimation of carvedilol in rat plasma (Hokama et al. 1999), human plasma (Yang et al. 2004; do Carmo Borges et al. 2005), and for its desmethyl metabolite in body fluids (Reiff 1987). Studies on fluorescence property of carvedilol and its estimation by fluorimetry have also been reported (Xu et al. 2005). However a survey of the literature did not reveal any UV spectrophotometric method for the analysis of carvedilol. In case of ezetimibe an extensive literature survey did not reveal any reported spectrophotometric analytical methods.

In the present study, two simple, rapid, precise, accurate and reproducible UV spectrophotometric methods for the estimation of ezetimibe and carvedilol in pure form and in its solid dosage forms were developed. The results of the analysis were validated by statistical methods (ICH guidelines 1996; USP 2000) and recovery studies. The developed methods were used to estimate the total drug content in two different commercially available tablet formulations of each drug.

2. Investigations, results and discussion

2.1. Method development and stability of drugs

To develop a rugged and sensitive UV spectrophotometric method for the analysis of ezetimibe and carvedilol in their respective pharmaceutical formulations different solvents systems were tried, e.g., high purity water, methanol, acetonitrile, 0.1 N NaOH, 0.1 N HCl, and various buffers like phosphate buffer (pH 5.2–8.0), acetate buffer (pH 3.6–5.6) and citrate buffer (pH 3.0–7.0) either alone or in combination (at a proportion 20 to 80% v/v) with aqueous or organic solvents. Solubility and bench-top stability studies of ezetimibe and carvedilol in various solvents indicated very good solubility and stability for both the drugs with varying proportions of acetonitrile with water (20 to 80% v/v). The UV spectrum obtained at different time intervals up to 24 h was found to be reproducible for acetonitrile-water system. In other solvent systems investigated, a marked difference in the UV spectrum of ezetimibe and carvedilol was observed within 2–6 h. Adequate amount of drug (in order to prepare a stock solution without attaining saturation solubility) could be solubilised at 20% v/v acetonitrile in water, therefore, a higher proportion of acetonitrile was deemed unnecessary. The final decision of using 20% v/v acetonitrile-water for analysis of these drugs was based on sensitivity of the assay, interference from blank in the analysis, suitability for drug content estimation and stability of the drug, ease of preparation, analysis time and cost in that order. Effect of various commonly employed tablet formulation additives on the absorbance of ezetimibe and carvedilol has been studied in the selected solvent system and no interference was observed.

2.2. Calibration and validation data for estimation of ezetimibe and carvedilol

The wavelength of detection (λ_{det}) in 20% v/v acetonitrile-water was found to be 232 nm for ezetimibe and 238 nm for carvedilol. The linearity range was found to be 2–50 μg/ml for ezetimibe at 232 nm and 2–20 μg/ml for carvedilol at 238 nm. The statistical analysis (ICH guidelines 1996; USP 2000) of data obtained for the estimation of ezetimibe and carvedilol in pure solution indicated high levels of precision and accuracy for the proposed methods as evidenced by the low values of standard deviation, standard error and coefficient of variation (Table 1).

The linear regression equations obtained were $Y = 0.0443 \cdot X + 0.0106$ for ezetimibe and $Y = 0.1080 \cdot X + 0.0340$ for carvedilol, where Y is the absorbance and X is the concentration (in μg/ml) of pure drug solution. Linearity of the regression equation and negligible scatter of points for the two drugs by the proposed methods were demonstrated from the highly significant ($p > 0.05$) correlation coefficient value (Table 2). The reported slope values without intercept on the ordinate, at 95% confidence limits, suggested that the calibration lines of ezetimibe and carvedilol solutions in 20% v/v acetonitrile-water did not deviate from the origin as the above-obtained values fall within the confidence limits (Table 2). Based on this evidence the linearity characteristics of the proposed methods can be practically considered as 0–50 μg/ml and 0–20 μg/ml respectively for ezetimibe and carvedilol. The precision of the fit was further confirmed from the low standard error values of the intercept, slope and the estimate.

