Original Article

Chemo metric assisted Spectrophotometric Method Development through QBD Approach for the estimation of Metoprolol succinate and Cilnidipine in combined solid dosage form

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Abstract

Regulatory agencies like USFDA has recommended implementation of Quality by design (QbD) a systematic process for pharmaceutical development along with its significance. Development of various pharmaceutical processes including analytical methods by applying Quality by design aids in ensuring the robustness of the method. QbD approached chemo metric assisted UV-VIS spectrophotometric analytical method was developed for the estimation of metoprolol succinate (MPS) and cilnidipine (CDE) from the combined dosage forms. Nature of spectra directed applicability of simultaneous equation and multicomponent methods for estimation of both drugs from the formulations; and 90 % alcohol was being the common solvent. For both this method 221 nm and 242 nm was the wavelength for measurement of absorbance of metoprolol and cilnidipine respectively. Effect of input variables on spectrum characteristics were studied for selection of critical parameters and developed method was validated as per ICH Q 2 R1 regulatory guidelines. Linearity of the drugs was ascertained over the conc range 1-40 µg/ml (microgram/ml) for MPS and 1-20 µg/ml for CDE. The percentage purity of assay found in method II was found 102.21 % for MPS and 101.28 % for CDE; and the accuracy study data of method I were varied from 0.4366 to 0.8989 for MPS and 0.5534 to 1.3027 for CDE. Precision study was shown acceptable data as % RSD in method I data varied from 0.7119 to 2.5465 for MPS and from 0.5902 to 0.5919 for CDE. The developed method is rigid, robust and efficient for the estimation of MPS and CDM from the composition of dosage form.

Keywords: Metoprolo succinate, Cilnidipine, QbD, ICH, Multicomponent, simultaneous equation method

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Introduction

Cilnidipine (CDE) chemically 1, 4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid 2-methyloxyethyl (2E)-3-phenyl-2-propenyl ester[1] is a dihydropyridine calcium channel blocker given orally in the management of hypertension[2].

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Reported analytical methods for estimation of cilnidipine were includes alone by UV spectrophotometric method[3], UV spectrometric methods with metoprolol [4],[5] alone UV-HPLC method [6], Stability indicating HPLC [7] QbD based HPLC [8] chromatographic methods [9-12] and have been found in the literature.

Metoprolol succinate (MPS) is a beta blocker; used in the treatment of hypertension, angina and to reduce myocardial infarction [2]. Chemically it is (RS)-1-(Isopropylamino)-3-[p-(2- Methoxyethyl) phenoxy]-2-propan-2-ol succinate [1].

Literature survey revealed that various analytical methods have been reported for estimation of MPS such as alone UV spectrophotometric method [2-16], with other drugs UV spectrophotometric method[17-22], bio analytical method [23], MPS alone by RP-HPLC [24], with other drug by RP-HPLC [25-30], stability indicating HPLC [31,32], designed and eco-

friendly TLC densitometry [33] and HPTLC [34] in combination with other drug. Cilnidipine and Metoprolol succinate are official in Indian Pharmacopoeia [35], whereas Metoprolol is official in BP[36]. Chemical structures of both drugs are shown in (Fig 1).

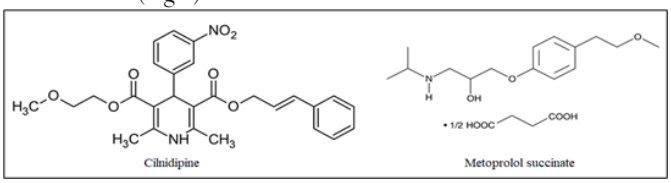

Fig 1: Chemical structure of Drug molecule

Quality by design concept is applied for the development of pharmaceutical processes to assure a predefined product quality. QBD concepts are mentioned in ICH guidelines Q 8(R2) (Pharmaceutical development), Q9 (Quality risk management), and Q10 (Pharmaceutical quality system) 37-39 shown in Fig 2. ICH guidelines Q8 (R2) [40] defines QBD as a "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". QBD approach in analytical method summarizes a complete understanding of how the analytical technique attributes and operating conditions affect the analytical performance. Factors to study in analytical quality by design (AQbD) approach may include the type of analytical technique chosen, reagents used and instrument parameters. QbD was applied to build rigid robust method through risk assessment at early stage and defining the design space at the later stage.

