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REVIEW ON RP-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR BIO ANALYSIS OF VERAPAMIL IN RAT PLASMA

VIVEK VIJAYAN
SPPSPTM, NMIMS University, Vile Parle West, Mumbai, Maharashtra-400056
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Abstract: A comparison of two RP-high-performance liquid chromatography (HPLC) methods to determine efficient sample extraction to determine efficient method for getting optimum retention time and recovery of verapamil from rat plasma.

Keywords: Verapamil; Rat plasma; HPLC; sample extraction

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Corresponding Author: MR. VIVEK VIJAYAN

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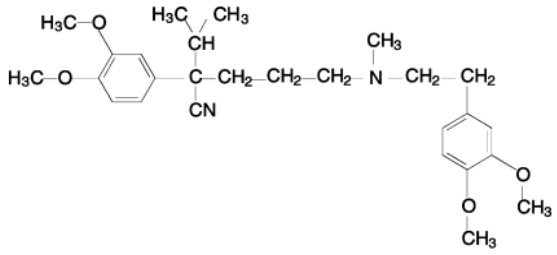
INTRODUCTION

Verapamil is a calcium channel blocking agent which has found widespread use in the management of supraventricular tachyarrhythmias, angina pectoris, ischemic heart disease, hyper-trophic cardiomyopathy and hypertension. For anal-ysis0 of drugs and their metabolites in biological samples, an efficient high-performance liquid chromatography (HPLC) combined with a various mode of detection, which is com-plicate to use, has been used. Because of rapid and extensive hepatic conversion to nor-verapamil, a biologically active N-demethylated metabolite, and a group of at least six major inactive derivatives. Hence, alternative routes of administration have been sought [1]. For analysis of drugs and their metabolites in biological samples, an efficient high-performance liquid chromatography (HPLC) combined with a various mode of detection, which is com-plicate to use, has been used. Because of undesirable sensitivity and selectivity, sample preparations including sample clean-up, and pre-concentrations are required prior to analysis [2]. High performance liquid chromatography (HPLC) has been the technique most often applied to the analysis of verapamil in serum or plasma [1].

In the majority of cases, the isolation of verapamil from the biological matrix has consisted of a liquid –liquid extraction from an alkaline sample into a volatile, water-immiscible, organic phase which, after evaporation to dryness is reconstituted in the mobile phase or in a solvent compatible with the mobile phase. Very often, however, the initial extraction is followed by a back-extraction into dilute aqueous acid, which is then injected onto the column. A more recent alternative to the isolation of verapamil from biological fluids has been liquid – solid extraction. When auto-mated, this technique is found more convenient to the slower and less precise liquid – liquid extraction [1].



STRUCTURE: VERAPAMIL

The purpose of this study is to compare and validate fully automated HPLC method with flourimetric detector for measuring verapamil in small volume of sample.

METHOD 1[1]: The purpose of this report is to describe a reversed-phase HPLC method with spectrophotometric detection that will quantify verapamil in a small volume of plasma with no

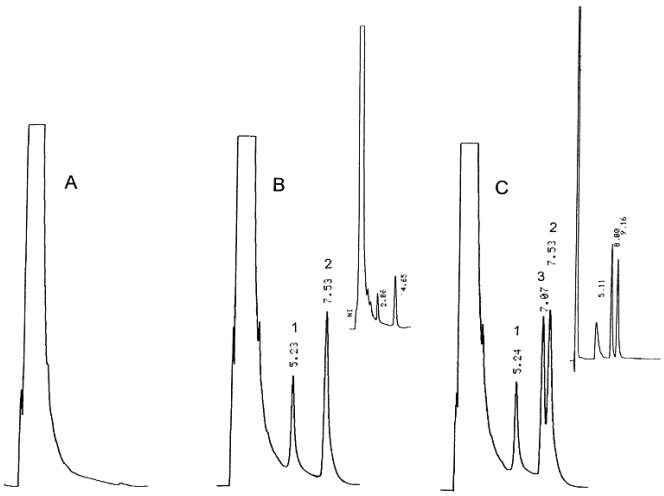
other sample preparation than a deproteinization step. This method was found to represent a simple, rapid, and accurate means of assessing the systemic absorption of verapamil from various sites in a rat model.