A one-way ANOVA test of linearity (Bolton 1997; Shah 1992) was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions of both the drugs. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5% significance level in case of proposed methods for the two drugs (Table 3).

The developed methods were further validated according to ICH Q2B guidelines (ICH 1996) and the results obtained are shown in Table 4. The LOD and LOQ were calculated by using the relation 3.3σ/S and 10σ/S respectively, where σ is the standard error of estimate and S is the slope. Calculated values of limit of detection (LOD) and quantitation (LOQ) for ezetimibe were found to be 0.43 and 1.29 μg/ml respectively. The LOD and LOQ of carvedilol were found to be 0.69 and 2.10 μg/ml respec-

Table 1: Calibration points of the proposed methods in estimation of standard solution of ezetimibe and carvedilol by UV spectrophotometry at 232 nm and 238 nm respectively

Ezetimibe				Carvedilol			
Conc. (μg/ml)	Mean absorbance*	CV%	Std. Error	Conc. (μg/ml)	Mean absorbance*	CV%	Std. error
2	0.1045 ± 0.0011	1.06	0.0017	2	0.2242 ± 0.0038	1.17	0.0009
10	0.4294 ± 0.0045	1.04	0.0024	5	0.5942 ± 0.0173	2.91	0.0014
20	0.8684 ± 0.0052	0.59	0.0042	10	1.1158 ± 0.0295	2.65	0.0068
40	1.7174 ± 0.0114	0.66	0.0076	15	1.6827 ± 0.0629	3.74	0.0036
50	2.1272 ± 0.0136	0.63	0.0069	20	2.1686 ± 0.0576	2.66	0.0085

* Average of nine determinations with standard deviations; CV – Coefficient of variation

Table 2: Regression analysis of data for the estimation from standard solution by the proposed method

Statistical Parameters	Ezetimibe	Carvedilol
Regression equation	$Y = 0.0443 \cdot X + 0.0106$	$Y = 0.1080 \cdot X + 0.034$
Correlation coefficient	0.9999	0.9990
Standard error of slope	2.2×10^{-3}	2.00×10^{-3}
Standard error of intercept on ordinate	6.17×10^{-3}	2.44×10^{-2}
Standard error of estimate	3.55×10^{-4}	2.12×10^{-3}
95% confidence interval of slope	4.49×10^{-2}, 4.37×10^{-2}	11.43×10^{-2}, 10.16×10^{-2}
95% confidence interval of intercept	2.65×10^{-2}, -5.25×10^{-3}	11.21×10^{-2}, -4.38×10^{-2}
Slope without intercept	0.0446	0.01103

* Based on seven calibration values: Y = Absorbance; X = Conc. of drug in μg/ml

Table 3: One-way ANOVA test for linearity of the proposed methods

Source of variation	DF	Ezetimibe			Carvedilol		
		MS	F_{Cal}	F_{Crit}[a]	MS	F_{Cal}	F_{Crit}[a]
Between groups	2	5.54×10^{-4}	8.82×10^{-4}	3.55	2.40×10^{-4}	4.25×10^{-4}	3.55
Within groups	18	6.286×10^{-1}			5.76×10^{-1}		
Total	20						

[a] Theoretical value of F (2, 18) based on one-way ANOVA test at 5% level of significance; MS – Mean sum of squares

Table 4: Validation report for the determination of ezetimibe and carvedilol standard solutions by the proposed methods