Fig 2: Analytical QbD approach

There are similar advantages of applying QbD principles to analytical methods as to manufacturing processes and product [41]. A QbD approach can be beneficial in the development of suitable, robust, low cost and eco-friendly (eco-friendly solvent, chemicals) method which is applicable at any stage of the lifecycle of the product. Also some regulatory guidelines have mentioned flexibility of changing analytical method without revalidation if the AQbD approach has been implemented during analytical method development. The first stage of AQbD approach is to fix an analytical target profile (ATP) for the method. ATP defines the goal of the analytical method development process and it is the sign of method performance [42, 43]. For analytical method validation ICH Q2 (R1) has given various method performance characteristics for an analytical method.

Thus a QbD based UV spectrophotometric was developed, QbD approach was implemented with the study of the effect of method input variables on spectral shape, intensity of absorbance, and absorbance maxima λmax and critical parameters were selected for the proposed method and method was validated as per ICH guidelines Q2 (R1).

MATERIALS AND METHODS

Instrumentation

Analysis was performed with a Shimadzu Double beam UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 2 nm and wavelength accuracy of ± 1 nm with 10 mm matched Quartz cells was used. Electronic balance Afcoset balance (The Bombay Burmah Trading corpo Ltd) with accuracy ±0.1 mg Model No. ER 200A was utilised for weighing and for degassing the solution Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used.

Reagents and Chemicals

Pharmaceutically pure sample of MPS was procured from Macleods Pharmaceuticals Ltd. Mumbai and CDE from Swapnroop drugs and pharmaceuticals, Aurangabad, Maharashtra, India procured as a gift samples and the commercial formulation Cilacar-M manufactured by J B Chemicals and Pharmaceuticals containing cilnidipine 10 and metoprolol 50 mg was procured from the local market.

AQbD approach application in method development

AQbD approach was applied to study the influence of input variable parameters on spectrophotometric analytical method performance shown in (Fig 3).

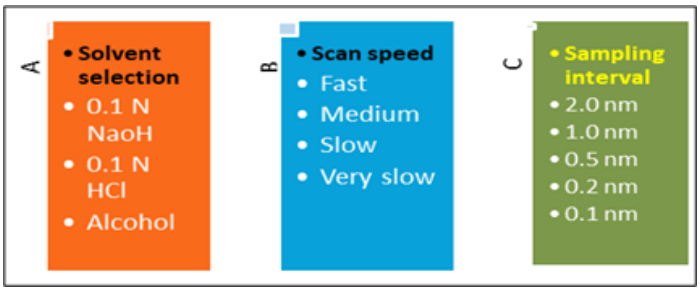

Fig 3: Diagram showing the relationship between input variable parameters and the spectrophotometric method performance characteristics

Solvent selection

MPS is freely soluble in water, ethanol, methanol and ethyl acetate; whereas CDE is very soluble in N, N-Dimethyl acetamide, freely soluble in acetone, soluble in methanol and practically insoluble in water. Although the solubility of the procured drugs were studied in alcohol 90%, 0.1 N HCl and 0.1 N NaOH separately; and found that CDE is insoluble in both these solvents however MPS is soluble in 0.1 N NaOH.

As both drugs have solubility in common solvent alcohol 90%, hence it was selected. Each solution with known conc of analyte was scanned in UV range of 400 nm to 200 nm. It was found that suitable solvent is alcohol 90% with respect to stable, robust and precise in producing result.

Preparation of stock solutions and standard solutions

10 mg each of drug CDE and MPS were separately and accurately weighed; and transferred into separate 25 ml volumetric flask. Dissolved into alcohol 90% solvent and volume was made to 25 ml with solvent. Subsequent standard solution of each drug with conc 16µg/ml was prepared by diluting aliquot 0.4 ml of stock solution to 10 ml with 50% alcohol into 10 ml capacity volumetric flask.

