Original Article

Synthesis, characterization, and determination of metabolite of verapamil hydrochloride by reversed-phase high performance liquid chromatography

Abstract

Aims: A suitable reversed-phase high performance liquid chromatography (RP-HPLC) method for detection and determination of laboratory synthesized metabolite norverapamil (NVER) present in the pharmaceutical formulations is the prime purpose of this study. The present study deals with synthesis, characterization, and development of simple, selective, rapid, and sensitive RP-HPLC method for simultaneous determination of verapamil (VER) and its synthetic metabolite NVER. **Materials and Methods:** A HIQ sil ODS C-18 column having 250 mm × 4.6 mm i.d. in isocratic mode with a mobile phase consisting methanol: Water (70:30 v/v, pH adjusted to 7.4 with dilute orthophosphoric acid (OPA) and triethylamine used as an organic modifier to avoid tailing effect). The flow rate was 1.0 ml/min and effluents were monitored at 222 nm. **Results:** The retention time of synthesized metabolite NVER and its parent drug VER were found to be 3.44 and 5.67 min, respectively. Valsartan (VAL) was used as the internal standard. The limit of detection were found to be 0.30 μg/ml for VER and 1.21μg/ml for NVER from physical mixture, and limit of quantitation 1.06 μg/ml for VER and 4.14 μg/ml for NVER. **Conclusions:** The method can be used for quantitation of synthesized metabolite NVER, in presence of the parent drug VER which could be useful in detection and determination of some impurities, such as NVER, described in European Pharmacopeia and others which can be toxic and often present in the pharma ceutical formulations.

Key words:
European pharmacopeia, norverapamil, reversed-phase high performance liquid chromatography, verapamil

Introduction

Verapamil (VER) (5-[(3,4-dimethoxyphenethyl) methyl-amino]-2-(3,4-dimethoxyphenyl)-2-isopropylvalero-nitrile hydrochloride [Figure 1] is a calcium channel-blocking agent which has found widespread use in the management of supraventricular tachyarrhythmias, angina pectoris, hypertrophic cardiomyopathy, and hypertension.

VER is widely and rapidly distributed throughout the body with a distribution half-life of 15-30 min. The majority of VER metabolized by *O*-demethylation and by *N*-dealkylation (25% and40%), respectively. Although the products of *O*-demethylation possess pharmacological activities similar to VER, they are rapidly metabolized to conjugates.[1]

Synthesis and characterization of metabolites of new drugs utilizing optimum time and material resources is one of the areas of current pharmaco economic interest. There is need to develop accurate, precise, selective, and sensitive analytical methods to quantify the metabolite norverapamil (NVER) in presence of VER. Adequate separation, selectivity and sensitivity of detection and accurate quantitation are always the prime concerns of pharmaceutical analyst in such work.

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Regulatory guidelines of the International Conference on Harmonization (ICH) have led to an increasing need for identification and quantification of trace impurities in drugs. The ICH defines impurities as any component of a pharmaceutical product which is not the chemical entity of active substance or excipients.[2]

Thus far, a large number of analytical methods such as liquid chromatography–mass spectrometry,[3-5] capillary zone electrophoresis,[6] adsorptive stripping voltammetry,[7] micellar liquid chromatography and fluorescence detection,[8] high performance liquid chromatography[9-16] methods have been described to analyze VER in body fluids. Few spectrophotometric methods have also been reported.[17-23] None of these reported methods were used for the determination of VER and its synthesized metabolite NVER by RP-HPLC.

The main purpose of the present study was to develop simple, selective, and sensitive high performance liquid chromatography (HPLC) method for synthesized metabolite NVER, in presence of the parent drug, which could be useful in detection and determination of some impurities, such as NVER described in European Pharmacopeia[24] and others, which can be toxic, often present in the pharmaceutical formulations.

Materials and Methods

Drug and chemicals

VER and valsartan (VAL) were supplied by Cipla Pharma Ltd., D-7, MIDC, Kurkumbh, India. HPLC grade methanol, orthophosphoric triethylamine were purchased from Loba Chemie, Jehangir Villa, 107, Wode House Road, Colaba, Mumbai India. Water used for analysis was double distilled using simple glass distillation assembly and filtered through 0.2 µm syringe filter. NVER was synthesized in laboratory.

