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ORIGINAL ARTICLE

Validated stability-indicating high performance thin layer chromatographic method for determination of Ivabradine hydrochloride in bulk and marketed formulation: An application to kinetic study

Mitesh H. Motisariya, Kalpana Govindbhai Patel *, Purvi A. Shah

Anand Pharmacy College, Quality Assurance and Pharmaceutical Chemistry Department, Near Townhall, Anand, Gujarat 388 001, India

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Abstract A sensitive, selective, precise and accurate stability-indicating high-performance thin layer chromatographic method for analysis of Ivabradine hydrochloride (IH) an anti anginal agent, both as a bulk drug and in formulations was developed and validated according to ICH guideline. Densitometric analysis of IH was carried out in the absorbance mode at 287 nm using ethyl acetate: 0.389 M ammonium acetate in methanol (1:5, v/v) as solvent system. This system was found to give compact spots for IH at an R_f value of 0.36 ± 0.01. Moreover, IH was subjected to acid and alkali hydrolysis, oxidation, accelerated humidity/temperature, wet heat treatment, and photo degradation. The drug undergoes degradation under mainly acidic and basic conditions. Also the degraded products were well resolved from the pure drug with significantly different R_f values. Linearity was found to be in the range of 1200–2800 ng/band. The LOD and LOQ for IH were 255.86 ng/band and 775.33 ng/band, respectively. "Bartlett's test" and "Lack of fit" applied on peak area for linearity, additionally proved validity of the developed method. Good accuracy and precision were obtained as revealed from %RSD value less than 2. Similarly, no interference was observed from common excipients in tablet formulation as well as degradation product, indicating specificity of the method. As the method could effectively separate the drug from its degradation product, it

* Corresponding author. Tel.: +91 2692250020.
E-mail addresses: kalpana_jpatel@yahoo.com, kalpanapatel.pharma@gmail.com (K.G. Patel).

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can be employed as a stability-indicating one. Moreover, proposed method was also utilized to investigate the kinetics of acidic degradation process at different temperatures and first order rate constant, half-life, shelf-life and activation energy were calculated.

1. Introduction

Ivabradine hydrochloride (IH), chemically 3-[3-({[(7S)-3, 4dimethoxybicyclo[4.2.0]octa 1,3,5-trien-7yl]methyl}(methyl)amino) propyl]-7,8dimethoxy-2,3,4,5-tetrahydro-1*H*-3 benzazepin-2-one (Fig. 1), is an anti-ischaemic agent with specific negative chronotropic action.[1] IH is a specific heart rate lowering agent, acting by reducing the rate of pacemaker activity in the sinoatrial node. Within the sinoatrial node, IH is a selective inhibitor of I_f, an important current involved in generating the early phase of spontaneous diastolic depolarization in pacemaker cells, thereby reducing the frequency of action potential initiation and lowering the heart rate.[2] IH thereby prolongs the duration of diastole, so as to improve the balance between myocardial oxygen supply and demand as well as coronary perfusion.[3] In context to this, IH decreases oxygen consumption thereby preventing symptoms and reducing morbidity and mortality in patients with coronary artery disease and angina.[4]

Literature reports, analysis of IH in human plasma and urine, rat and dog plasma by high performance liquid chromatography with fluorescence detection and liquid chromatography–mass spectrometry method.[5,6] Moreover, determination of IH in marketed formulation by simple HPLC and UV spectrophotometric methods, dissolution profile study of immediate release tablet formulation of IH by HPLC are also available in literature.[7–10] Also, stability indicating HPLC method has been reported for the determination of IH in bulk and dosage form.[10] Literature reviewed reveals no information related to the stability-indicating methodology by high performance thin layer chromatography (HPTLC) for the determination of IH in pharmaceutical dosage forms. Accordingly, the purpose of the present study is to put ICH recommendations into practice by subjecting IH to a variety of suggested stress test conditions along with kinetic study to establish inherent stability of the drug and to develop the validated stability indicating HPTLC assay.

According to the International Conference on Harmonization (ICH) guidelines, requirements for establishing SIMs have become more clearly mandatory.[11] Moreover, kinetic studies and accelerated stability experiments play a significant role for solving problems encountered in quality control and to predict the expiry dates of pharmaceutical products. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, light, oxygen, pH and moisture. Stress testing can help in identifying degradation products and provide important information about the intrinsic stability of drug substances.[12]

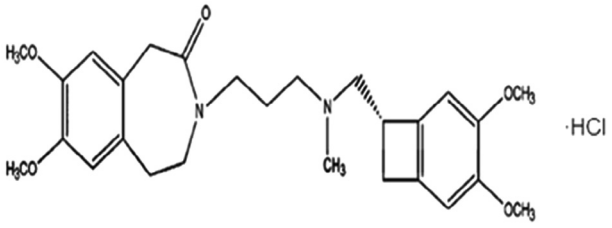

Figure 1 Chemical structure of Ivabradine hydrochloride.

Although LC procedures are accurate and effective means for analysis, they are time consuming; in addition, one of the major drawbacks is the use of large amounts of solvents and expensive instrumentation. As a consequence, rapid, efficient, and inexpensive analytical procedures that meet the continuous need for high-throughput assays of the drugs in quality control laboratories are highly demandable. Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today, TLC and HPTLC are rapidly becoming routine analytical techniques due to their several advantages over other methods.[13] The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering the analysis time, sample clean up and cost per analysis.[14] Also, HPTLC plates are pre-coated with smaller size particles, have narrow particle size distribution, and possess smooth surfaces and thinner layers. Other characteristics of HPTLC compared with TLC include smaller sample volume, economy, faster separation, more reproducible results and lower detection limits. Furthermore, it provides processing of samples and standards at the same time and so could be used for the quantitative determination of such chemical compounds with high degree of accuracy and precision. Thus, separation and quantification can provide results that are either superior or comparable with other analytical methods such as HPLC.[15]

An ideal stability indicating method hence was developed by HPTLC that is capable of quantifying IH and can also resolve IH from its degradation products. Furthermore, the proposed method was used to study the degradation kinetics profile of IH under acidic condition at different temperature and degradation kinetic parameters like activation energy, degradation rate constant, t_{90} (where 90% of original concentration of drug is left) and t_{50} (half-life) were computed from Arrhenius plot. The proposed stability indicating method is simple and allows rapid analysis for stability studies and quality control analysis of drug in bulk and dosage form.

2. Experimental

2.1. Materials

Pharmaceutical grade of Ivabradine hydrochloride (IH) was kindly supplied as a gift sample by Biocon Ltd., Andhra Pradesh, India, used without further purification. All solvents and chemicals used were of analytical grade, purchased from Merck Specialities Pvt. Ltd., India. Marketed tablet dosage forms used in this study were IVABRAD5