Selection of wavelength and conc range

From UV spectra it was found that CDE has measurable absorbance at 242 nm (Fig 4) and less interference was observed by MPS; similarly MPS has maximum absorbance at 221 nm (Fig 5) and measurable interference having constant absorptivity by CDE was accounted. Chemometric method i.e. simultaneous equation method was applied and which was reasonable remedy to overcome interference at each other's absorbance, and other method was multicomponent. From the nature of spectra to study linearity, working conc range 1 to 20µg/ml for CDE and 1 to 40µg/ml for MPS was selected. Also combined drug solution was prepared simulated to marketed formulation. Selected critical parameters based upon above discussion, observations are listed in Table No 1 and by using these; method was validated as per ICH guidelines and by analysing marketed preparations.

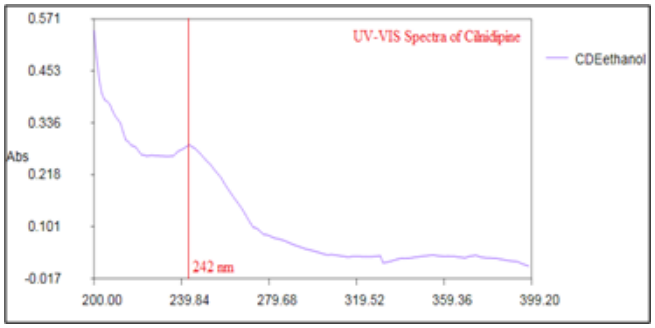

Fig 4: UV spectra of Cilnidipine in ethanol

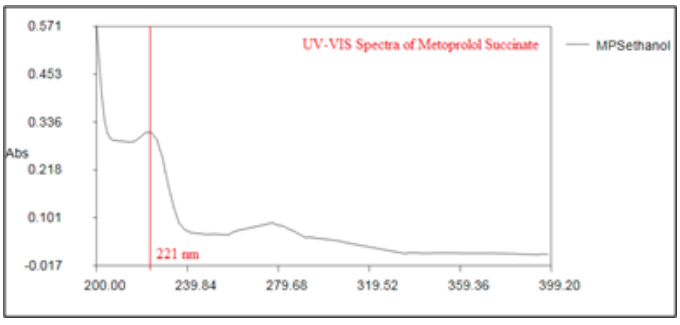

Fig 5: UV spectra of metoprolol succinate in ethanol

Experimental Method for estimation

From the overlain spectra simultaneous equation method was applicable for estimation of both the analytes from the combined dosage form.

Method-I: Simultaneous equation method for estimation of MPS and CDE

MPS was shown maximum absorbance i.e. λmax at 221 nm where measurable interference with constant absorptivity by CDE found and CDE has maximum absorbance i.e. λmax at 242 nm where negligible interference by MPS observed. At 242 nm the λmax of CDE, MPS was shown consistency in the absorptivity; hence two wavelengths 221 and 242 nm were considered as 1 and 2 respectively for the said method to estimate MPS and CDE. The equation A= abc was applied for x (MPS) and y (CDE) determination. Conc of working standard solutions of MPS and CDE containing 12 mcg/ml conc were separately prepared and used for the method.

$$Cx = \frac{A2\ .ay1 - \ A1.ay2}{ax2.ay1 - \ ax1.ay2}$$

$$Cy = \frac{A1\ .ax2 - \ A2.ax1}{ax2.ay1 - \ ax1.ay2}$$

Where,

C X = Conc of MPS in sample solution
A1 and A2 = absorbance of sample solution at 1 and 2 wavelength
ay1 and ay2 = absorptivity of CDE at 1 and 2 wavelength of standard solution
ax1 and ax2 = absorptivity of MPS at 1 and 2 wavelength of standard solution
Cy = Conc of CDE in sample solution
As = Absorbance of Sample solution at 2 wavelength

Method-II: Multicomponent method

After the interpretation of spectra multicomponent method was found suitable to estimate both drugs so wavelengths 221 nm and 242 nm selected. The characteristics of spectra was guided that the use of mixed standards were found appropriate than the use of pure standards. Mixed standards containing different proportions of both the drugs were rationally experimented keeping in view the conc of the drugs in the available formulations. Six mixed standards were selected for quantitative analysis shown in Table No 1.