Equipments

SHIMADZU-QP 3010 mass spectroscopy interfaced with gas chromatography via electron impaction source was used for mass analysis and detection. A flow rate of 1 ml/min was used for sample analysis using helium as mobile phase. The electron impaction source temperature was maintained at 280 nm. The detector used was gas chromatographic real analyzer. The column with 300 m length and 0.2 mm internal diameter was used.

The Bruker NMR spectrometer used for analysis (model AVNCE-300 MHz). The NMR spectra of VER and NVER were recorded using Dimethyl Sulfoxide (DMSO) as solvent.

Chromatographic conditions

HPLC was performed using a JASCO HPLC comprising of a pump PU-2080, Rheodine manual injector with a fixed 20 µl external loop and UV-2070 detector. The chromatographic separations were performed on a HIQ sil ODSC-18 column (250 mm × 4.6 mm i.d., particle size 5 µm). Mobile phase comprising of methanol: Water (70:30) with triethylamine and 0.01M OPA as a mobile phase. The aqueous phase was eluted at a flow rate of 1ml/min and effluent was monitored at 222 nm. Quantitation was achieved by measuring the peak area ratios of the drug to the internal standard. The mobile phase was prepared daily, filtered, and degassed by ultrasonication before use.

Experimental

Synthesis of metabolite

In a flask containing 0.5 g of VER (0.001mol), 1.12 g of hydroxylamine hydrochloride and 0.45 ml of triethylamine were added. To this a mixture 3 ml of ethanol and 1.53 ml of water was added and the reaction mixture was exposed to microwave irradiation for 12 min at 100°C [Figure 2]. The mixture after cooling was poured in ice-cold 1N HCl. This solution was washed with two 5 ml portions of chloroform, the pH was adjusted to 7by careful addition of 6 N NaOH and the resulting mix was extracted with two 5 ml portions of chloroform. The final organic phase was dried over $MgSO_4$, filtered and concentrated to an oily, brown solid.

Method validation

Calibration procedure

Standard working solutions of 1000 µg/ml of VER, NVER,

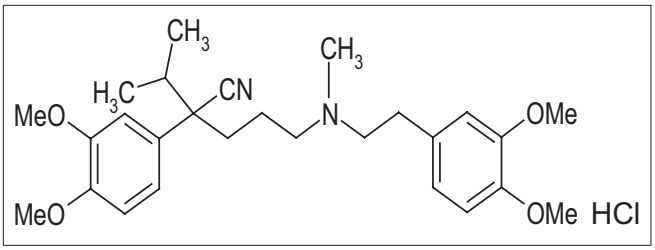

Figure 1: Strucure of verapamil hydrochloride

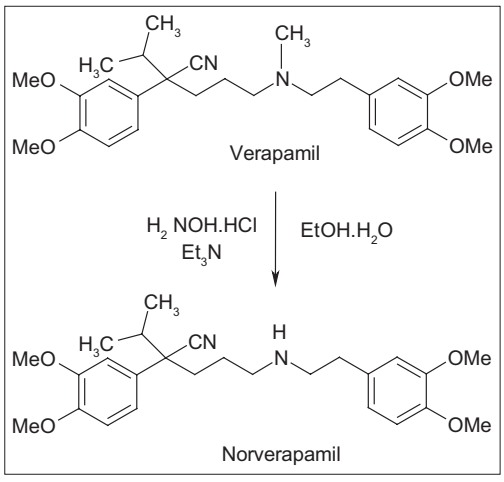

Figure 2: Reaction involved in the synthesis of norverapamil

and VAL were prepared using mobile phase as a solvent. Required volume of solution from standard working solution was taken to get final dilutions of required strength for calibration curves and volume was made up with mobile phase. The analytical responses of VER and NVER were found to be linear in concentration range of 10-60 µg/ml, respectively. The laboratory samples were prepared using stock solutions of drug and metabolite covering entire range of calibration curve. Amount of NVER present in laboratory sample was calculated using calibration curve data.

