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International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 865-870, April-June 2010

Application of RP-HPLC and UV-Visible Spectroscopy for the Estimation of Atenolol and Verapamil in Tablets Before and After the Expiry Period

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ABSTRACT: The stability and the quality of drug products are assured by continual testing and systematic evaluation using different analytical techniques. The product retains and exhibits its maximum potency during its shelf life. Once the drug crosses its expiry period, it deteriorates, not only decreasing its therapeutic activity but also rendering it toxic. In the present investigation RP-HPLC and UV-Visible spectroscopic methods are employed for estimation of cardiac drugs Atenolol and Verapamil in tablet dosage form before expiry period and 10-12 months after its expiry. Chromatography was carried out on a C-18 column using a mobile phase of .02M KH_2PO_4 buffer solution: methanol: acetonitrile (60:30:10 v/v) for Atenolol. The flow rate was 1ml/min with detection at 275nm. For Verapamil, a mobile phase of 0.15M sodium acetate buffer: acetonitrile (70:30v/v) was used. The flow rate was 2ml/min with detection at 278nm.The calibration curves obtained using HPLC method was linear in the range 640 - 960µgml^{-1} for Atenolol and160 – 240 µgml^{-1} for Verapamil. The calibration curves obtained using UV-Visible spectroscopy was linear in the range 50-150 µgml^{-1}for Atenolol and 20-100 µgml^{-1} for Verapamil.

Assays of Atenolol found using HPLC technique before expiry was 49.87 mg/tablet and after expiry was 44.75mg/tablet. For Verapamil before expiry it was 39.80 mg/tablet and after expiry 33.41mg/tablet. This was substantiated using UV-Visible spectroscopy. Recovery studies have been carried out to ensure the accuracy of the procedure adopted in each case. The high recovery percentage highlights the accuracy of the method followed.

Keywords: HPLC, UV-Visible spectroscopy, Atenolol, Verapamil, assay.

Introduction

Atenolol is chemically (R,S)-4-(2-hydroxy-3-isopropyl-aminopropoxy) phenylacetamide, is a beta-adrenoceptor antagonist. It finds it official place in the Indian Pharmacopoeia [1] and the British Pharmacopoeia [2]. Atenolol is used to treat angina (chest pain) and hypertension (high blood pressure). It is also used to treat or prevent heart attack. There are various methods employed for the determination of Atenolol in single-dosage formulations. They include UV spectrophotometry, spectrofluorimetry, high performance liquid chromatography and gas-liquid chromatography [3-9].

Verapamil, chemically Valeronitrile,5-((3,4-Dimethoxyphenethyl) Methylamino)-2-(3,4-Di methoxy -phenyl)-2-Isopropyl is a calcium ion influx inhibitor (slow-channel blocker or calcium ion antagonist) indicated for the management of hypertension, angina and arrhythmia. Many analytical methods of analysis of Verapamil have been reported by HPLC technique [10, 11]. The simultaneous HPLC analysis of enantiomers of

Verapamil and its metabolite in human plasma has been reported [12-13].

The present study aims at quantifying the degradation of Atenolol and Verapamil in their tablet dosage form before and 10-12 months after the expiry period using HPLC method and UV-Visible spectroscopy.

2. Experiment

2.1 Instrumentation

An isocratic high performance liquid chromotograph Shimadzu HPLC with single LC-20AT pump, variable wavelength programmable UV-Visible detector SPD-20A prominence with 20µL Rheodyne 7725 loop injector was used. The HPLC system is equipped with the spinchrome software to acquire, store and analyze the data. The UV-Visible spectrometric analyses have been carried out with Shimadzu-1601 series double beam spectrophotometer.

2.2 Solvents and Chemicals

The tablets of Atenolol and Verapamil belonging to the 'to-be-discarded' lot of drugs were procured from a few pharmaceutical firms in Chennai. These tablets have crossed their expiry period by 10-12 months. Also similar formulations within the expiry period were received from the same firms. Working reference standards of the drugs were obtained from Dr. CEELAL Analytical Lab, Chennai where the analysis was carried out. All chemicals used were of analytical or HPLC grade. KH_2PO_4 buffer (SISCO Research Laboratories), methanol (Leonid Chemicals Pvt. Ltd.) and acetonitrile (Ranbaxy Fine Chemical Ltd.) were used.