Sample solutions were prepared in the MPS: CDE ratio of 1: 2, 4: 3, 3: 1, 8: 1 and sampling wavelength and conc of each drug in the six mixed standards were provided to the instrument using multicomponent mode of the instrument. Subsequently all the mixed standards were scanned in the range of 400 to 210 nm. The instrument's multicomponent mode collected and computed spectral data from the mixed standards employing matrix equations; and used for quantitative analysis of the samples. The conc of each of the drug in the sample solutions were computed and printed out by the instrument.

Table No 1: Selected critical parameters for UV-VIS analytical method of MPS and CDE

Parameter	Selected variables for simultaneous equation method					
	MPS			CDE		
Wavelength	221			242		
Solvent	50% ethanol			50% ethanol		
Scan speed	Fast			Fast		
Sampling interval	0.5 nm			0.5 nm		
Selected Mixed standard solutions for multicomponent method						
Standard No and conc of drug	1	2	3	4	5	6
Conc of MPS in µg/ml	0	8	16	24	32	40
Conc of CDE in µg/ml	20	16	12	8	4	0

Experimental method for estimation

Selected critical parameters should meet the performance characteristics of the analytical method so as to attain analytical target profile of the method. An ICH guideline Q2 R1 was applied to study methods performance with critical parameters in order to implement AQbD approach. The method was validated as per ICH guidelines.

System suitability

System suitability is studied to demonstrate the suitability of the developed procedure under consideration for the analytical method. Six replicates of working standard solutions with conc16 µg/ml and 12 µg /ml each of MPS and CDE respectively were prepared separately and absorbance was recorded, SD calculated and % RSD of the response was.

Linearity

The linearity of an analytical method is its ability to obtain response i.e. absorbance which is directly proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range of 1-40µg/ml for MPS and 1-20 µg/ml for CDE and scanned in 400 to 200 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at their respective wavelength; i.e. 221 for MPS and 242 nm for CDE in spectrum order. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Assay of formulation

Assay was carried out by proposed methods and assay was validated by statistical parameters.

Estimation of formulations by simultaneous equation method

Tablet powder equivalent to 10 mg MPS and 2.5 mg CDE was weighed and transferred into 25 ml volumetric flask. Dissolved into 90% alcohol, mixed well for 10 mins and volume was made to 25 ml with the solvent. Solution was filtered through what man filter paper and aliquots of solution were further diluted with the 50% alcohol to obtain tablet sample solution. Solution was scanned in the range of 400 to 200 nm to obtain absorbance of tablet solution at 221 nm and 242 nm in spectrum order. Obtained absorbance were utilised to estimate unknown conc of formulation; and results were statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Accuracy and Precision

The accuracy of an analytical method expresses the closeness of an agreement between test result and true result. Accuracy study was performed by recovery study i.e. standard addition method; diluted standard solutions of MPS and CDE were prepared and standard solutions added in 80,100 and 120% proportionate to the tablet solution. Three replicates at each of these three levels were prepared and measured and % of conc, SD and RSD were calculated.

The precision study was carried out by performing assay of tablet six times; also the reproducibility in result was studied by inter day and intraday precision.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of MPS and CDE by the proposed method were determined using calibration graph method and calculated as $3.3\sigma/s$ and $10\ \sigma/s$ for LOD and LOQ respectively; σ is the standard deviation of calibration curve and s is the slope of regression line.

