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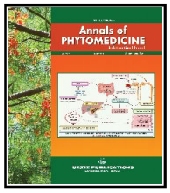

Review Article : Open Access

Analytical and spectrophotometric study of calcium channel blocker with green solvents using UV-spectrophotometer: A comprehensive review

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Abstract

Calcium channel blockers such as felodipine, nifedipine, verapamil hydrochloride, azelnidipine, cilnidipine, and amlodipine besylate are analyzed by UV-spectroscopy with the help of green solvents. Green solvents are the solvents that are less toxic, and less hazardous than the non-ecofriendly solvents (di-isopropyl ether,1,4-dioxane, pentane, hexane, *etc*.). However, the calcium channel blocker drugs are readily soluble in some green solvents such as ethyl acetate, acetone, ethanol, *etc*. Felodipine dissolves in acetone, ethanol, and DMSO (dimethyl sulfoxide). Nifedipine is practically insoluble in water but soluble in ethanol and water mixture. Verapamil dissolves in acetone. Azelnidipine dissolves in acetone and ethyl acetate (freely soluble) and water (sparingly soluble). By using a UV-spectrophotometer, the drugs were analyzed and the λ_{max} of the drugs were obtained. Then, the method was validated according to the ICH guidelines. The purpose of this article is to review the analytical methods developed for the analysis of the calcium channel blockers in green solvents and validated as per the ICH guidelines.

1. Introduction

Calcium channel blockers, a medications that antagonize or disrupt the movement of calcium ions across the channels. These are the medications used for the treatment of cardiovascular diseases like hypertension, blood pressure, arrhythmia, anginal pectoris, *etc*. These drugs are majorly classified into dihydropyridines (felodipine, nifedipine, azelnidipine, *etc*.), phenyl alkylamines (verapamil), and benzothiazepine (diltiazem) based on their chemical structure (Akella Anuradha *et al.,* 2023). The ca^{2+} entry of the l-type calcium channel present in the cardiac and the smooth muscles leads to the activation of the myosin which leads to the vascular contraction of the smooth muscles, but calcium channel blocker drugs bind to the channel and block the entry of ca^{2+} ions, resulting in the vasodilation and relaxation of smooth muscles. Felodipine is used for managing and treating hypertension by reducing blood pressure and lowering the risk of motility and cardiovascular morbidity. The mechanism of action for felodipine explains that the result shows that both systolic and diastolic blood pressure drops and heart rate increases (reflex tachycardia) (Dhritimoni Devi *et al*., 2023). Felodipine is consumed orally (2.5 mg, 5 mg, 10 mg) and easily absorbed. The bioavailability of felodipine is about 20% due to the extensive first-pass metabolism. Metabolism takes place in the liver and weakly inhibits CYPEA4 (cytochrome P450 3A4) and CYP2D6 enzymes (Indian Pharmacopoeia, 2011).

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Azelnidipine exhibits a prolonged hypotensive effect and shows strong anti-arteriosclerosis action in blood vessels due to high vascular tissue affinity. Azelnidipine also shows the properties of cardioprotective, neuroprotective, and arteriosclerosis. Its half-life ranges between 16 and 28 h (Narendra Nyola and Govinda Samy Jeyabalan, 2014). Nifedipine blocks the l-type calcium channel and increases the oxygen supply to the heart which helps in the treatment of angina pectoris. It is mainly used to treat myocardial infections. It belongs to the class-II classification of bio-pharmaceutical system which has low solubility and high permeability. Nifedipine is metabolized into an inactive state of 2,6-dimethyl-4-(2-nitrophenyl)-5-methoxycarbony-l-pyridine-3-carboxylic acid. It has a half-life of 2 h.

Verapamil HCl is used for the treatment of vasospastic angina, unstable and chronic stable angina, and arrhythmia (Rakesh Kumar *et al*., 2020). It is not used for patients who have left-ventricle dysfunction, because verapamil HCl may increase the risk of exacerbating the condition verapamil blocks the l-type calcium channel at a specific area of alpha-1 sub-unit. It controls the conduction of the heart due to prolonging the refractive period and slows down the conduction at AV nodes. Cilindipine and bisoprolol fumarate are a combination of ca^{2+} channel blockers and β-blockers (The Merck Index, 2001). These combined drugs dilate the blood vessels which helps in increasing the efficacy of pumping of the heart which reduces the risk of stroke and cardiac arrest (Indian Pharmacopoeia, 2018). Amlodipine besylate has antioxidant properties and the ability to enhance the production of nitric oxide which is mainly for the vasodilation action and reduces blood pressure. It is also used to treat coronary artery diseases, angina pectoris, and prinzmetal angina. Amlodipine metabolized into pyridine derivatives (Varuni, *et al*., 2023) as per Table 1. Compared to other dihydropyridine classes of calcium channel blockers it has a prolonged half-life of 30-50 h (Walters and Redman, 1984).