2.3 Standard stock solutions

The stock solution of the drugs Atenolol and Verapamil were prepared by dissolving 25mg of each of the pure drug in two separate 100ml volumetric flasks containing 50ml of methanol each, filtered and sonicated for about 15 minutes.

2.4 Working standard solution

The working standard solution was prepared by taking 5ml of the standard stock solution in two differed 25ml standard flasks and adding 20ml of the mobile phase. This solution is used as the working standard for analysis of all samples.

2.5 Sample preparation

The currently marketed pharmaceutical forms of Atenolol and Verapamil and their expired ones were assayed. Twenty tablets were pulverized and the powder amount equivalent to 25mg of the drug was weighed accurately, transferred to a volumetric flask, dissolved in 50ml of HPLC grade methanol and sonicated for about 15 minutes. The solution was filtered through a 0.45 µm membrane filter to separate insoluble portion. 5ml of the filtrate was taken in a 25ml standard flask and made up to the volume with the suitable mobile phase and mixed well. The same procedure was carried out for the expired tablets. Each of these solutions of 20 µL was injected five times into the column and analyzed.

The recovery studies were carried out on the unexpired drugs of Atenolol and Verapamil by adopting the standard addition technique. The standard pure sample of each of the drug corresponding to 100, 110, and 120% of the label claim were added to the pre-analyzed sample to determine whether the excipients present in the formulation lead to positive of negative interferences [14]. Each set of additions was repeated five times. From the HPLC curves and UV-visible spectra of the two drugs, the amount of drug was quantified.

2.6 Chromatographic System and Conditions

The Phenomenex C-18 110A column of dimension (250 x 4.6mm with particle size 5µm) was used. A mobile phase of .02M KH_2PO_4 buffer solution: methanol: acetonitrile (60:30:10 v/v) was used for Atenolol. The flow rate was 1ml/min with detection at 275nm. For Verapamil, a mobile phase of 0.15 sodium acetate buffer: acetonitrile (70:30v/v) was used. The flow rate was 2ml/min with detection at 278nm. Each of the mobile phases was filtered through a 0.45 µm millipore membrane filter and degassed. A Rheodyne 7725 loop injection with 20 µL was used for the injection of the samples. Every filtrate was injected five times into the column. The composition and the flow rate of the mobile phase were programmed for the motor pump and delivered at a constant rate. The baseline was continuously monitored during this period. The peak area was recorded for every concentration by selecting the UV detector wavelength suitably so that there was less interference from mobile phase and to obtain highest sensitivity.

2.7 UV-Visible Spectroscopic studies

For UV-Visible spectroscopic studies, the stock solution of the drugs were prepared by dissolving 25mg of each of the pure in 20mL of methanol in separate flasks. This stock was further diluted with methanol, filtered and sonicated to obtain solution required of drug concentration. With methanol as

reference, the maximum UV absorbance of these samples was noted at their corresponding λ_{max} with the double beam spectrophotometer. For both the drugs the absorbance values for various concentration was noted and the linear behavior studied. Each drug concentration in the chosen range was scanned five times to ensure the absorbance values. The drug content of the samples was calculated from their respective regression curves obtained from standard solutions by using these absorbance values.

3. Results and discussion

3.1 Linearity

Linearity was evaluated by analysis of working standard solutions of Atenolol and Verapamil for five different concentrations [14, 15]. The plot of peak area versus the respective concentration of Atenolol and Verapamil were found to be linear in the concentration range of 640 - 960µgml^{-1} and 160 – 240 µgml^{-1}. Using the plot of UV-Visible absorbance versus the concentration, Atenolol was found to be linear in the range 50-150 µgml^{-1} and the concentration of Verapamil was found to be linear in the range 20-100 µgml^{-1}. Regression analysis was done to calculate the calibration equations and correlation coefficients. The regression data obtained for the two drugs are listed in table 1.

