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UV - Spectrophotometric Method Development and Validation for Determination of Raloxifene in Pharmaceutical Dosage Form.

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Research Article

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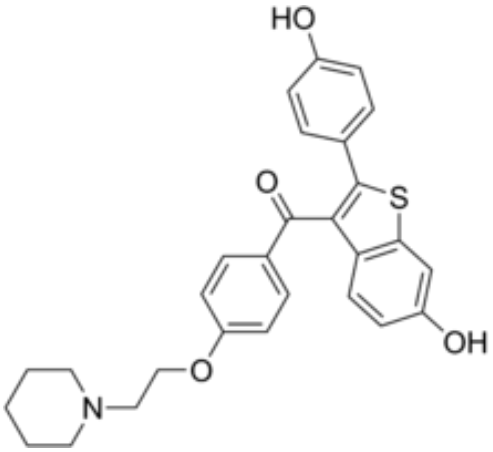
ABSTRACT

A simple, precise and economical second order derivative method has been developed for the estimation of Raloxifene in bulk and pharmaceutical formulations. In this method Raloxifene showed sharp peak at 288 nm when n= 1 and linearity was measured at 288 nm. It obeyed Beer's law in the concentration range of 2-12 mcg/ml. The LOD and LOQ were found to be 0.32 mcg/ml and 0.98 mcg/ml respectively. A recovery of Raloxifene in tablet formulation was observed in the range of 99.38-100.60%. The proposed method is precise, accurate and reproducible and can be extended to the analysis of Raloxifene in bulk and pharmaceutical formulations.

INTRODUCTION

Raloxifene[10,11] is a Antihypocalcemic agent. Chemical name of Raloxifene is [6-hydroxy-2-(4-hydroxyphenyl) - benzothiophen-3-yl]-[4-[2-(1-piperidyl)ethoxy]phenyl] -methanone. It has a molecular formula of $C_{28}H_{27}FNO_4S$and a molecular weight of 478.583 g/mol.



Literature survey reveals that several analytical methods have been reported for the estimation of Raloxifene by UV[1,2,3,4,5], RP-HPLC[6,7],UPLC-MS[8] and LC-MS[9] methods. Apart from above one spectroscopic method such as UV/Vis, difference spectrophotometric method, RP-HPLC etc., were reported for this compound.

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UV spectrophotometric method was reported for the quantitative determination of Raloxifene in pharmaceutical dosage forms. The developed method was simple, precise, specific and accurate and the statiscal analysis proved that method is reproducible and selective for the analysis of Raloxifene in bulk drug and tablet formulations.

EXPERIMENTAL

Instruments and reagents

A Elico-210 UV/VIS spectrophotometer was used with 1 cm matched quartz cell. All the chemicals used were of analytical grade. Methanol was procured from Loba Chem. Ltd., Mumbai. An analytically pure sample of Raloxifene was obtained from Hetero Drugs Limited as a gift sample.

Preparation of working standard drug solution

The standard Raloxifene (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with Methanol to obtain final concentration of 1000 mcg/ml and the resulting solution was used as working standard solution.

Analysis of marketed formulations

For the estimation of Raloxifene in tablets formulations by this method. 5 branded tablets were weighed and triturate to fine powder. Tablet powder equivalent to 10 mg of Raloxifene was weighed and transfer into 100 ml volumetric flask than dissolved with Methanol and further diluted with Methanol. It was kept for ultrasonication for 30 min; this was filtered through Whatman filter paper No. 41 and then final dilution was made with Methanol to get the final stock solution of 100 mcg/ml. From this stock solution, various dilutions of the sample solution were prepared and analysed.

Spectroscopic method

The spectra showed sharp peak at 288 nm when n=1 and linearity was measured at 288 nm (Fig 1). The absorbance difference at n=1 ($dA/d\lambda$) is calculated which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 2-12 mcg/ml and scanned in the spectra. The calibration curve of $dA/d\lambda$ against concentration of the drug showed linearity. Similarly absorbance of sample solution was measured and amount of Raloxifene was determined from standard calibration curve.

RESULTS AND DISCUSSION

As the drug Raloxifene showed a broad spectrum, the spectroscopy method applied has the advantage that it locate the hidden peak in the normal spectrum, when the spectrum is not sharp and it also eliminate the interference caused by the excipients and the degradation products present, if any, in the formulation.

