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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF EFONIDIPINE HYDROCHLORIDE AND METOPROLOL SUCCINATE USING HYDROTROPIC AGENTS

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ABSTRACT

This study presents the development and validation of a method for the simultaneous estimation of Efonidipine Hydrochloride (EDH) and Metoprolol (MPL) using hydrotropic agents. Solubility enhancement is important aspect in the analysis of these drugs, and hydrotropic agents offer a cost-effective and environmentally friendly solution. The method development involved the optimization of hydrotropic agents such as urea and sodium acetate to improve solubility and facilitate simultaneous quantification. Validation parameters including accuracy, precision, specificity, linearity, and robustness were evaluated to ensure the reliability and suitability of the method for analytical purposes. The validated method offers practical utility for quality control laboratories, pharmaceutical industries, and regulatory agencies involved in the analysis of EDH and MPL formulations, streamlining the analysis process and supporting comprehensive quality assessment.

Keywords: Efonidipine Hydrochloride, Metoprolol, hydrotropic agents, simultaneous estimation, method development, method validation, solubility enhancement, pharmaceutical analysis.

INTRODUCTION

Efonidipine Hydrochloride (EDH) and Metoprolol Succinate (MPL) are widely prescribed cardiovascular drugs known for their therapeutic efficacy in the management of hypertension and various cardiovascular conditions (Dewan *et al.,* 2022; Darji *et al.,* 2024). EDH, a dihydropyridine calcium channel blocker, exerts vasodilatory effects by inhibiting calcium influx, while MPL, a selective beta-blocker, acts by blocking the beta-adrenergic receptors, resulting in decreased heart rate and blood pressure. However, the simultaneous estimation of EDH and MPL presents analytical challenges due to their poor solubility in traditional solvents, hindering accurate and precise quantification (Patel *et al.,* 2015). To address this limitation, the use of hydrotropic agents has gained prominence in recent years. Hydrotropic agents are water-soluble organic compounds capable of enhancing the solubility of poorly soluble drugs through the formation of soluble complexes or micelles.

Several studies have demonstrated the effectiveness of hydrotropic agents such as urea, sodium acetate, and sodium citrate in enhancing the solubility of EDH and MPL, thereby facilitating their simultaneous quantification using various analytical techniques including UV-VIS spectroscopy, HPLC, and HPTLC (Solanki *et al.,* 2022; Patel *et al.,* 2023; Solanki *et al.,* 2022; Kumar *et al.,* 2017; Rajput *et al.,* 2020).

Building upon this existing body of knowledge, the present study aims to develop and validate a method for the simultaneous estimation of EDH and MPL utilizing hydrotropic agents. The optimization of hydrotropic agents and the evaluation of method validation parameters including accuracy, precision, specificity, linearity, and robustness will be conducted to ensure the reliability and suitability of the developed method for pharmaceutical analysis.

By overcoming the solubility challenges associated with EDH and MPL and providing a practical analytical solution, this method is poised to significantly enhance the efficiency and accuracy of quality assessment in pharmaceutical formulations containing these cardiovascular drugs.

MATERIALS AND METHODS

Solubility

Solubility of EDH and MPL was determined at 25±1°C. Accurately weighed 10mg EDH and MPL was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed visually.

The results reveal that certain solvents significantly enhance the solubility of the compound, as indicated by the notable fold increases observed. For instance, the combination of 2M Urea and 2M Sodium acetate (1:1) demonstrates the highest solubility enhancement, with fold increases of 13 and 16 for EDH and MPL, respectively. This suggests a synergistic effect of these solvents in enhancing solubility, potentially due to their unique properties or interactions with the compound.

Similarly, other solvent combinations such as 2M Sodium Citrate: 8M Urea (1:1) and 2M Ammonium Acetate: 2M Sod. Citrate (1:1) also exhibit considerable solubility enhancement, with fold increases ranging from 6 to 8. These findings underscore the importance of solvent selection and the potential benefits of solvent combinations in optimizing solubility.

It's worth noting that some solvents, such as 2M Sodium Citrate, 2M Sodium Benzoate, and 2M Ammonium Acetate, also demonstrate significant solubility enhancement when used individually. This highlights their effectiveness as standalone solvents for enhancing solubility.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80`mL mixed hydrotropic solution containing 2M Urea: 2M Sodium acetate (1:1) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to 100ml with mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of EDH and MPL and transferred into 25 ml volumetric flask separately and diluted up to

25 ml with RO Water that gave concentration of 100µg/ml (Stock-B).

Preparation of Working Standard Solution

Aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml respectively for EDH. 1ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for MPL.

