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STUDIES ON LINEARTY AND ASSAY USING RP-HPLC AND UV-VISIBLE SPECTROSCOPY FOR THE DRUGS OXCARBAZEPINE AND PIOGLITAZONE BEFORE AND AFTER EXPIRY PERIOD

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ABSTRACT

The standard for stability and quality of drugs for approval of marketing, are assured by testing and systematic evaluation using different analytical techniques. The drugs are tested in pharma analytical lab involving analysts associated spectra, chromatogram etc , and the results are analyzed leading to fixation of expiry dates. Once the drug crosses its expiry period, its potency is lost and it deteriorates, not only decreasing in therapeutic activity but also turning to be toxic. In the present investigation RP-HPLC and UV-Visible spectroscopic methods are employed for estimation of drugs oxcarbazepine and pioglitazone 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 0.05M KH_2PO_4 and 0.5 ml Triethylamine buffer solution: methanol: acetonitrile (60:20:20 v/v) for oxcarbazepine. The flow rate was 1ml/min with detection at 254nm. For pioglitazone a mobile phase of 0.02 M KH_2PO_4 buffer: acetonitrile (50:50v/v) was used. The flow rate was 2ml/min with detection at 270nm.The calibration curves obtained using HPLC method was linear in the range 160 - 240µgml^{-1} for oxcarbazepine and 170-280 µgml^{-1} for pioglitazone. The calibration curves obtained using UV-Visible spectroscopy was linear in the range 10-30 µgml^{-1} for oxcarbazepine and 10-34 µgml^{-1} for pioglitazone. Assays of oxcarbazepine found using HPLC technique before expiry was 152.05mg/tablet and after expiry were 138.50mg/tablet. For pioglitazone, before expiry it was 30.85mg/tablet and after expiry 27.60mg/tablet. This was substantiated using UV-Visible spectroscopy.

INTRODUCTION: Oxcarbazepine is chemically 10, 1 l-Dihydro-10-oxo-5*H*-dibenz [*b,f*]azepine-5-carboxamide, and is used in the treatment of seizure disorders [1].

There are various methods employed for the determination of oxcarbazepine in single- dosage formulations.

They include UVspectrophotometry, spectro-fluorimetry, high performance liquid chromatography and gas-liquid chromatography [2- 8].

Pioglitozone, is chemically (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxbenzylthiazolidine-2, 4-dione. It is a drug that reduces the amount of glucose in the blood. It is in a class of anti-diabetic drugs called thiazolidinediones that are used in the treatment of type II diabetes [9].

Many analytical methods of analysis of pioglitozone have been reported by HPLC technique in their tablet dosage form before and 10-12 months after the expiry period using HPLC method and UV-visible spectroscopy [10-11].

EXPERIMENTAL:

Instrumentation: An isocratic high performance liquid chromatograph 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 spin chrome software to acquire, store and analyze the data. The UV-Visible spectrometric analyses have been carried out with Shimadzu-1601 series double beam spectrophotometer [12- 13].

Solvents and Chemicals: The tablets of oxcarbazepine and pioglitazone 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 Pharma Analytical Lab, Pondicherry where the analysis was carried out.

Standard Stock Solutions: The stock solution of the drugs oxcarbazepine and pioglitazone 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.

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

Sample Preparation: The currently marketed pharmaceutical forms of oxcarbazepine and pioglitazone 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.

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 0.05M KH_2PO_4 and 0.5ml triethylomine buffer solution: methanol: acetonitrile (60:20:20 v/v) was used for oxcarbazepine. The flow rate was 1ml/min with detection at 254nm. For pioglitazone, a mobile phase of 0.02 KH_2PO_4 buffer: acetonitrile (50:50v/v) was used. The flow rate was 2ml/min with detection at 270nm.

Each of the mobile phases was filtered through a 0.45µm millipore membrane filter and degassed. A Rheodyne 7725 loop injection with 20µL 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.

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 of required 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 using these absorbance values.

