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NOTE

Enzymatic Method for the Determination of Paroxetine by Oxidative Coupling with Orcinol

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A novel enzymatic method for routine determination of paroxetine in pharmaceutical formulations and bulk dosage forms is proposed. This enzymatic method is based on the formation of coloured species on oxidative coupling of paroxetine with orcinol which undergoes prior oxidation in the presence of Horse radish peroxidase. The resulting pink coloured chromogen shows maximum absorption at λ_{max} 750 nm. Results of analysis were validated statistically and by recovery studies. Assay and recovery studies were also performed. The molar absorptivity was found to be 0.67×10^4 L mol^{-1} cm^{-1} and the % RSD of the method was found to be 0.17.

Key Words: Horse radish peroxidase, Orcinol, Paroxetine.

Paroxetine, chemically is (3S-*trans*)-3-((1,3-benzodioxol-5-yloxy)methyl)-4-(4-phenyl)piperidine derivative which is chemically unrelated to the tricyclic or tetracyclic antidepressants and is used for the treatment of panic attacks and it is marketed under trade names such as paradise, paxil, paxil CR and pexeva (GSK). Only a few methods *viz.*, liqiud chromatography coupled to tandem mass spectrometry, HPLC[1-5], LC-MS[6], GC-MS[7], high performance liquid chromatography-electro spray ionization mass spectrometry (HPLC-MS/ESI)[8] appeared in the literature for the determination of paroxetine in bulk and pharmaceutical formulations. As the drug has recently come into existence, the number of available procedures that could be of utility in quality control analysis is less and hence the author has proposed these methods for the routine analysis of paroxetine in pharmaceutical formulation.

After due calibration of the instrument, spectral and absorbance measurements are made using ELICO UV-visible spectrophotometer model EL-179 (Mumbai, India).

All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Freshly prepared solutions were always used for analysis. In the proposed method orcinol reagent (0.1 %), hydrogen peroxide (3.0 %), phosphate buffer (0.5 M) and crude enzyme Horse radish peroxidase were used.

Standard and sample solution of paroxetine: About 100 mg of paroxetine (formulation) was accurately weighed on a digital single pan balance and dissolved in 100 mL of water in a volumetric flask to prepare a solution that has a concentration equal to 1 mg/mL standard solution and further dilutions were made with the same solvent (100 µg/mL) for this method.

Enzyme extraction: A turnip radish weighing 40 g was pealed washed and cut into 1" cubes. Then ground with 200 mL of phosphate buffer. The resulting solution was subjected to centrifugation and later filtered using Whatman filter paper. The crude enzyme was collected and stored at 4 ºC.

Assay procedure: 10 mL of buffer solution was transferred to a series of 50 mL volumetric flask. To each tube 2 mL of orcinol solution, 1 mL of hydrogen peroxide and 1 mL of enzyme was added. The resulting solution was incubated for 10 min and the drugs paroxetine in different aliquots (0.5-2.5 mL, 100 mcg/mL) were added. The absorbance of the pink coloured chromogen was measured at 750 nm against the reagent blank. The amount of paroxetine was computed from the calibration curve.

The results of analysis for the proposed enzymatic method were validated through systematic statistical analysis and results are tabulated. The statistical analysis values are reported in Table-1 and assay and recovery results for these methods are tabulated in Table-2.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PAROXETINE

Parameters	Proposed method
λ_{max} (nm)	750
Beer's law limit (µg/mL)	5-25
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.0558
Molar absorptivity (L mol^{-1} cm^{-1})	0.67214
Correlation coefficient (r)	0.9983
Regression equation (Y)*	
Slope (a)	0.12890
Intercept (b)	0.00077
% RSD**	0.17000
% Range of errors (95 % confidence limits)	
0.05 significance level	±0.14210
0.01 significance level	±0.21030

*Y = a + bx, where 'Y' is the absorbance and x is the concentration of paroxetine in µg/mL.
**For six replicates.

TABLE-2
ESTIMATION OF PAROXETINE IN PHARMACEUTICAL FORMULATIONS

Formulations	Labeled amount (Mg/vial)	% Recovery by proposed method
Tablet 1	200 mg	98.34
Tablet 2	200 mg	99.28
Tablet 3	200 mg	99.22
Tablet 4	200 mg	100.30

The proposed method is based on the mechanism of oxidative coupling where in the initial reaction, the chemical reagent orcinol undergoes oxidation in the presence of horse radish peroxidase and then the oxidized reagent couples with the drug paroxetine to form a pink coloured chromogen that exhibits maximum absorption at a wavelength of 750 nm. The results of analysis indicating various parameters were studied by systematic statistical analysis. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and sandell's sensitivity for this method is presented in Table-1. The regression analysis using the method of least squares was made for the slope (a), intercept (b) and correlation coefficient (r) obtained from different concentrations was summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1.The accuracy of this method in the case of formulations was thoroughly studied by recovery experiments and the results are presented in Table-2. An additional check on the accuracy of this method was analyzed by adding known amounts of pure drug to pre-analyzed formulations.

Conclusion

The novel enzymatic method proposed is simple, sensitive and reproducible based on the principle of absorption visible spectrophotmetry for the determination of paroxetine in pharmaceutical formulations. The method is reliable and can be employed for the determination of Paroxetine in various pharmaceutical formulations and bulk dosage forms.

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