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Estimation of Verapamil in Guinea Pig Serum by Reversed Phase High Pressure Liquid Chromatography

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The purpose of the investigation was to develop a high performance liquid chromatography assay procedure for quantitative estimation of verapamil in guinea pig serum. The procedure employs less sample volume, simple mobile phase, UV detector and readily available internal standard i.e. propranolol. The procedure is sensitive at lower blood concentrations and permits rapid analysis of upto five samples per hour. In conclusion, a specific and sensitive method has been developed for verapamil assay in guinea pig serum using μ bondapak C_{18} column and UV detector, which has an added advantage over, published methods.

Verapamil is a calcium channel blocker, which inhibits the calcium flux across cell membrane, has been shown to have antianginal, antihypertensive and antiarrythemic properties[1]. Numerous analytical methods have been reported for quantitative estimation of verapamil in dosage forms and biological fluids like plasma, urine and saliva[2,3]. Earlier investigation on verapamil had shown human plasma concentrations of 23.5 ng/ml[4]. These methods either use large quantities of serum, fluorimetric detector, special column, complex mobile phase, no internal standard or having long retention time. Hence, a simple and quick method of estimating verapamil was developed using small sample volume, simple mobile phase, most commonly available μ bondapak C_{18} column, UV detector and readily, available internal standard i.e. propranolol.

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MATERIALS AND METHODS

Verapamil hydrochloride was gifted by Torrent Pharmaceuticals Ltd., Ahmedabad. Propranolol, which was used as an internal standard, was gifted by Cipla Limited, Mumbai. Acetonitrile and methanol HPLC grade were purchased from S.D. Fine Chemicals, Boisar. Water HPLC grade was purchased from CDH (P) Ltd., Mumbai. Sulfuric acid used was of AR grade. Albino Guinea pigs were purchased from lucky Zoological House, Model Town, New Delhi. The HPLC system used was a Waters HPLC pump 501 equipped with a Rheodyne 7115 sample injector with a 20 μl sample loop (Rheodyne Cotani, Ca, USA)[5-7]. The column used was μ bondapak C_{18}[8,9] containing an irregular 10 μm shaped silica gel (3.9 mm I.D. and 300 mm length). The UV detector was Waters 486 tunable absorption detector. The detector was set at a wavelength of 278 nm and was used together with Waters 486 data modules.

UV absorbance curve of verapamil hydrochloride in mobile phase showed λmax. at 230 nm and 278 nm. UV absorbance curve of propranolol in mobile phase showed λmax. at 292 nm. Therefore a common wavelength of 278 nm was selected for analysis of both verapamil and propranolol. A stock solution of verapamil hydrochloride was prepared by dissolving 10 mg of drug in 10 ml of

acetonitrile. This solution was diluted appropriately to prepare standard solutions of verapamil hydrochloride in serum and water, which were used to construct a calibration curve. A standard solution of verapamil hydrochloride (100 ng) was injected into the HPLC system to determine the retention time under the chromatographic condition used in experiment. Stock solution of propranolol was also prepared by dissolving 10 mg in 10 ml acetonitrile. This solution was diluted appropriately for using as an internal standard. A standard solution (100 ng/ml) was injected into the HPLC system to determine the retention time.

Serum was separated from blood by centrifugation for 10 min at 2500 rpm. A sample of serum (0.2 ml) was pipetted into glass stoppered borosil glass tubes with the help of micropipette. Then 200 µl internal standard (propranolol) was added to the serum. The mixture was vortexed for 30 sec and incubated for 37°. The mixture was deproteinized by diluting twenty-fold with absolute methanol. The mixture was vortexed and allowed to stand for 5 min, centrifuged at 2500 rpm for 10 min and filtered to remove particulate matter of > 0.45 µm. The filtrate was evaporated to dryness and the residue was resolubilized in 0.5 ml of mobile phase.

The mobile phase used for elution was prepared by mixing water (adjusted to pH 2.4 with sulfuric acid) and acetonitrile in ratio of 55:45. The mixture was filtered and degassed under vacuum before use. The flow rate was 1.2 ml/min and the column temperature was ambient. The pressure was maintained between 905 to 945 psig. The UV detector was set at 278 nm.