RESULTS AND DISCUSSION:

Linearity: Linearity was evaluated by analysis of working standard solutions of oxcarbazepine and pioglitazone for five different concentrations [14-15]. The plot of peak area versus the respective concentration

of oxcarbazepine and pioglitazone were found to be linear in the concentration range of 160-240 µgml^{-1} and 170-280 µgml^{-1}. Using the plot of UV-Visible absorbance versus the concentration, oxcarbazepine was found to be linear in the range 10-30 µgml^{-1} and the concentration of pioglitazone was found to be linear in the range 10-34 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 METHOD

Parameter	HPLC method		UV-Visible spectroscopy method	
	Oxcarbazepine	Pioglitazone	Oxcarbazepine	Pioglitazone
Detection wavelength	254nm	270nm	254nm	270nm
Linearity Range	160-240 µgml^{-1}	170-280 µgml^{-1}	10-30 µgml^{-1}	10-34 µgml^{-1}
Regression equation (Y = a + bx)	17.47x-451.5	18.75x-388.0	0.03x+0.051	0.025x-0.022
Slope (b) (mean)	17.47	18.75	0.03	0.025
Intercept (a) (mean)	451.5	388.0	0.051	0.022
Correlation Coefficient n = 5	0.9999	0.9997	0.9998	0.9998

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

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 oxcarbazepine and pioglitazone. 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.**

TABLE 2: EVALUATION OF OXCARBAZEPINE AND PIOGLITAZONE IN THE CURRENT AND EXPIRED PHARMACEUTICAL FORMULATIONS USING HPLC AND UV-VISIBLE SPECTROSCOPY

Technique	S.No.	Oxcarbazepine		Pioglitazone	
		Amount (mg per tablet) Label claim = 150mg per tablet		Amount (mg per tablet) Label claim = 30mg per tablet	
		Current	Expired	Current	Expired
HPLC	1	152.05	138.50	30.85	27.60
	2	152.12	138.45	30.75	27.55
	3	152.09	138.35	30.79	27.43
	4	152.25	138.14	30.87	27.31
	5	152.20	138.25	30.65	27.27
	Mean assay	152.142	138.338	30.782	27.432
	Mean assay (%)	101.38	92.33	102.83	92.0
	R.S.D (%)	0.1042	0.1275	0.5571	0.84
UV-Visible Spectroscopy	1	152.59	138.45	30.33	27.56
	2	152.54	138.40	30.43	27.52
	3	152.33	138.35	30.24	27.40
	4	152.45	138.42	30.15	27.35
	5	152.50	138.32	30.40	27.45
	Mean assay	152.482	138.33	30.31	27.456
	Mean assay (%)	101.72	92.30	101.26	91.86
	R.S.D (%)	0.0664	0.0668	0.505	0.625

Fig. 1 shows overlay of chromatograms of oxcarbazepine before and after expiry and **Fig. 2** shows overlay of UV-Visible spectrum of pioglitazone before and after expiry.