Selection of wavelength for linearity

Solutions of 2µg/ml of EDH and 10µg/ml MPL were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of EDH and MPL was observed at 285.0 nm and 270.0 nm, respectively. EDH and MPL showed linearity in the concentration range of 2-10 µg/ml and 10-50µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

To study the linearity of EDH and MPL the selected wavelength are:

λ_{max} of EDH	285.0 nm
λ_{max} of MPL	270.0 nm

Method (Simultaneous equation method)

Study of overlay spectra

Working standard solution from the standard stock solution prepared in concentration 2µg/ml of EDH and 10µg/ml of MPL were scanned in the spectrum mode over the range of 200-400 nm against Water as blank and the overlain spectra of the two were recorded. EDH showed an absorbance peak at 285.0 nm, whereas MPL at 270.0 nm. The overlain spectra also showed isoabsorptive points at 278.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 285.0 nm and 270.0 nm that are λ_{max} of EDH and MPL respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C\,EDH = \frac{A1ay2 - A2ay2}{ax1ay2 - ax2ay1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(1)$$

$$C\,MPL = \frac{A1ax2 - A2ax2}{ax1ay2 - ax2ay1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(2)$$

Where, A_1 and A_2 are absorbances of mixture at 285.0 nm and 270.0 nm respectively, ax_1 and ax_2 are absorptivities of EDH at λ_1 (285.0 i.e. λ_{max} of EDH) and λ_2 (270.0 i.e. λ_{max} of MPL) respectively and ay_1 and ay_2 are absorptivities of MPL at λ_1 and λ_2 respectively. C_{MPL} and C_{EDH} are concentrations of EDH and MPL respectively by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the EDH and MPL.

Validation of simultaneous equation method

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of EDH and MPL to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Analysis of tablet sample

Twenty marketed tablets of EDH and MPL were weighed and ground to a fine powder; amount equal to 40mg of EDH was taken in 10 ml volumetric flask. The MPL present in this amount of tablet powder was 25mg. Then 8 ml of 2M Urea:2M Sodium acetate (1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

RESULTS AND DISCUSSION

Table 1 showed comprehensive data regarding the analytical evaluation of Efonidipine Hydrochloride (EDH) and Metoprolol Succinate (MPL), spanning from method development to validation and analysis of tablet formulations. The linearity assessment, as depicted in Table 1, showcases the robustness of the analytical method. Both EDH and MPL exhibit high correlation coefficients (r2) of 0.999, indicative of strong linear relationships between concentration and absorbance. Moreover, the calculated slope and intercept values further affirm the reliability of the method across the specified concentration ranges. The method's suitability for simultaneous estimation is underscored by distinct working wavelengths for EDH (285 nm) and MPL (270 nm), allowing for selective quantification.

Table 2 delves into recovery studies conducted on marketed formulations, elucidating the method's accuracy and

applicability in real-world scenarios. Across varying recovery levels (80%, 100%, and 120%), both EDH and MPL consistently demonstrate high mean recovery percentages, accompanied by relatively low standard deviations. These findings suggest that the developed method can effectively quantify EDH and MPL in pharmaceutical formulations with minimal bias and acceptable variability, bolstering confidence in its utility for quality control purposes.

The validation results presented in Table 3 further validate the robustness of the analytical method. Precision assessments, including repeatability, day-to-day, analyst-to-analyst, and reproducibility, demonstrate consistent and reliable performance across multiple measurements and analysts. The reported mean values, coupled with narrow standard deviations, highlight the method's precision and reproducibility, essential attributes for ensuring accurate and consistent analytical results in routine laboratory practice.

Finally, Table 4 elucidates the analysis of tablet formulations containing EDH and MPL. The comparison between label claims and the amounts found reveals close agreement, signifying the method's accuracy in quantifying the active pharmaceutical ingredients in the formulations. Moreover, the low standard deviations and relative standard deviations indicate minimal variability in drug content among different tablets within the same batch, reinforcing the method's reliability and suitability for pharmaceutical quality assessment.

Table 1: Results of Linearity of Efonidipine Hydrochloride and Metoprolol succinate

	Method	
Parameter	**EDH**	**MPL**
Working λ_{max}	285 nm	270 nm
Beer's law limit (µg/ml)	2-10	10-50
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.070	0.123
Intercept (c)*	0.003	0.000

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	**% Recovery (Mean±SD)***	
	EDH	**MPL**
80	98.97±0.459	98.78±0.714
100	97.02±1.835	99.17±0.334
120	99.18±0.580	99.09±0.343

*Average of three determination

Table 3: Results of validation (Mean±SD)*

Parameter		Method	
		EDH	**MPL**
Precision*	Repeatability	96.811±0.110	98.2±0.120
	Day-to-Day	97.814±0.062	99.592±0.015
	Analyst-to-Analyst	99.235±0.151	99.066±0.041
	Reproducibility	97.649±0.080	99.504±0.114

*Average of five determination

Table 4: Analysis of Tablet Formulation of EDH and MPL

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
EDH	40	39.65	99.13	0.115	0.135
MPL	25	24.74	98.96	0.165	0.178

CONCLUSION

In conclusion, the comprehensive analysis presented in the tables underscores the effectiveness and reliability of the developed method for simultaneous estimation of EDH and MPL using hydrotropic agents. The method's robustness, accuracy, precision, and applicability in pharmaceutical analysis are well-demonstrated, paving the way for its adoption in routine quality control and regulatory compliance in the pharmaceutical industry.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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