Accuracy

The accuracy of the method was determined by calculating percentage recovery of VER and NVER. Recovery studies were carried out by applying the method at three levels, i.e., 80%, 100%, and 120%. Known amounts of standard VER and its metabolite were added to pre-analyzed samples. And they were subjected to analysis by the proposed HPLC method.

Precision

Intraday and interday precision study of VER and NVER was carried out by estimating the corresponding responses thrice on the same day and on 3 different days for the concentration of 50 µg/ml for VER and NVER.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the following formulae: LOD=3.3(SD)/*S* and LOQ=10(SD)/*S*, where SD=standard deviation of response (peakarea) and S=average of the slope of the calibration curve.

Robustness

Under simulated chromatographic conditions (flow rate, pH, and mobile-phase component ratio), the reproducibility of results was observed to be reasonably good. These changes produced no significant impact on percentage recoveries of drugs. The results of the robustness study indicated that the developed method is robust and is unaffected by small variations in the chromatographic conditions.

System suitability parameters

System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. These parameters were determined on freshly prepared standard stock solutions of VER and its metabolite NVER.

Results and Discussion

The use of HPLC methods for simultaneous determination and quantitation of drug with its metabolite has received considerable attention in the recent past and its importance in the quality control of drugs. Various regulatory authorities like ICH, United State Food Drug Administartion (USFDA), Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's). Investigation of impurity profile of an active pharmaceutical ingredient is of crucial importance for medical safety reasons. Therefore, a lot of effort during a development of pharmaceutical dosage form is focused to minimize the impurities.[25] HPLC has been used widely in the field of impurity profiling; the wide range of detectors, and stationary phases along with its sensitivity and cost-effective separations have attributed to its varied applications. Synthesized metabolite NVER described in European Pharmacopeiais as an impurity which can be toxic, often present in pharmaceutical formulations of VER,[24] being, therefore, mandatory to perform impurity profiling of VER.

In present work, synthesis and characterization of NVER and the development of an analytical method for simultaneous estimation of NVER along with VER were carried out.

For the synthesis of NVER specificity of reaction was targeted. After extensive review of literature and preliminary experimentation with respect to hetero-atom de-alkylation reactions we selected hydroxylamine hydrochloride and triethylamine for synthesis of NVER. The synthesized metabolite NVER was purified by pH partitioning and characterized by spectroscopic techniques Fourier transform infrared spectra [Figure 3], mass spectra [Figure 4], and nuclear magnetic resonance spectra [Figure 5].

In the development of this method, various mobile-phase compositions containing methanol–water in different ratios was tried but the resolution was not found to be satisfactory giving a tailing effect. To overcome this problem, triethylamine was used as organic modifier. This reported method was found to estimate the VER along with metabolite in a run time of 10 min. Finally, the mobile phase comprising of methanol–water (70:30) with triethylamine and 0.01M OPA

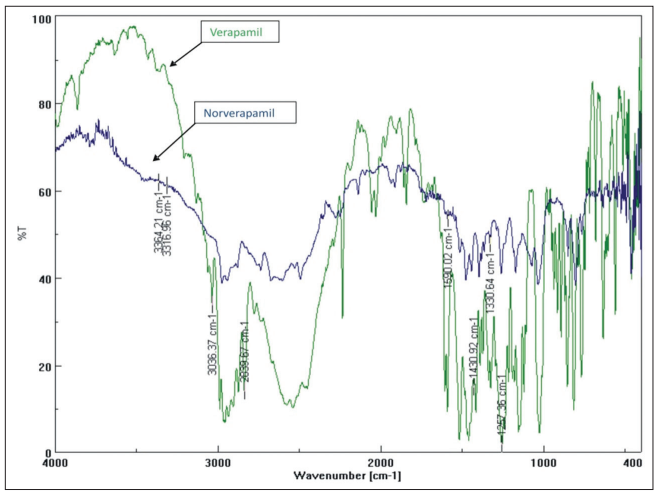

Figure 3: Fourier transform infrared overlain spectra of verapamil and norverapamil