Robustness and Ruggedness

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

RESULTS AND DISCUSSION

Method development comprises numerous steps, and of which solvent selection, selection of method for measurement are significant one. Uses of aqueous solvents, eco-friendly solvents like hydrotropic have got remarkable weightage due to low cost, readily available and environmentally sound. Drugs underlying analysis must have appreciable solubility in the selected solvent. Chemical structure of the drug and physico-chemical properties available in the literature guides about use of appropriate solvent in the method.

From UV spectra two wavelengths were selected as 221 nm (λmax of MPS) and 242 nm (λmax of CDE) shown in Fig 6 for calculation of both drugs in combined solution shown.

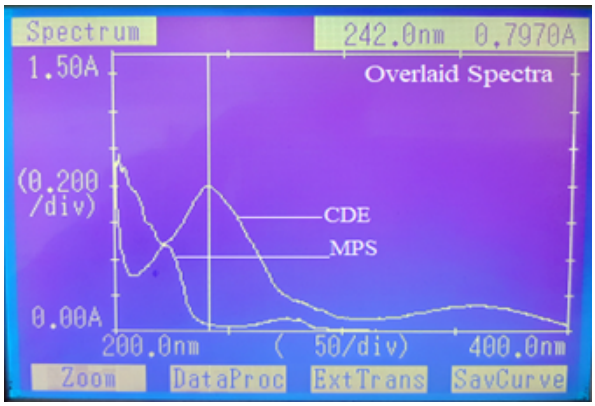

Fig 6: Overlaid spectra of MPS and CDE

System Suitability

The absorbances of six replicates of standard solutions of tabulated respective conc are reported in Table No 2. The SD and % RSD was found for MPS and CDE and meets the system suitability requirements indicates method was suitable for analysis.

Table No 1: Selected critical parameters for UV-VIS analytical method of MPS and CDE

Sr No	Conc in µg /ml	Absorbance of MPS*	Conc in µg /ml	Absorbance of CDE*
1	16 µg /ml	0.49618	10 µg /ml	0.81052
	SD RSD	0.08377 0.06886	SD RSD	0.050141 0.02514

*Mean of six determinations

Linearity

The calibration curve of both drugs was found to be linear in the conc range of 1-40 µg/ml for MPS and 1-20 µg/ml for CDE as shown in Fig 7. The regression equation of line and its parameters slope, r2 value and intercept are tabulated in Table No 3, which proved the linear relationship between conc and obtained response.

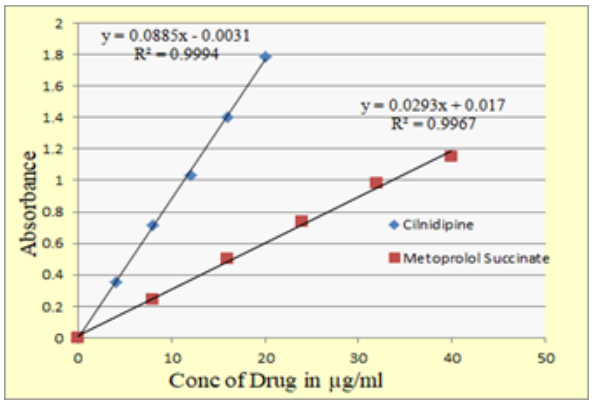

Fig 7: Calibration curve of MPS and CDE

Table No 3: Parameters of regression equation obtained in Microsoft excel

Parameters	MPS	CDE
Detection wavelength	221	242
Beer's law limit (µg/ml)	1–40 µg/ml	1–20 µg/ml
Correlation coefficient (r^2)	0.9967	0.9994
Regression equation (y = mx + c)	Y = 0.0293X + 0.017	Y = 0.0885X - 0.0031

Assay

The assay was carried out by the proposed method. The overlaid spectra obtained in multicomponent method are shown in Fig 8. The spectrum of formulation by method II was shown in Fig 9. The assay of formulation was carried out by proposed method and calculated % of nominal conc and RSD was found within acceptable limits are summarized in Table No 4. The results indicated app