2. Benefits of green analytical chemistry

UV-spectroscopy is a technique mainly based on the measurement of the interaction of electromagnetic radiation with quantized matter at specific energy levels. Light is an electromagnetic wave that has a certain energy and wavelength, the wavelength between 200-800 nm is used in UV-Vis spectroscopy. When the sample is absorbed the light transition (change of an electron from one to another energy level) will take place. Four types of transitions are possible, π-π^*, n-π^*, σ-σ^* and n-σ^* (Gedil *et al.,* 2004). The principle of Beer-Lambert's law was majorly used in spectroscopy for the calculation of absorbance maxima (λ_{max}), which states that absorbance directly relates to the concentration of absorbing material and path length of the light (Amisha Sharma *et al.,* 2021). UV-spectroscopy is widely used in many areas of science such as bacterial culture, drug identification, nuclei acid purity checks, and quantitative and qualitative control in industries and chemical research centers. Green solvents are those solvents that are less toxic and eco-friendly compared to hazardous solvents which help in the reduction of the environmental impacts created by the hazardous solvents (methanol, dichloromethane, hexane, pentane, *etc.*), which are discarded after every experiment. Green solvents should follow the twelve principles of green chemistry as per Figure 1.

The twelve principles are as follows: prevent waste, atom economy, less hazardous chemical synthesis, designing safer chemicals, safer solvent and auxiliaries, design for energy efficiency, use of renewable feedstocks, reduce derivatives, catalysis, design for, PHJ degradation, real-time pollution prevention and safer chemistry for accident prevention (Hymavathi, 2021). Green solvents are majorly classified into water, alcohol, liquid polymers, ionic liquids, fluorinated solvents, and supercritical fluids (carbon dioxide). When the pressure is applied above the critical temperature and pressure to gases like carbon dioxide, it gets converted into liquid this phenomenon is known as supercritical fluid (Hassan *et al.,* 2022). Ethyl lactate is an ester of lactic acid derived from the processing of corn. Because of their biodegradable, non-corrosive, non-carcinogenic, and recyclable properties, they are utilized majorly in coating industries. (Ahmed Inas, 2015).

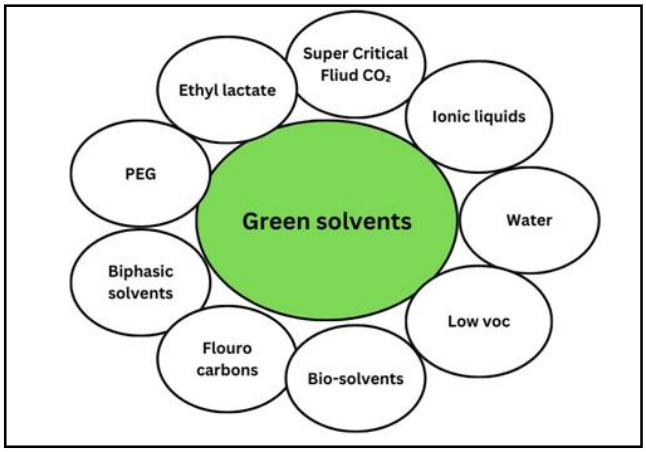

Figure 1: Classification of green solvents.

Table 1: Literature review of the following drugs

S.No.	Title of the article	Journal name	Reference	Point of review
1.	Development and validation of UV-spectrophotometric method for estimation of felodipine using green solvent in tablet formulation.	ACTA Scientific Pharmaceutical Sciences	Sandeep Sonawane *et al.* (2018)	UV-spectrophotometric estimation using green solvent
2.	Analytical method development and validation of azelnidipine by UV-visible spectroscopy.	World Journal of Pharmaceutical Research	Imdad Husen Mukeri *et al.* (2021)	UV-spectrophotometric estimation using green solvent
3.	Development and spectrophotometric method validation of nifedipine in solid dosage form.	Asian Journal of Research in Chemistry and Pharmaceutical Sciences	Varsha Jakune *et al.* (2020)	UV-spectrophotometric estimation using green solvent
4.	Spectrophotometric determination of verapamil hydrochloride using bromocresol green.	DER-Pharmachemica	Marta Sulyma *et al.* (2018)	UV-spectrophotometric estimation using green solvent
5.	Development and validation of Q-absrobance ratio UV-spectro-photometric method for simultaneous determination of bisoprolol fumarate and cilindipine in marketed formu-lation besicor C5.	Asian Journal of Pharmaceutics	Mishra P. (2022)	UV-spectrophotometric estimation using green solvent
6.	New eco-friendly validated spectro-photometric method for the estimation of amlodipine besylate in bulk drug using ninhydrin.	Asian Journal of Biomedical and Pharmaceutical Science	Patil *et al.* (2013)	UV-spectrophotometric estimation using green solvent

