Synthesis and Characterization of Norverapamil and Quantification of Verapamil and Nor-Verapamil in Plasma

N. M. Bhatia, P. A. Pathade, H. N. More, P. B. Choudhari, S. D. Jadhav, and M. S. Bhatia

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy near Chitranagari, Kolhapur-416 013 (M.S.) India

Received August 24, 2011; in final form June 20, 2012

Abstract—Verapamil is a calcium channel blocking agent which has found widespread use in the management of supraventricular tachyarrhythmias, angina pectoris, hypertrophic cardiomyopathy and hypertension. It is converted to its biologically active metabolite nor-verapamil in liver by cytochrome P450. In present communication, synthesis and characterization of nor-verapamil and development of reverse phase high performance liquid chromatographic method for the quantification of nor-verapamil along with verapamil in plasma has been carried out. The characterization of nor-verapamil was carried out using GC-MS, FT-IR and NMR spectroscopy. The separation was carried out with an isocratic JASCO RP-HPLC system using 5 µm KYA TECH HiQ Sil C18 HS column (250 mm × 4.6 mm internal diameter) as a stationary phase and methanol : water : 0.01 M orthophosphoric acid : triethylamine [70 : 30 : 2 : 0.5, v/v/v/v] as mobile phase. The flow rate was maintained at 1.0 mL/min and UV detection at 222 nm. The calibration for verapamil and nor-verapamil were found to be linear over concentration range of 50–300 ng/mL with correlation coefficient (n = 6) of 0.9995 and 0.9997, respectively. This method was validated according to USFDA guidelines. The method was found to be simple, accurate, precise sensitive and selective for the determination of verapamil and nor-verapamil in plasma and thus useful in bioequivalence studies of verapamil.

Keywords: synthesis, nor-verapamil, RP-HPLC, verapamil

DOI: 10.1134/S1061934813100031

Verapamil (VER) is a calcium channel blocking agent which has found widespread use in the management of supraventricular tachyarrhythmias, angina pectoris, hypertrophic cardiomyopathy and hypertension. It is converted to its biologically active metabolite nor-verapamil in liver by cytochrome P450. Because of rapid and extensive hepatic conversion by cytochrome P450 (CYP) to nor-verapamil (N-VER) in liver to a biologically active N-demethylated metabolite i.e., N-VER and a group of at least six major inactive derivatives. N-VER has a plasma half-life about 5 to 13 h [1].

A voltammetric behavior of VER in urine and pharmaceutical formulations was studied using adsorptive stripping technique [2]. The study enantioselective binding of VER to plasma lipoproteins by capillary electrophoresis–frontal analysis was also reported [3]. A few spectrophotometric methods for the determination of VER in pharmaceutical formulations were reported [4–9]. Multidimensional on-line sample preparation of VER and its metabolites by a molecularly imprinted polymer was coupled to liquid chromatography–mass–spectrometry. A new molecularly imprinted polymer (MIP) material selective for VER was synthesized and utilized for on-line metabolic screening of this common calcium antagonist in biological samples [10]. Aerobic biodegradability of the calcium channel antagonist VER and identification of a microbial dead-end transformation product was studied by LC/MS/MS [11]. Direct determination of VER in urine and serum samples by RP-HPLC method including micellar liquid chromatography and fluorescence detection have been described [12]. To avoid interferences from the sample matrices, capillary electrophoresis, reverse phase high performance liquid chromatography (RP-HPLC) methods for the isolation and quantification of VER and procured metabolites in biological fluids have been described [13–19].

In the present communication, we have succeeded in synthesis, and characterization of N-VER as well as in developing a simple, accurate, precise, sensitive and selective RP-HPLC method for the quantification of N-VER in presence of the parent drug VER form plasma.