Table 1: Regression Characteristics by HPLC and UV-Visible Spectroscopic methods

Parameter	HPLC method		UV-Visible spectroscopy method	
	Atenolol	Verapamil	Atenolol	Verapamil
Detection wavelength	275nm	278nm	274nm	278nm
Linearity Range	640 - 960µgml^{-1}	160 – 240 µgml^{-1}	50-150 µgml^{-1}	20-100 µgml^{-1}
Regression equation (Y = a + bx)	2.5346x-108.82	7.6189x+97.226	.005x-.0025	0.0115x+0.0058
Slope (b) (mean)	2.5346	7.6189	0.005	0.0115
Intercept (a) (mean)	108.82	97.226	-0.0025	0.0058
Correlation Coefficient n = 5	0.9986	0.9995	0.9990	0.9999

The results show that within these concentration ranges there was excellent correlation between peak-area ratio and concentration of each drug.

3.2 Assay

The validated HPLC method and the UV-Visible spectroscopic method were used in the analysis of the drug content of the current and the expired tablets of Atenolol and Verapamil. The area under the curve due to the drug and hence the drug content per tablet was computed from the formula given below.

Drug content = (Peak area for sample / Peak area for standard) x Conc. Of standard x Conc. Of sample x purity of standard x Avg. mol. wt. of sample.

Using the UV-Visible spectroscopic method the maximum UV absorbance of the current and expired samples was noted at their corresponding λ_{max}. With methanol as reference, the drug content of the samples was calculated from their respective regression curves obtained from standard drug solutions by using these absorbance values. The statistical parameter and results are reported in table 2.

Fig 1. Overlay of chromatograms of Atenolol before and after expiry

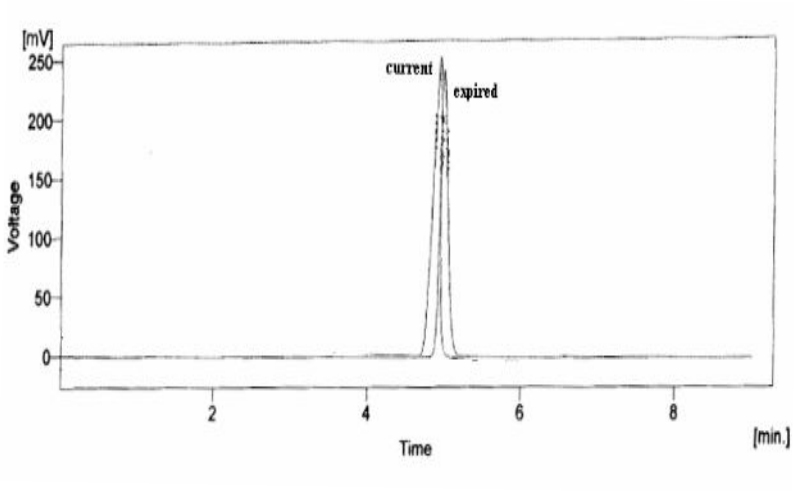
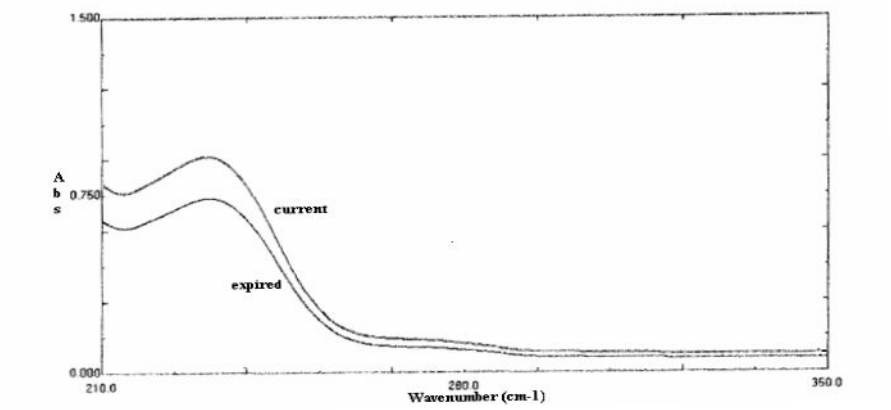


Table2: Evaluation of Atenolol and Verapamil in the current and expired pharmaceutical formulations using HPLC and UV-Visible spectroscopy