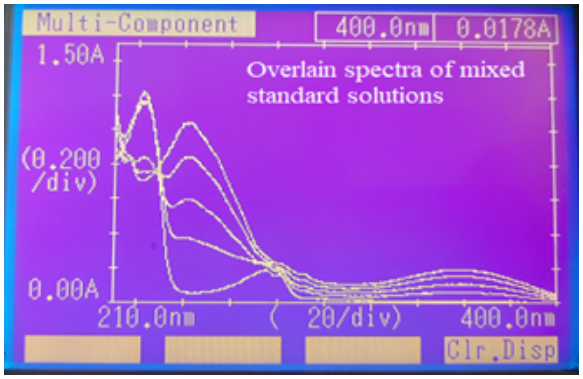

Fig 8: Overlaid spectra obtained in multicomponent method

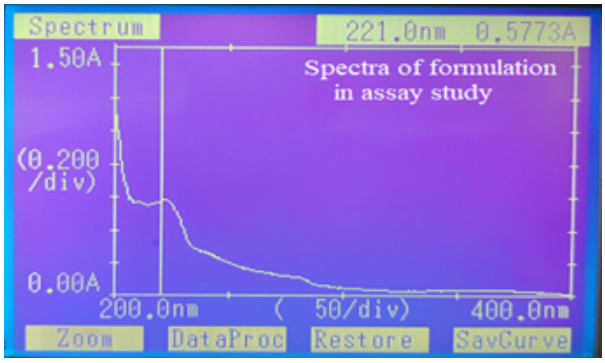

Fig 9: Spectra of formulation obtained in the assay

Table No 4: Results of assay of formulation by proposed method

Formulation	Drug	Label Claim (mg/Tablet; n=6)	Amount found/mg	Drug Content %	Std Deviation	% RSD
Method-I	MPS	40	40.346	100.865	1.3471	1.3354
	CDE	10	10.118	101.181	0.6098	0.6032
Method-II	MPS	40	41.102	102.215	0.5913	0.6331
	CDE	10	10.128	101.281	0.4270	0.4013

Accuracy and Precision

The results of accuracy are summarised in Table No 5 a and b, the obtained results were within acceptable limit; and methods accuracy was justified by calculating % drug content.

The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarised in Table No 5 a and b.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) and Robustness and Ruggedness

The LOD and LOQ of CDE and MPS were found in acceptable limits by the proposed method.

Robustness was studied and capacity of analytical procedure to measure analyte was remain unaffected by small but deliberate variations in method parameter. The analytical method was found rugged during development; similarity the result was produced by performing the analysis by different analyst.

Table No 5a: Results of accuracy and precision

Sr. No.	Parameter	Level of study	Drug Name	S.D.	% RSD
1 Method I	Precision	Intraday Precision	MPS	0.71192	0.70798
			CDE	0.59025	0.59421
		Inter day precision	MPS	2.54653	2.49253
			CDE	0.59194	0.58338
2	Accuracy study of ASM and CDE	80%	MPS	0.56161	0.56785
		100%		0.43661	0.46693
		120%		0.89891	0.93561
		80%	CDE	0.89001	0.93521
		100%		1.30271	1.53918
		120%		0.55349	0.59278

Table No 5b: Results of accuracy and precision

Sr. No.	Parameter	Level of study	Drug Name	S.D.	% RSD
1 Method II	Precision	Intraday Precision	MPS	0.83632	0.90531
			CDE	0.42701	0.40132
		Inter day precision	MPS	0.59132	0.63318
			CDE	0.36317	0.33841
2	Accuracy study of ASM and CDE	80%	MPS	1.15765	1.55372
		100%		1.41352	1.13099
		120%		1.42982	1.42964
		80%	CDE	0.84344	0.83741
		100%		0.48751	0.39116
		120%		1.02151	1.02064

CONCLUSION

Both the drugs were estimated from the combined formulation by simultaneous equation method. Results were found within acceptable limits, statistical data obtained were shown rigidity of the method. The validated method was employed alcohol as solvent thus become ecofriendly. The proposed method is precise, accurate, robust and reproducible hence can be routinely used for simultaneous estimation of cilnidipine and metoprolol succinate from combined dosage form.

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