3. Experimental methodology

3.1 Drug-Felodipine

Patel *et al.* (2020) have developed and validated the method using a series of solutions of drugs exhibiting a concentration of 5-50 µg/ml using ethanol as a solvent. The absorbance value obtained was 363.5 nm using ethanol as a blank and spiking a known amount of drug into the solutions to determine accuracy. A known amount of API (80%,100%, and 120%) was dissolved in solution to ensure accuracy. After reanalyzing by the recommended method and the percentages were obtained as %RSD of 0.261, 0.80, 0.02 which are less than the standard deviation of 2. Precision was examined by inter-day and intra-day and the absorbance of six distinct solutions of concentration 50 µg/ml was recorded and the value obtained was less than the standard deviation of 2%. Ruggedness of the sample was confirmed in different laboratories, by different analysts using specified parameters. Robustness was performed at the wavelength 263.5 nm and 264 nm. The standard deviation and RSD (relative standard deviation) obtained were 0.003 and 0.75%, respectively.

3.2 Drug-Azelnidipine

Basavaiah *et al.* (2005) developed and validated the method by measuring the absorbance of azelnidipine at a concentration between 2-14 µg/ml using methanol as blank. The absorbance obtained was 257 nm. Accuracy was established for the solutions 80,100,120% of the standard were added and added along with the known concentrations of azelnidipine dissolved with methanol in a volumetric flask. The absorbance was measured at 257 nm and % the RSD value obtained was in the range between 1.09%-0.83% which is less than 2%. For repeatability, the middle concentration of 15 µg/ml, and the repeatability process was carried out six times, and the %RSD was found to be 1.13% along with the %RSD $\leq$2. The precision was examined by inter-day and intra-day methods at different concentrations of 5,15 and 25 µg/ml. The %RSD value obtained is in the range of 1.03%-1.70% for intra-day and 1.26%-1.67% for inter-day was below the normal range of %RSD $\leq$2. Limit of detection (LOD) is used to determine the lowest amount of analyte in a sample. LOD is calculated by using the formula, LOD = (3.3 × SD)/Slope, and the value was found to be 0.77. One of the parameters of quantitative assays for low concentrations of a substance in a sample is the limit of quantification (LOQ). LOQ is calculated by using the formula, LOQ = 10 × (σ/ S), and the value was found to be 2.36. Robustness was performed at wavelengths 255, 256, and 357 nm at a concentration of 15 µg/ml and the %RSD value is 1.37% - 1.92% which is less than 2%.

3.3 Drug-Nifedipine

Khopade *et al.* (2000) developed and validated the method using a series of solutions of drugs exhibiting a concentration ranging between 20-100 µg/ml using methanol as solvent. The absorbance was measured at the wavelength of 249 nm. Precision is performed by inter-day and intra-day methods at the concentration of 40 µg/ml. %RSD for inter-day was obtained as 0.518% and 0.223% for intra-day which is less than the normal limit of 2%, and hence validated. LOD is used to determine the lowest amount of analyte. LOD is calculated by, LOD = 3.3 × SD/Slope, and the obtained value is 0.041 µg/ml. LOQ is used to identify the impurities of the products. It is calculated by using the formula, LOQ = 10 × SD/Slope, and the value obtained is 0.12 µg/ml. Robustness determines the small variation of an analytical. The %RSD value was found to be 0.768% and 1.142% and it was found within the limit. The ruggedness of the sample was confirmed in different laboratories, by different analysts using specified parameters and the %RSD obtained was 0.508% and 0.592%, respectively. Since the result was within the limit and validated.

3.4 Drug-Verapamil HCL

Mohammed *et al.* (2017) developed and validated the method by preparing the solution of drugs with concentrations ranging between 1.9648-4.4208 mg/ml using acetone as a solvent and bromocresol green as an indicator which produces the color. Absorbance and color were produced at the wavelength 409 nm. The precision was calculated using nine replicates of the sample, the result obtained from the assay, $\Delta_x$exceeded the maximum uncertainty RSD of 80 mg and was found to be 8.74×10^{-2} and for 40 mg 3.33×10^{-2}. In ruggedness, the sample solution was stable for at least 45 min and the parameters did not change even after adding different reagents (± 10%).