Validation parameter	Ezetimibe			Carvedilol		
Accuracy	100.18 ± 1.87			98.5123 ± 1.98		
Precision	Conc.	Intraday	Interday	Conc.	Intraday	Inter-day
	2	1.70	2.79	2	1.15	2.02
	10	1.01	0.21	5	0.80	3.08
	20	0.07	0.44	10	0.71	2.72
	40	0.35	0.91	15	0.69	3.81
	50	0.32	0.23	20	0.34	2.69
	% RSD (Mean ± SD)	0.85 ± 0.61	0.88 ± 0.93	% RSD (Mean ± SD)	0.74±0.29	2.86 ± 0.65
Specificity	A 20 μg/ml solution of ezetimibe will show an absorbance of 0.8684 ± 0.0052 at 232 nm			A 10 μg/ml solution of carvedilol will show an absorbance of 1.1158 ± 0.029 at 238 nm		
Linearity	2–50 μg/ml			2–20 μg/ml		
Limit of detection	0.43 μg/ml			0.69 μg/ml		
Limit of quantitation	1.29 μg/ml			2.10 μg/ml		
Robustness (% RSD)	<1.0%			<1.4%		

tively. Both methods were robust as the change in percentage of acetonitrile from 15 to 25% v/v resulted in a change in precision of less than 1.0% and 1.4% respectively for the estimation of ezetimibe and carvedilol.

2.3. Drug content estimation and recovery studies

The two methods were employed for the estimation of ezetimibe and carvedilol in two of their respective pharmaceutical formulations (in duplicate at three concentration levels). A pure drug solution (100 μg/ml) prepared in 20% v/v acetonitrile-water was used as in-house control. The results of drug content estimation and analytical recovery studies are presented in Table 5. The estimated drug content with low values of standard deviation again established the precision of the proposed methods and, therefore, suggested non-interference from the formulation matrix present in the formulations evaluated. The accuracy of the results of estimation was further tested by recovery experiments. The accuracy of analytical recoveries obtained was 99.74 ± 0.23% and 99.87 ± 0.27% in the two selected formulation matrices for ezetimibe. For carvedilol the accuracy of analytical recoveries were obtained as 98.88 ± 0.01% and 99.96 ± 0.02%. Accurate analytical recovery values for both the drugs further suggested absence of interference from the formulation matrix on the UV absorbancy profile of the two drugs by the proposed methods.

2.4. Forced degradation studies

The proposed method of estimation of ezetimibe and carvedilol can be considered to have stability indicating potential. The UV spectrum of ezetimibe upon forced degradation in 0.5N sulphuric acid and heating at 80 °C for 20 min showed a drastic change in the profile with absorbance drastically reducing at λ_{det} of the drug. Similarly for carvedilol degradation using 0.5 N hydrochloric acid and 0.5 N sodium hydroxide significantly altered the UV absorbancy profile of the drug.

In conclusion the proposed methods for the estimation of ezetimibe and carvedilol were found to be accurate, precise, easy and stability indicating for routine analysis in quality control laboratories and formulation design and development laboratories. There is no extraction or complicated sample preparation step involved thus decreasing the error and time involved in drug content estimation. The sample recoveries for all formulations were in good agreement with their respective label claims suggesting non-interference of formulation excipients, present in analyzed formulations, in the estimation. Furthermore, the assay

Table 5: Assay and recovery result from two commercially available formulations

Sample	Ezetimibe			Carvedilol		
	Label claim (mg/tab)	Assay* (%)	Analytical Recovery* (%)	Label claim (mg/tab)	Assay* (%)	Analytical recovery* (%)
Pure drug solution[φ]	–	100.21 ± 0.86	99.97 ± 0.17	–	100.54 ± 0.03	100.29 ± 0.09
Brand 1	10	98.11 ± 0.75	99.74 ± 0.23	3.125	98.70 ± 0.04	98.88 ± 0.01
Brand 2	10	99.01 ± 0.33	99.87 ± 0.27	12.5	99.42 ± 0.03	99.96 ± 0.02

* Mean of triplicate determination
[φ] 100 μg/ml

methods developed can be used as stability indicating methods due to the high stability of analyte in the solvent systems used and detection of any degradation at the selected wavelength of analysis.