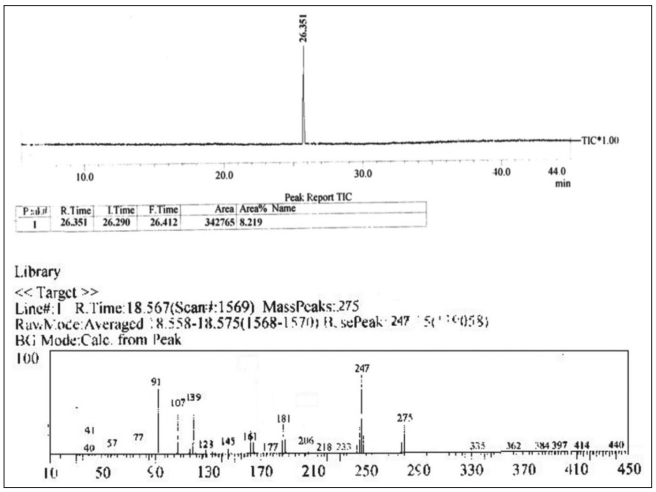

Figure 4: Mass spectra of norverapamil

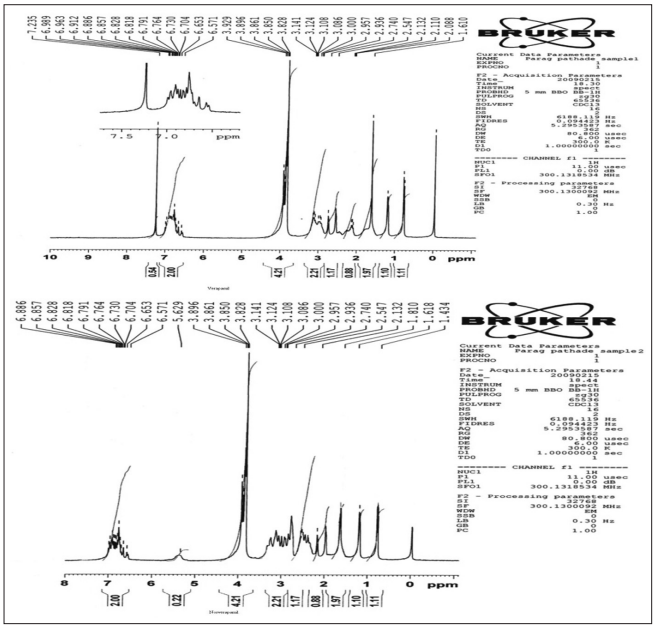

Figure 5: Nuclear magnetic resonance spectra of verapamil and norverapamil

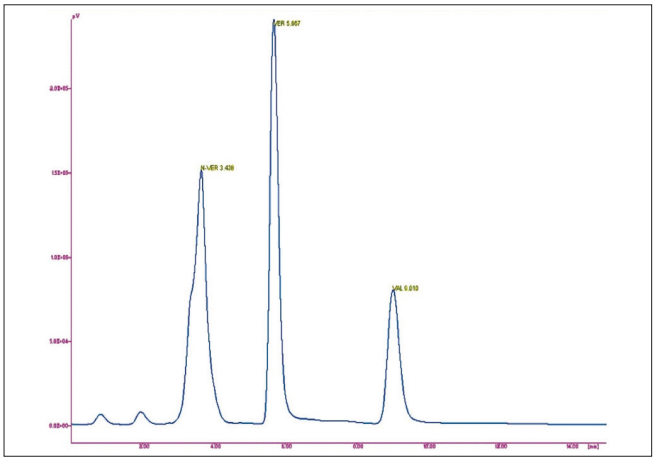

Figure 6: Chromatogram of physical mixture

as a mobile phase at 1ml/min was optimized, which gave two sharp, well-resolved peaks for NVER, VER, and VAL elutes in 3.44, 5.67, and 9.01 min, respectively [Figure 6].