3.5 Drug-Cilnidipine and bisoprolol fumarate

Patel *et al.* (2012) developed and validated the method of a series of five solutions of concentration 2-10 µg/ml of cilnidipine and bisoprolol fumarate using ethanol as solvent and were analyzed in triplicate. The absorbance was obtained at 224.5 nm and 232 nm. Intra-day and inter-day method was done for both accuracy and precision and were examined using concentrations of 4 µg/ml and 8 µg/ml for both bisoprolol fumarate (bf) and cilnidipine (cld), respectively. The accurate percent recoveries were in the range between 99.3-99.75% for bisoprolol fumarate and 99.48-100.06% for cilnidipine. Standard solutions were analyzed to determine the concentrations of LOD and LOQ. LOD for bisoprolol fumarate was found to be 0.0943 µg/ml and 0.0751 µg/ml and for cilnidipine, it was found to be 0.0355 µg/ml and 0.1141 µg/ml at 224.5 and 232 nm, respectively. LOQ for bisoprolol fumarate was found to be 0.2860 µg/ml and 0.8983 µg/ml and for cilnidipine 0.1610 µg/ml and 0.3457 µg/ml at 224.5 nm and 232 nm, respectively.

3.6 Drug-Amlodipine besylate

Rahman *et al.* (2005) synthesized this method with the help of standard stock solution with different concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 were taken in a volumetric flask and required amount of ninhydrin was added followed saturated sodium bicarbonate solution. Volume was made up to the mark with the help of 0.1M HCl to get the concentration range of 50-300 µg/ml and was boiled in the water bath for 15 min and cooled. The maximum absorbance was measured at 566 nm. Precision was carried out with the help of inter-day and intra-day methods for 7 days using concentrations (100,120 and 150 µg/ml).

Only very few methods are available for the estimation of calcium channel blockers using green solvents. Implementation of these green techniques is very essential and crucial for the safety of analysts and the environment.

Patitapabana *et al.* (2015). The intra-day values obtained for the used concentrations are as follows: %RSD is 0.4992 for 100 µg/ml, 0.5259 for 120 µg/ml, and 0.5711 for 150 µg/ml, respectively. For inter-day, %RSD is 0.5391 for 100 µg/ml, 0.7262 for 120 µg/ml and 0.7100 for 150 µg/ml. Accuracy was determined by recovery studies

which were carried out by adding different amounts of samples 80%,100%, and 120% of the sample and the results obtained are as follows: %RSD is 1.43, 1.32, and 1.75, respectively. LOD and LOQ are determined by using the standard deviation of the response and the slope approaches as given in the ICH guidelines. The LOD and LOQ obtained are, 0.09 µg/ml and 0.2729 µg/ml, respectively.

Prabhakar and Giridhar (2003) developed simple, accurate, and reproducible spectrophotometric methods have been developed for the simultaneous estimation of telmisartan and amlodipine besylate in combined tablet dosage forms. The method involves determination using the simultaneous equation method, the sampling wavelengths selected are telmisartan = 297 nm and amlodipine besylate is 238 nm. Over the concentration ranges of 8 - 48 µg/ml for telmisartan and 1 - 6 µg/ml for amlodipine besylate, respectively. The method was validated for linearity, accuracy, precision, robustness, and application for assay as per ICH guidelines. The proposed method is simple, economical, accurate, and precise, and could be successfully employed in routine quality control for the simultaneous analysis of telmisartan and amlodipine besylate.

The above development techniques and methods used for the estimation of the UV-spectrophotometric method using green solvents in both chemical and pharmaceutical sectors for the calcium channel blockers such as felodipine, azelnidipine, nifedipine, verapamil HCl, bisoprolol fumarate and cilnidipine and amlodipine besylate.

4. Discussion

As per U.S Tri EPA exposure to non-green solvents leads to an increase in chest tightness, nausea, and certain asymptomatic conditions. Further prolonged exposure causes severe effects such as hemorrhage, ataxia *etc*. Due to the more wastage in to environment, it imparts toxicity in water bodies and aquatic animals and the microbiological environment also will be affected.

Challenges and limitations including finding the proper solvent for analysis without interferences and solubility is a major task. As green solvents are economical and widely available cost factor does not affect the selection.

In the future, a detailed study of the different calcium channel blockers with green solvents such as ethanol gives a wide scope for routine analysis and quality control of these drugs in the industry.

5. Conclusion

This review provides an overview of the importance of green solvents and the methods developed for the estimation of different calcium channel blockers. The employment of these methods brings a safe environment for the analyst in the estimation of calcium channel blockers compared to other analytical approaches that are already designed. These methods improve environmental and analyst safety, reduce waste, and save energy throughout the process.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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