3. Experimental

3.1. Materials

Ezetimibe and carvedilol were obtained as gifts from Dr. Reddy's Laboratory, Hyderabad, India. Spectroscopic grade acetonitrile were purchased from Merck, India. High quality pure water was prepared using Millipore purification system (Millipore, Molsheim, France, model Elix SA 67120). Two commercially available tablet formulations of ezetimibe (EZEDOC, Lupin Laboratory, Aurangabad, India and ZETEZE, Ranbaxy Laboratory, Gurgaon, India) and carvedilol (CARCA 3.125 and CARCA 12.5, Intas Pharmaceutical, Ahmedabad, India) were selected on a random basis from the market. These tablets contain common additives like diluents (lactose), disintegrating agents (starch), glidants and lubricants (magnesium stearate, talc etc.).

3.2. Instrument

A UV-visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V-570) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cell pair with Jasco spectra manager software was used for all absorbance measurements.

3.3. Method development

To develop a rugged and suitable UV spectrophotometric method for the analysis of ezetimibe and carvedilol in formulations different solvents systems were used. The criteria employed for assessing the suitability of a particular solvent system for analysis of each drug were solubility of the drug, cost, time required for analysis, sensitivity of the assay, solvent noise, preparatory steps involved and use of the same solvent system for extraction of the drug from the formulation excipient matrix for estimation of the drug content.

3.4. Stability of drugs in the solvents investigated

The stability of the drugs was assessed under bench top conditions in various solvent systems used for method development with respect to any change in the UV absorbancy profile of the drug with time. Various solvent systems employed for this purpose include, high purity water, methanol, acetonitrile, 0.1 NaOH, 0.1 N HCl, and various buffers like phosphate buffer (pH 5.2–8.0), acetate buffer (pH 3.6–5.6) and citrate buffer (pH 3.0–7.0) either alone or in combination with aqueous or organic solvents (at a proportion 20 to 80% v/v). For all the above conditions a 100 μg/ml stock solution of the drug in acetonitrile was diluted using the investigated solvents to get a concentration of 10 μg/ml and scanned for UV absorbancy at different intervals of time.

3.5. Preparation of standard curve

Since both ezetimibe and carvedilol are poorly water-soluble drugs the stock solution of each of the drugs was prepared by dissolving 10 mg of the drug in 20% v/v acetonitrile-water in a 100 ml volumetric flask. The λ_{det} of ezetimibe and carvedilol in the above media was determined by scanning a suitable dilution of the stock using UV spectrophotometer. From the stock solution, various dilutions were made in 10 ml volumetric flasks to obtain dilutions of concentration 2, 10, 20, 40, 50 μg/ml for ezetimibe and 2, 5, 10, 15, 20 μg/ml for carvedilol. The absorbance was measured on scanning UV spectrophotometer as listed in section 3.2. The results are presented in Table 1 for both the drugs. The results of regression analysis and test for linearity are presented in Table 2 and 3 respectively.

3.6. Method validation

Following method validation parameters were determined by analyzing the required test solutions as per the procedure given in section 3.4.

Accuracy and precision: Five separate (10 μg/ml) standard and test solutions of ezetimibe and carvedilol were prepared in duplicate from freshly prepared stock solution and analyzed.

Linearity: Five separate series of solutions of the drug, in the concentration range 2–50 μg/ml for ezetimibe and 2–20 μg/ml for carvedilol, were prepared from the stock solution and analyzed.

Specificity: Series of five solutions of the drug at 10 μg/ml each of ezetimibe and carvedilol were prepared from the respective stock solutions meant for method validation and analyzed.

Limit of detection (LOD) and quantitation (LOQ): LOD and LOQ were calculated on the basis of response and slope of the regression equation. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected for the two drugs.

Robustness: Robustness of the method was determined by varying the relative percentage of acetonitrile from 15–25% v/v in the solvent system used.