EXPERIMENTAL

Reagents and chemicals. Methanol, orthophosphoric acid, triethylamine, ethanol, hydrochloric ac-
id, sodium hydroxide, chloroform and hydroxylamine hydrochloride of HPLC grade were obtained from Loba Chemie Pvt. Ltd. Mumbai, India. VER and valsartan (VAL) were obtained as gift samples from Cipla India Pvt. Ltd., Kurkumbh, Mumbai.

**Chromatographic conditions.** The separation was carried out with an isocratic JASCO RP-HPLC system using 5 µm KYA TECH HiQ Sil C18 HS column (250 mm × 4.6 mm internal diameter) as a stationary phase and methanol : water : 0.01 M orthophosphoric acid (OPA) : triethylamine [70 : 30 : 2 : 0.5, v/v/v/v] as mobile phase. The pump used in this HPLC system was PU 2080 pump (Dual piston with gear driven pump). The 20 µL sample solutions of analyte were injected to chromatographic system using Rheodyne Injector. The flow rate of 1 mL/min and detection wavelength of 222 nm was chosen for analysis. The UV detector used in this HPLC system was Czerny turners mount monochromater with deuterium lamp as light source. The chromatographic and the integrated data were recorded using Hercule 2000 (interface) computer system. Data processing was carried out using Borwin® Version 1.5 software.

**Synthesis of 2-(3,4-dimethoxyphenyl)-5-[2-(3,4dimethoxyphenyl)] ethyl amino-2-isopropyl pentanenitrile (N-VER).** In a flask containing 0.5 g of VER, 1.12 g of hydroxylamine hydrochloride and 0.45 mL of triethylamine were added. 3 mL of ethanol and 1.53 mL of water were added to this mixture and reaction mixture was exposed to microwave irradiation for 12 min at 100°C. The mixture after cooling was poured in ice-cold 1 M hydrochloric acid. This solution was washed with two 5 mL portions of chloroform, pH was adjusted to 7 by addition of 6 M sodium hydroxide and resulting mixture was extracted with two 5 mL portions of chloroform. The final organic phase was dried over MgSO₄, filtered and concentrated to an oily and brown solid. The synthesis of N-VER is as shown in Fig. 4.

**Nuclear magnetic resonance spectroscopy (NMR).** The NMR spectrometer used for analysis was of Bruker Company and model Varian mercury 300 (AVNCE-300 MHz). The detector and magnet used in NMR spectroscopy are Rₓ coil and superconductivity magnet, respectively. The NMR spectra of VER and N-VER were recorded using CDCl₃ as a solvent and are reported in Fig. 3.

**Fourier transform infrared spectroscopy (FT-IR).** The FT-IR spectrophotometer used is of JASCO Company and of model FT/IR 4000. The light source, optical system and interferometer in FT/IR 4000 are high intensity ceramic lamp—standard (halogen lamp—optional), single beam optical system and 45° interferometer. The comparative FT-IR spectrum of VER and N-VER is as shown in Fig. 4.

**Preparation of standard stock solutions.** Standard stock solutions of VER, N-VER and VAL were prepared by dissolving accurately weighted 50 µg of VER, N-VER and VAL into 50 mL volumetric flasks using mobile phase and volume was made up to get concentration of 1000 µg/mL. These solutions were then sonicated for 10 min.

The individual analyte stock solutions prepared [1000 µg/mL each] were added separately to drug-free plasma in volumes not exceeding 2% of the plasma volume. The plasma samples were stored in the freezer at −17°C and allowed to thaw at room temperature before processing. The plasma samples were centrifuged at 4000 g for 10 min. An aliquot (1 mL) and acetonitrile (2 mL) were pipetted into a 10 mL polypropylene tube. The mixture was vortex mixed briefly and after standing for 5 min at room temperature the mixture was centrifuged at 4000 g for 20 min. The supernatants were stored as standards stock solutions of these analytes in freezer for bio-analysis.

**Linearity study of VER and N-VER.** For each drug, appropriate aliquots stored in freezer were pipetted out from each standard stock solution into a series of 10 mL volumetric flasks and to each flask 0.1 mL of internal standard solution of VAL were added and then final vol-