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Simultaneous Estimation of Tramadol Hydrochloride and Paracetamol by UV Spectrophotometric Method from Tablet Formulation

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Abstract: A simple, accurate, precise and economical procedure for simultaneous estimation of tramadol hydrochloride and paracetamol in two component tablet dosage form has been developed utilizing concept of internal standard addition. The method is based upon determination of tramadol hydrochloride at 270.5 nm and paracetamol at 243.5 nm, in distilled water. Tramadol hydrochloride and paracetamol at their respective λmax 270.5 nm and 243.5 nm shows linearity in the concentration range of 20-100 µg/ml and 3-15 µg/ml respectively. The method was validated statistically. Recovery study was performed to confirm the accuracy of the method.
Key Words: Tramadol hydrochloride, Paracetamol, Simultaneous equation method, Area under curve method, Recovery study.

Introduction And Experimental

Tramadol hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Tramadol hydrochloride possesses agonist actions at the µ-opioid receptor and effects reuptake at the noradrenergic and serotonergic systems. Tramadol is a compound with µ-agonist activity. Chemically it is [2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol]. It is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Paracetamol is official in Indian Pharmacopoeia and British Pharmacopoeia. [16] The I.P. & B.P. both suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations. Tramadol is available in the form of oral drops, tablets, capsules and injections[1]. There are various methods available for estimation of tramadol hydrochloride like UV spectrophotometric[2,3], spectrofluorometry[4], HPLC[5] , gas chromatography[6], GC-MS and LC-MS[7], capillary electrophoresis[8], HPTLC[9], HPTLC-densitometry, [10,11] etc. Paracetamol estimated simultaneously with other drugs by UV and

RP-HPLC methods. [13, 14, 15] [However some of these methods are costlier and time consuming. To overcome these difficulties spectrophotometric analysis serves to be the quickest, promising and reliable method for routine analytical needs. The aim of the present study is to develop a new simple, rapid, reliable and precise UV spectrophotometric method for analysis of tramadol from tablet formulation; method is based on measurement of UV absorbance of tramadol hydrochloride in methanol diluted with distilled water.

Apparatus:

Spectral runs were made on a Shimadzu UV-Visible spectrophotometer, model- 1700 (Japan) was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells. Glasswares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents and Solution:

All the reagents used in this assay were of analytical grade and the reagent solutions were prepared using preanalysed distilled water. Tramadol and Paracetamol pure drugs were obtained as a gift sample from Aristo Pharmaceuticals Limited, Mumbai. Tablets of

tramadol hydrochloride and paracetamol combined dosage form were purchased from local market for analysis. Distilled water was used as a solvent for the spectrophotometric estimation.

Determination of λmax:
Weighed an accurate amount 10mg of tramadol hydrochloride was dissolved in 20ml distilled water and diluted upto 100ml by distilled water to obtain a 100mcg/ml concentration of tramadol hydrochloride in solution. Weighed an accurate amount 10mg of paracetamol was dissolved in 20ml distilled water and diluted upto 100ml by distilled water to obtain a 100mcg/ml concentration of paracetamol. This solutions was subjected to scanning between 200 – 400 nm and absorption maxima at 270.5 nm and 243.5nm for tramadol hydrochloride and paracetamol respectively were determined.

Standard Stock Solution:
A stock solution containing 100mcg/ml of pure drugs were prepared by dissolving accurately weighed an accurate amount 10mg of tramadol hydrochloride was dissolved in 20ml distilled water and diluted upto 100ml by distilled water to obtain a 100mcg/ml concentration of tramadol hydrochloride in solution. Weighed an accurate amount 10mg of paracetamol was dissolved in 20ml distilled water and diluted upto 100ml by distilled water to obtain a 100mcg/ml concentration of paracetamol.

Working standard solution:
Stock solutions were as such used as working standard solutions.