An isocratic system consisting of a Series 3B liquid chromatograph (Perkin-Elmer, Norwalk, CT), a Spectroflow 783 programmable absorbance detector (Kratos Analytical, Ramsey, NJ), and a LCI-100 laboratory computing integrator (Perkin-Elmer). Samples were introduced automatically with a WISP 710B sample processor (Waters Associates, Milford, MA) or manually through a Model 7125 sample injector fitted with a 100ml loop (Rheodyne, Cotati, CA).

The deproteinizing solution was a mixture of acetonitrile – perchloric acid in a an 8:2 ratio. The internal standard solution was prepared by dis-solving dextromethophan hydrobromide in ex-tracting solution to a concentration of about 43 mg/ml.

The mobile phase was a mixture of methanol – acetonitrile – water (10:30:60), to which 0.1 ml of acetic acid and 0.2 ml of triethylamine were added in succession, filtered and degassed prior to use. The flow rate was 1.5 ml/min. Separations were accomplished at ambient temperature on an analytical Microsorb-MV Cyano column (1504.6 mm i.d., 5mm particle size, Rainin Instrument Company, Woburn, MA) protected by an Adsor-bosil CN guard column (Alltech Associated, Deerfield, IL). A Microsorb-MV C18 (1004.6 mm i.d., 3mm particle size) column protected by an Adsorbosil C18 guard column was used in studies involving the separation of verapamil from norverapamil. Detection was at 235 nm and 0.05 AUFS.

Portions of plasma sample and deproteinizing solution, in a ratio of 4:1, were added to a 2ml polypropylene micro centrifuge tube with a snap-cap. After capping, the tube contents were vortex mixed for 30 s, and the suspension was centrifuged at 4000 rpm for 10 min. A 100ml portion of the clear supernatant was injected into the liquid chromatograph.



A. Blank rat plasma,

B. Rat plasma containing internal standard and verapamil

Elution time were 5.2 & 7.5 min resp. at 1ml/min.

Elution time were 2.9 & 4.65 min resp. at 1.5 ml/min.

C. Rat plasma containing internal std, nor-verapamil and verapamil.

Elution time were 5.2, 7.07 & 7.5 resp.

All chromatograms were obtained on a cyanopropylsilane column except for that in the inset of 1C, which was derived using a C18 column. In all cases, the flow rate was 1 ml/ min, except in the case of the inset of 1B, which was obtained at 1.5 ml/min. Key: 1, dextromethorphan, the internal standard; 2, nor-verapamil; 3, verapamil.

The HPLC method with spectrophotometric detection described here analyses verapamil in rat plasma in less than 200µl of sample, and in as little as 6 min, with reasonable sensitivity, and good accuracy and precision. The sample preparation consists of a simple one-step deproteinization, the internal standard is readily available, and the mobile phase can be prepared by the simple admixing of its components. This method should be useful in pharmacokinetic studies involving small animal models and numerous samples.

METHOD 2[2]: The purpose of this study is to develop and validate a fully automated reverse-phase coupled column microbore-HPLC method with fluorometric detector for measuring verapamil in micro-volume without sample preparation. The procedures includes pre-column for clean-up, enrichment of compounds followed by intermediate column for sample concentration or extraction, finally transferred to the analytic column for subsequent HPLC analysis by means of an automated column-switching valve.

The coupled column HPLC system used was a NANOSPACE SI-1 microbore system equipped with a six-port switching valve unit (Shiseido Co., Tokyo, Japan). The system is designed for semi-microcolumn LC by reducing any possible dead spaced volume in the entire system be-cause the dead volumes in the connections between columns and any switching valves can negatively affect the separation efficiency. The column used for the sample clean-up step was an MF-Ph precolumn, Capcell Pak (4.0 mm i.d.×20 mm, Shiseido Co., Tokyo, Japan). A C-18 column, Capcell Pak UG120 (2.0 mm i.d.×35 mm, Shiseido Co., Tokyo, Japan) was used for the primary separation of compound from plasma using the mobile phase A at 0.5 ml/min; an analytical C-18 column, Capcell Pak UG120 (1.5 mm i.d.×250 mm, Shiseido Co., Tokyo, Japan) was employed in the analysis of verapamil by using the mobile phase B at a flow rate of a 0.15

ml/min. The column temperature was maintained constant at 45◦C. The column effluent was monitored using a luorescence detector with an excitation wavelength of 280 nm and an emission wavelength of 313 nm. Mobile phase A (50 mM ammonium phosphate, pH 4.5) and mobile phase B (50 mM ammonium phosphate:acetonitrile = 70:30 v/v) were filtered and degassed through a 0.22µm Magna-R filter (Whatman International Co., Maidstone, UK).