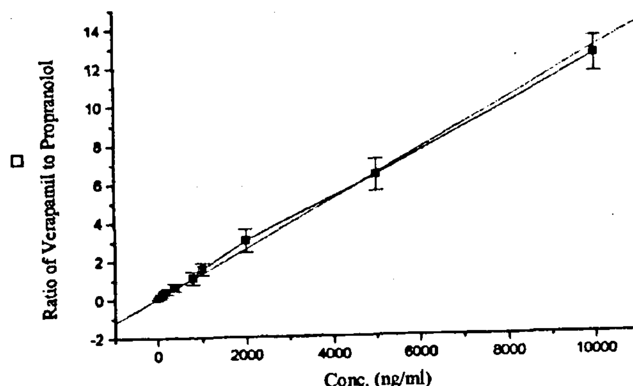

Fig. 1 : Serum concentration Vs ratio of verapamil to propranolol (internal standard). -■- Represents ratio of verapamil to propranolol. Vertical bars represent standard deviation

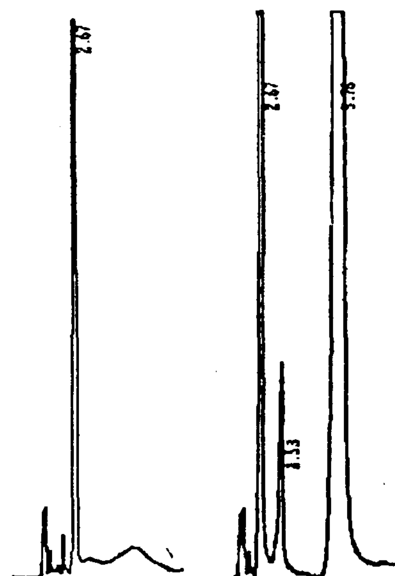

Fig. 2 : High Pressure Liquid Chromatographs showing peaks of Guinea pig serum with a retention time of 2.67, Propranolol with 3.53 and Verapamil with 5.76. Conditions: mobile phase, water (adjusted to pH 2.4 with sulfuric acid) and acetonitrile in ratio of 55:45; flow rate 1.2 ml/min; sample size 20 µl; wavelength 273 nm.

RESULTS AND DISCUSSION

Fig. 2 shows high pressure liquid chromatographs of guinea pig serum, propranolol and verapamil. The retention time for guinea pig serum was 2.67, for verapamil was 5.76 and for propranolol was 3.53 min, respectively. The major metabolites of verapamil cannot be quantitated due to non-availability of pure metabolic products. It is clear from fig. 2 that there is no overlapping or interference among all the 3 peaks.

Standard curve was prepared by adding known amounts of verapamil (in concentration range of 50-10,000 ng/ml) and propranolol (200 ng/ml) to blank serum and determining the peak area ratio of verapamil/internal standard. Fig. 1 shows peak area ratio of drug to internal standard as a function of the concentration added. The verapamil standard curves were found to be linear and pass through the origin. The equation for straight line was $Y = 0.0010 X$. The value of R^2 being 0.9710 and standard error of Y estimate - 1.0749.

The reproducibility of the method was determined by multiple analysis of plasma samples spiked with an aliquot of standard verapamil and propranolol solution. Table 1 shows variation for within-day analysis and between-day analysis. The recovery in serum was determined at

TABLE 1 : COEFFICIENT OF VARIATION OF VERAPAMIL IN GUINEA PIG SERUM

Concentration (ng/ml)	n	Coefficient of Variation	
		Within-day Analysis	Between day-Analysis
50	5	3.61	4.07
100	5	4.21	4.94
200	5	4.29	3.98
400	5	5.26	4.89
800	5	2.31	3.14
1,000	5	3.32	3.93
2,000	5	4.08	3.52
5,000	5	3.79	3.32
10,000	5	4.27	3.92

'n' indicates the number of samples

TABLE 2 : RECOVERY % OF VERAPAMIL AND PROPRANOLOL

	Concentration	n	Recovery % ±S.E.
Verapamil	50	5	60.24±1.9
	200	5	61.24±2.8
	500	5	63.29±1.6
Propranolol	200	5	62.49±3.2
	500	5	60.69±2.8
	1000	5	61.04±1.9

'n' is the no. of samples and S.E. denotes standard error

three levels of verapamil and propranolol. The results were given in Table 2. These results indicate that the recovery of both verapamil and propranolol exceeds 60%.

In conclusion, a specific and sensitive method has been developed for verapamil assay in serum using µ bondapak C_{18} column and UV detector, which has some advantages over published methods. Extraction procedure is simple and quick and five samples can be worked upon per hour.

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