Linearity and Calibration:
The aliquots working standard solution was diluted serially with sufficient distilled water to obtain the concentration range of 20 – 100 mcg/ml for tramadol hydrochloride and 3 – 15mcg/ml for paracetamol. A calibration curve for tramadol and paracetamol were obtained by measuring the absorbance at the λmax of 270.5 nm and 243.5nm respectively and vise-versa. And the following equations are set. Statistical parameters like the slope, intercept, coefficient of correlation, standard deviation, relative standard deviation, and standard error were determined.

$$A_1 = 0.000697\, C_1 + 0.06926\, C_2 \quad \ldots.(1)$$
$$A_2 = 0.006191\, C_1 + 0.01746\, C_2 \quad \ldots.(2)$$

Analysis of Marketed Tablet Formulation:
Accurately weighed the 20 tablets and powdered. The powder equivalent to 442mg of tablet was transferred to 100ml volumetric flask which contains 37.5mg of tramadol hydrochloride and 325mg of Paracetamol, to make 1:3 ratio of Paracetamol and tramadol 937.5mg of pure tramadol is added to it. This mixture was sonicated for 15 minutes and filtered through Whatman filter paper No. 41. From which six dilutions containing 2mcg/ml of Paracetamol and 6mcg/ml of tramadol hydrochloride were made and the absorbances taken at 243.5nm and 270.5nm. And by using above equations concentrations of Paracetamol (C_1) and tramadol hydrochloride (C_2) were determined.

Recovery studies:
Recovery studies were performed to judge the accuracy of the method. 0.5ml of standard formulation (100mcg/ml) was taken in three 10ml volumetric flask and to it 80%, 100% and 120% (i.e. 0.4ml, 0.5ml, 0.6ml) of working standard solution (100mcg/ml) added respectively and made the volume upto the mark. The respective absorbances at 243.5nm and 270.5nm were recorded against the blank. The amount of added concentration was determined from the obtained absorbance values and percent recovery was determined for each formulation. [12]

Robustness:
The evaluation of robustness was performed for system suitability to ensure the validity of analytical procedure. This was done by varying the instrument, analyst, and time of study. The analysis was performed on Shimadzu UV-Visible spectrophotometer, model-1700 (Japan) and UV-Visible Spectrophotometer model -1800 (Japan). Interday and intraday analysis was performed by changing the analyst.

Results
The UV scan of standard solution between 200 – 400 nm showed the absorption maxima at 270.5nm for tramadol hydrochloride and for paracetamol at 243.5nm. The Beer's law was verified from the calibration curve by plotting a graph of concentration vs absorbance. The plots are shown in fig. 1 and 2. Regression analysis showed very good correlation. The calibration plot revealed zero intercept which is clear by the regression analysis equation $Y = mX + C$. (Where Y is absorbance, m is the slope and X is the concentration in mcg/ml) as obtained by the least square method. The results thus obtained are depicted in Table No. I. The results of analysis for assay and recovery study for tablet formulation was studied and shown in Table No. II, III and IV. No significant variations were observed on intraday and interday analysis. Also no significant variations were observed on changing the instrument.

Discussion
The spectrum of tramadol hydrochloride and paracetamol in distilled water showed the absorption

maxima at 270.5 nm and at 243.5nm respectively. No effect of dilution was observed on the maxima, which confirmed the maxima at 270.5nm for tramadol hydrochloride and at 243.5nm for paracetamol. The statistical analysis of data obtained for the calibration curves of tramadol hydrochloride and paracetamol in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 0 – 20 mcg/ml for tramadol hydrochloride and 3-15mcg/ml for paracetamol. The plots clearly showed a straight line passing through origin. The estimated method was validated by low values of % RSD and standard error, indicating accuracy and precision of the methods. Excellent recovery studies further proves the accuracy of the method. Robustness of the method was studied by varying the instrument, time of study and analyst. Reproducibility of the results confirmed the robustness of the method.