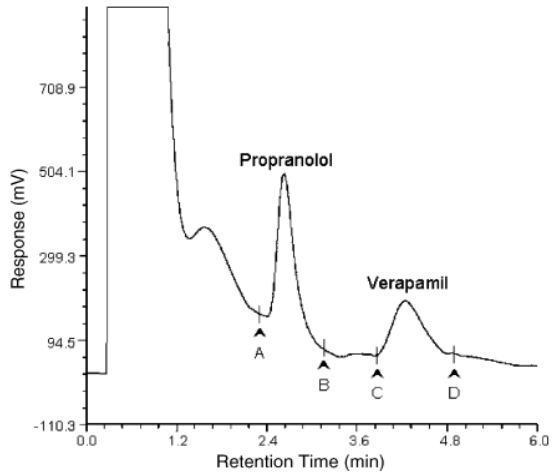


Fig 2: Separation of verapamil and propranolol-spiked plasma on MF Ph-1 precolumn and determination of switching time (min) for valves (2.35, 3.10, 3.85 and 4.85); conditions: mobile phase A, flow rate: 0.5 ml/min, injection volume: 10µl.

A diagram of the different column-switching positions and time sequences of column-switching procedure in the HPLC system is shown, respectively.

• Step 1 (0–2.35 and 3.10–3.83).

Plasma sample was introduced onto precolumn where plasma proteins, verapamil and internal standard were separated using mobile phase A at flow rate of 0.5 ml/min. The intermediate column and analytical column were equilibrated using mobile phase B at a flow rate of 0.15 ml/min.

• Step 2 (2.35–3.10 and 3.85–4.85 min).

When the valve status was changed to B, target drug-containing fraction separated in precolumn was focused on to the top an intermediate column using mobile phase A at flow rate of 0.5 ml/min. The analytical column was equilibrated using mobile phase B.

• Step 3 (4.85–25.0 min).

The compounds trapped in the top of intermediate column were transferred to the analytical column and analysed by fluorescence detector at an excitation wavelength of 280 nm and an emission wavelength of 313 nm.

Coupled column technique is a useful sample preparations system that can directly analyze complex biological samples in the small volume without any loss in sensitivity and chromatographic efficiency obtained by semi-microcolumns. Coupled column devices have been proved to simplify the HPLC analysis of drugs in biological samples, by facilitating the total automation of the chromatographic pro-cess, then increasing the speed and work capacity.

To update, an increasing number of HPLC methods with on-line sample clean-up by solid-phase extraction using coupled column devices have been developed. The principle of coupled column technique for sample clean-up. The other compounds of the biological matrix are eluted to waste, whereas the cut-off effluent containing the analytes is diverted to the intermediate or analytical column, where they are separated for identification and/or quantitation. Zone cutting technique probably is one of the most useful and versatile of the entire coupled column techniques. Therefore, we used two zones cut-ting techniques for propranolol and verapamil fraction in system that is shown in Fig. 2.

The principle of coupled column technique for sample clean-up is to trap the fraction of the sample that contains the analytes in the precolumn. The other compounds of the biological matrix are eluted to waste, whereas the cut-off effluent containing the analytes is diverted to the intermediate or analytical column, where they are separated for identification and/or quantitation.

An automated microbore-HPLC method using coupled column technique without sample preparation has been developed for simple, specific and accurate analysis of verapamil in small volume of rat plasma. The total analysis time of this method was 25 min and sample volume was only 10µl and superior to other extractive spectrophotometer methods in respect to automated simplified, sensitivity, specific and inexpensive experimentation. The suitability of this method was confirmed in the pharmacokinetic study for verapamil in rats.

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