Technique	S.No.	Atenolol		Verapamil	
		Amount (mg per tablet) Label claim = 50mg per tablet		Amount (mg per tablet) Label claim = 40mg per tablet	
		Current	Expired	Current	Expired
HPLC	1	49.97	44.73	39.85	33.45
	2	49.85	44.82	39.75	33.60
	3	49.76	44.56	39.62	33.26
	4	49.92	44.97	39.90	33.24
	5	49.87	44.65	39.87	33.49
	Mean assay	49.87	44.75	39.80	33.41
	Mean assay(%)	99.75	89.49	99.50	83.52
	R.S.D(%)	0.14	0.32	0.25	0.43
UV-Visible Spectroscopy	1	49.49	47.35	39.45	38.20
	2	49.12	47.47	39.20	38.26
	3	49.40	47.72	39.89	38.37
	4	49.80	47.82	39.70	38.45
	5	49.27	47.21	39.50	38.15
	Mean assay	49.45	47.51	39.55	38.41
	Mean assay(%)	98.83	95.03	98.87	96.02
	R.S.D(%)	0.47	0.47	0.59	0.43

Fig 2. Overlay of UV-Visible spectrum of Verapamil before and after expiry



The results were in close agreement to the label claim of the currently used pharmaceutical preparation of Atenolol and Verapamil and the relative standard deviation observed for both the drugs were very low. Whereas the results obtained for the expired tablets of Atenolol and Verapamil clearly showed that there has been a marked deterioration in the quantity of drugs in these tablets.

3.3 Precision Evaluation

To check the accuracy and reliability of the developed methods analytical recovery experiments were carried out by standard addition method at 100, 110 and 120% level. The accuracy of the method adopted has been expressed in terms of percentage recovery. The percentage of recovery was calculated from the ratios of the amount added and the amount of drug found by both HPLC and UV-Visible spectroscopic methods, by using the formula given below.

% Recovery = $Y/(X+X_1)$

Where, Y is the amount of drug found by the proposed method, X the amount of pre-analyzed sample, and X_1 the amount of standard drug added. The results are shown in table 3.

4. Conclusion

A simple, fast and accurate HPLC method has been adopted to quantify the amount of drugs Atenolol and Verapamil present in pharmaceutical formulations before and after 10-12 months of expiry period. The regression of each of the drug concentration over the mean peak area obtained by HPLC method has been used to quantify the amount of drug present in the tablet forms. The HPLC results indicate that Atenolol contains 99.75% of drug in the pharmaceutical dosage form, whereas the drug present is only 89.45% of the labeled amount, eleven months after expiry period. In the case of Verapamil, the drug content reduces to 83.52% after the expiry period from the actual drug content of 99.50%. Hence it is very clear that the drugs tend to deteriorate and lose their efficacy after the stipulated shelf life. The percentage recoveries for Atenolol and Verapamil found using HPLC method were %and %. The percentage recoveries found using UV-Visible spectroscopy were %and %.UV-Visible spectroscopic analysis for the same samples confirms the results obtained by the former method. Thus HPLC and UV-Visible spectroscopy can be successfully employed for the estimation of drugs in pharmaceutical dosage form. It is highly suitable for the routine analysis of the drugs to monitor the quality control of the drug products.

Table 3: Recovery Studies for precision evaluation in Atenolol and Verapamil

Method	Drug	Excess Drug (%)	Amount found (mg)	Recovery (%)
HPLC	Atenolol	100	49.83	99.66
		110	49.94	99.88
		120	50.89	99.78
			Mean	99.77
			R.S.D %	0.11
	Verapamil	100	40.31	100.78
		110	39.72	99.30
		120	39.65	99.13
			Mean	99.74
			R.S.D %	0.74
UV-Visible spectroscopy	Atenolol	100	50.35	100.70
		110	49.33	98.66
		120	49.86	99.72
			Mean	99.70
			R.S.D %	1.02
	Verapamil	100	39.58	98.95
		110	39.74	99.35
		120	40.22	100.55
			Mean	99.62
			R.S.D %	0.68

(These results show that the method is precise and accurate.)

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