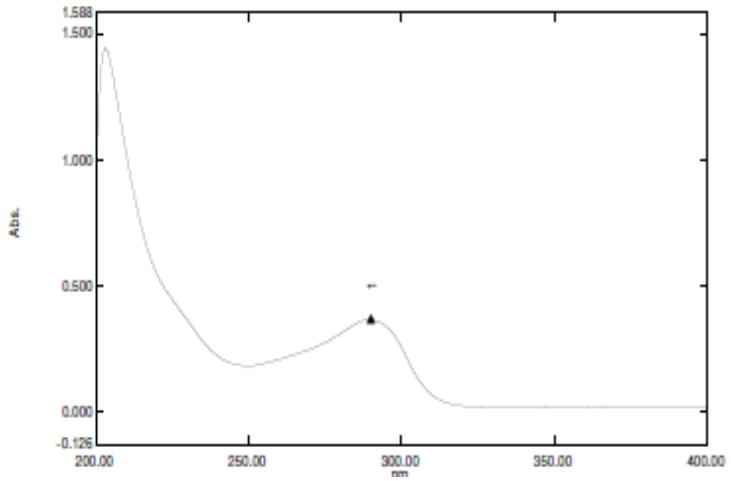

Figure 1: Spectrum of Raloxifene at 288nm

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The spectra showed sharp peak at 288 nm when n=1 and linearity was measured at 288 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 2-12 mcg/ml with r^2= 0.999 and given in Table 1.

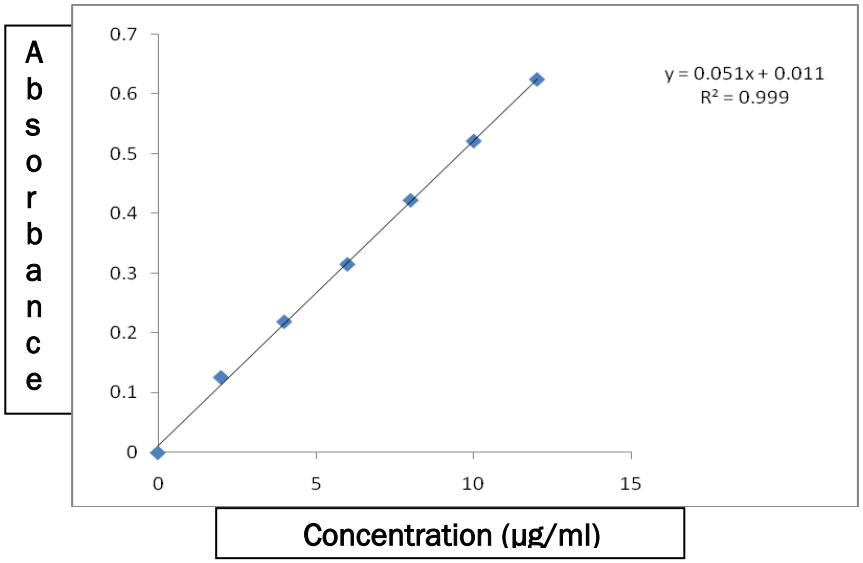

Figure 2: Calibration Curve for Raloxifene at 288 nm

Table1: Results of calibration curve at 288 nm

SL.NO.	Conc. (mcg / ml)	Absorbance at 288 nm
1	0	0
2	2	0.126
3	4	0.219
4	6	0.315
5	8	0.422
6	10	0.521
7	12	0.624

Precision

From stock solution (1000mg/ml) take 10ml and make up the dilution to 10ml with Methanol. The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation.

Table 2: Intraday precision results for Raloxifene

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.509
2	10	0.513
3	10	0.506
4	10	0.499
5	10	0.502
6	10	0.508
	STDEV	0.005037
	AVG	0.506167
	%RSD	0.99
	SE	0.002

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Table 3: Inter day precision results for Raloxifenee

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.514
2	10	0.512
3	10	0.509
4	10	0.503
5	10	0.509
6	10	0.507
	STDEV	0.003847
	AVG	0.509
	%RSD	0.75
	SE	0.001

Acceptance criteria:

% RSD of the six replicate injections should not more than 2.0%.

Recovery Studies

Recovery studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to the previously analysed tablet powder sample and shown in Table 4. The percentage recovery value indicates non interference of the excipients used in formulation.

Table 4: Recovery study Data

Sample (%)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered* S(µg/ml)	%Recovery ± STDEV*	%RSD
80%	1	9	9.03	100.6±0.001528	0.32
100%	1	11	10.98	99.89±0.005033	0.89
120%	1	13	12.92	99.38±0.005686	0.87

**Average of six determinations.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Wavelength

The solution was prepared and observed in replicate for three times with (±2) wavelength i.e. 286nm and 292nm respectively.

Table 5: Robustness summary

S. No	Condition	Modification	Mean Absorbance ± STDEV	% RSD (for Absorbance
1	Wavelength (nm)	286	0.491667 ±0.004509	0.009
2		292	0.499±0.006	0.120

*Average of three determinations

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

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Table 6: Ruggedness studies of Raloxifene by UV- visible spectroscopic method

S. No	Analyst-1		Analyst-2	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.499	10	0.511
2	10	0.507	10	0.503
3	10	0.502	10	0.498
	STDEV	0.004041	STDEV	0.006557
	AVG	0.502667	AVG	0.504
	%RSD	0.80	%RSTDEV	1.30
	SE	0.002	SE	0.003

Acceptance criteria

% RSD should not more than 2.0%.

CONCLUSION

A spectrophotometric method for quantifying Ralista-60mg in formulation samples has been developed and validated. The proposed method is precise, accurate and reproducible and can be extended to the analysis of Raloxifene in bulk and tablet formulations.

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