3.7. Estimation of drug content in commercial tablets

Two commercially available tablet brands of ezetimibe and carvedilol were selected from the Indian market for estimation of total drug content per tablet. For each brand, 20 tablets were weighed and finely powdered. An accurately weighed aliquot amount (equivalent to 10 mg of the drug on the basis of label claim) was transferred to a series of 100 ml volumetric flasks (three in each case) and dissolved in 20 ml of acetonitrile and filtered through Whatman filter paper no.1 and was diluted with 20% v/v acetonitrile to produce a concentration of 100 μg/ml. This was diluted at three different concentration levels within the range of linearity and the absorbance value was measured. From the absorbance value, the drug content per tablet (on an average weight basis) was calculated. The results are shown in Table 5.

3.8. Recovery studies

As an additional check on the accuracy of the developed assay methods, analytical recovery experiments were performed by adding known amounts of pure drug to pre-analyzed samples of commercial dosage forms. The percentage analytical recovery values are also listed in Table 5.

3.9. Forced degradation studies

To verify the method for its stability indicating potential, ezetimibe was forcefully degraded using 0.5 N sulphuric acid and heated at 80 °C for 20 min and carvedilol was degraded using 0.5 N hydrochloric acid and 0.5 N sodium hydroxide. The degraded solutions were scanned against the control.

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References

Bolton S, Pharmaceutical Statistics: Practical and Clinical Application, 3rd edition, Marcel Dekker, New York, 1997, pp 153, 216–269.

Catapano AL (2001) Ezetimibe: a selective inhibitor of cholesterol absorption. Eur Heart J Suppl 3(suppl-E): E6–E10.

Clader JW (2004) The discovery of ezetimibe: a view from outside the receptor. J Med Chem 47: 1–9.

do Carmo Borges NC, Mendes GD, de Oliveira Silva D, Marcondes Rezende V, Barrientos-Astigarrage RE, De Nucci G (2005) Quantification of carvedilol in human plasma by highperformance liquid chromatography coupled to electrospray tandem mass spectrometry: application to bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci 822: 253–262.

Dulin B, Abraham WT (2004) Pharmacology of carvedilol. Am J Cardiol 93: 3–6.

Hokama N, Hobara N, Kameya H, Ohshiro S, Sakanashi M (1999) Rapid and simple micro-determination of carvedilol in rat plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 732: 233–238.

International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonized Tripartite Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption at step 4 of the ICH process on November 1996 by the ICH Steering Committee, Published by IFPMA, Switzerland.

Reiff K (1987) High-performance liquid chromatograpic method for the determination of carvedilol and its desmethyl metabolite in body fluids. J Chromatogr B Biomed Sci Appl 413: 355–362.

Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Layoff T, Vishwanathan CT, Cork CE, McDowall RD, Pitman KA, Spector S (1992) Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies. Pharm Res 9: 588–592.

United States Pharmacopoeia, United States Pharmacopoeial Convention, 24th Edition, Rockville, MD, 2000, pp 2149–2157.

van Heek M, Davis H (2002) Pharmacology of ezetimibe. Eur Heart J Suppl 3(suppl-J): J5–J8.

van Heek M, France CF, Compton DS, McLeod RL, Yumibe NP, Alton KB, Sybertz EJ, Davis HR Jr (1997) *In vivo* metabolism-based discovery of a potent cholesterol absorption inhibitor, SCH58235, in the rat and rhesus monkey through the identification of the active metabolites of SCH48461. J Pharmacol Exp Ther 283: 157–163.

Xu LX, Hui N, Ma LY, Wang HY (2005) Study on fluorescence property of carvedilol and determination of carvedilol by fluorimetry. Mol Biomol Spectroscopy 61: 855–859.

Yang E, Wang S, Kratz J, Cyronak MJ (2004) Stereoselective analysis of carvedilol in human plasma using HPLC/MS/MS after chiral derivatization. J Pharm Biomed Anal 36: 609–615.