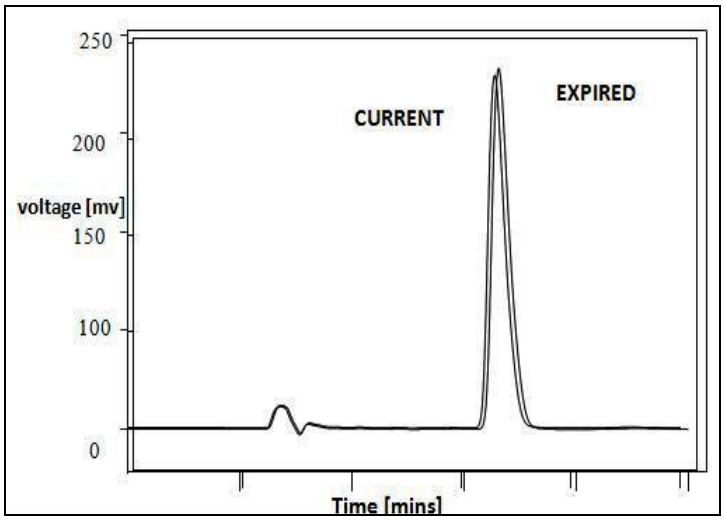

FIG. 1: OVERLAY OF CHROMATOGRAMS OF OXCARBAZEPINE BEFORE AND AFTER EXPIRY

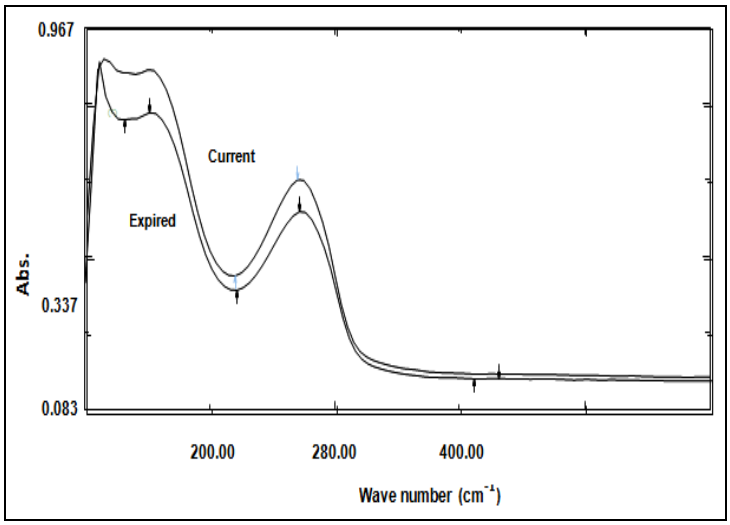

FIG. 2: OVERLAY OF UV-VISIBLE SPECTRUM OF PIOGLITAZONE BEFORE AND AFTER EXPIRY

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

CONCLUSION: A simple, fast and accurate HPLC method has been adopted to quantify the amount of drugs oxcarbazepine and pioglitazone 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 oxcarbazepine contains 102.83% of drug in the pharmaceutical dosage form, whereas the drug present is only 92.0% of the labeled amount, after expiry period.

In the case of pioglitazone, the drug content reduces to 91.86% after the expiry period from the actual value of 101.26%. Hence it is very clear that the drugs tend to deteriorate and lose their efficiency after the stipulated shelf life. 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.

REFERENCES:

1. Venkatesh, S., G.O.Reddy, B M. Reddy, M. Ramesh and A.V.N.Appa Rao, Antihyperglycemic activities of *Caralluma attenuate*. *Fitoterapia*, 2003; 74:274-279.
2. Suba.V, Murugesan.G, Arunachalam.S.G, Mandal and Saha B.P., Hypoglycemic potential of *Barleria lupulina* extract in rats. *Fitoterapia*, 2004; 75:1-4.
3. WHO, The WHO Expert Committee on Diabetes mellitus. Geneva: World health organization, Technical Report Series, 1980; 646.
4. Upathaya, V and K. Pandey, Ayurvedic approach to *Diabetes Mellitus* and its management by Indigenous Resources. In: *Diabetes Mellitus* in Developing countries-Bajaj, J.S(Ed), *Interprint,* New Delhi, 1984; 375-377.
5. Levis R.D and Silyn- Roberts H, Entrainment of the ultradian leaf movement Rhythm of *Desmodium gyrans* by temperature cycles, res: *J. Interdicippl* cycle 1987; 18,192-203.
6. Gamble JS, The flora of Presidency of Madras, Vol II: Botanical survey of India. Calcutta, 1958.
7. Elmahmood A.M, Doughari J.H, Ladan N and Amey, Antimicrobial screening of Stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. Afri. J. Pharm. 2008; 2 (5): 089-094
8. Kudi, A.C., J.U.Umoh, L.O.Eduvic, J.Getu, Screening of some Nigerian Medicinal plants for Antibacterial activitiy. *J.Ethnopharm*. 1999; 67:225-228
9. Ogundipe, O.O., J.O.Moody, T.O.Fakeye and O.B.Ladip, Antimicrobial activity of *Mallotus oppositifolium* extractives.*Afr.J.Med.Sci*.Vol.29:3/4, 2000; 281-283.
10. Harbone JB, *Phytochemical methods*. London: Chapman and Hill, 1973.
11. Carrel A and A.Hatmann: Cicatrization of wounds, The relation between the size of the wound and the Rate of its Cicatrization.*J.Expero.Med*: 1916; 24:429.