UV overlain spectra of both VER and NVER showed that both the drugs absorbed appreciably at 222 nm, which was selected as the detection wavelength. The method was validated by using ICH Q2R1 (Q2B) guidelines. The calibration curve for VER and Never [Figure 7] was found to be linear over the range of 10-60 µg/ml, as shown in Table 1 with correlation coefficient (n=6) of 0.9995 and 0.9997, respectively.

The recovery study of VER in presence of NVER was found to be in range of 99.12-100.02, proving accuracy of the developed method [Table 2].

Intra-and inter-precision studies proves the repeatability and reproducibility of method and are shown in Table 2. The limit of detection were found to be 0.30µg/ml for VER and 1.21 µg/ml for NVER and the limit of quantitation 1.06 µg/ml for VER and 4.14 µg/ml for NVER from physical mixture as shown in Table 3. The results for validation

Table 1: Linearity study of verapamil and norverapamil

Concentration ($\mu g\ ml^{-1}$)	Response factor verapamil	Response factor norverapamil
10	0.3667	0.3575
20	0.6965	0.6656
30	1.0607	1.0216
40	1.3978	1.3504
50	1.6846	1.6603
60	2.0351	1.9475
Correlation coefficeint	0.9995	0.9997

Table 2: Results of recovery and precision study

Parameter	% Label claim estimated* (Mean±%S.D.)		% R.S.D.	
	Recovery study			
Analyte (verapamil)	99.12±100.02		100.02	
	Precision study			
	Repeatability			
Analyte	Verapamil	Norverapamil	Verapamil	Norverapamil
Laboratory sample	99.32±1.34	98.54±1.42	1.34	1.42
	Intermediate precision			
Day 1				
Morning	99.90±0.58	99.64±1.17	0.58	0.63
Evening	98.83±0.62	99.18±1.12	1.17	1.12
Day 2				
Morning	98.94±0.78	99.13±1.16	0.79	1.16
Evening	99.87±1.41	99.79±1.39	1.42	1.39

*Average of nine determination; SD – Standard Deviation; %RSD – Relative standard deviation

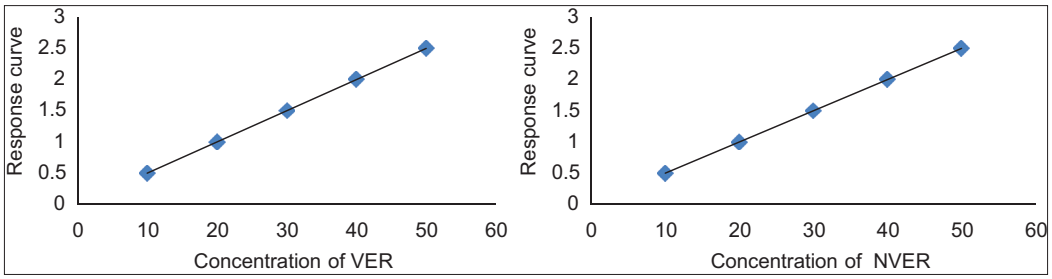

Figure 7: Calibration curve for verapamil and norverapamil

Table 3: System suitability parameters in physical mixture

Parameters	Verapamil	Norverapamil	Valsartan
Theoretical plates number (N)	1374.54	1937.96	1012.36
Resolution (RS)		5.40	
		4.89	
Retention Time in minutes (α)	3.44	5.67	9.01
Tailing factor (T)	1.47	1.13	1.87
Asymmetry	1.58	1.60	1.41
Calibration Curve (μg/ml)	25-60	10-80	50
Limit of Detection (μg/ml)	0.3028	1.2176	–
Limit of Quantitation (μg/ml)	1.0609	4.1418	–

and system suitability test parameters are summarized in Table 3.

Conclusion

The reported HPLC method is simple, selective, rapid, and sensitive for quantitation of synthesized metabolite NVER, in presence of the parent drug VER, which could be useful in detection and determination of some impurities, such as NVER described in European Pharmacopeia and others which can be toxic and often present in the pharmaceutical formulations.

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