Conclusions

From the results and discussion the method described in this paper for the determination of tramadol hydrochloride and paracetamol from tablet formulation is simple, accurate, sensitive reproducible and economical. The proposed method utilizes inexpensive solvents. The proposed method could be applied for routine analysis in quality control laboratories.

Table No. I: Optical characteristics and precision

Optical characteristics	Paracetamol	Tramadol
Absorption maxima	243.5nm	270.5 nm
Beer's law limit	2-20mcg/ml	0 – 20 mcg/ml
Coefficient of Correlation	0.999721	0.999879
Regression equation	Y=0.06926X+0.008329	Y=0.006191X+0.002685
Slope	0.06926	0.006191
y intercept	0.008329	0.002685
Molar absorptivity (lit/mole/cm)	9135.7055	1796.041431
Sandell's sensitivity (mcg/Sq.cm/0.001)	0.016547	0.166945

Table No. II: Results of Analysis of tablet.

Formulation		Tablet	
% Estimated	270.5nm	P	107.84
	270.5nm	T	102.62
	243.5nm	P	100.70
	243.5nm	T	103.83
Amount found in mg	270.5nm	P	323.375
	270.5nm	T	37.34
	243.5nm	P	322.368
	243.5nm	T	37.14

Table No. III: Results of Analysis of tablet.

% COV	270.5nm	0.05932
	243.5nm	0.01204
% Limit of detection (%LOD)	P	0.1957
	T	0.03974
% Limit of quantitation (%LOQ)	P	0.5932
	T	0.1204

COV - Coefficient of variation

Table No. IV – Recovery study data

%			80	100	120
Conc. In mcg/ml		P	9	10	11
		T	27	30	33
Amount Found in mcg/ml	P	270.5nm	8.674	10.254	10.957
	T	270.5nm	26.1	30.75	32.88
	P	243.5nm	8.538	10.264	11.008
	T	243.5nm	25.5	30.79	33.02
% Recovery	P	270.5nm	96.37	102.54	99.60
	T	270.5nm	96.66	102.5	99.63
	P	243.5nm	94.86	102.64	100.08
	T	243.5nm	94.44	102.63	100.06
S.D	243.5nm		0.0001	0.0004	0.000263
	270.5nm		0.000265	0.000122	0.000449
% RSD	243.5nm		0.01204	0.04007	0.02456
	270.5nm		0.05932	0.0231	0.07956

S.D. – Standard Deviation, RSD – Relative Standard Deviation

Fig. 1: Calibration curve of Tramadol in distilled water

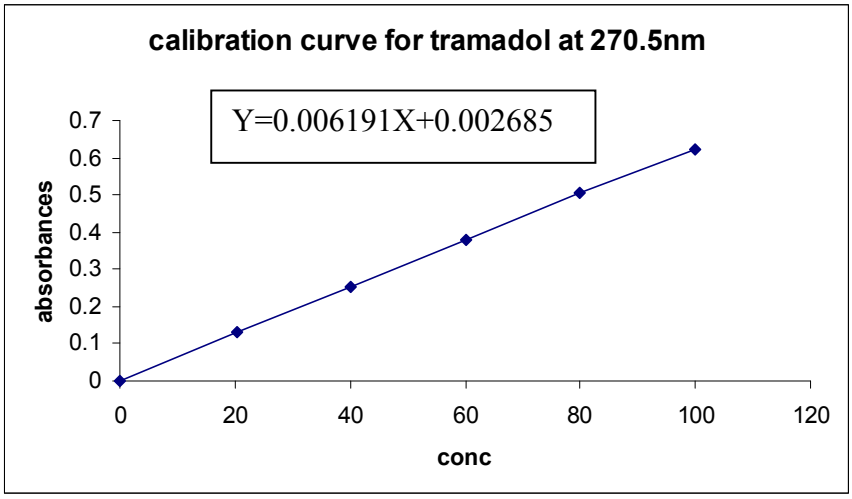
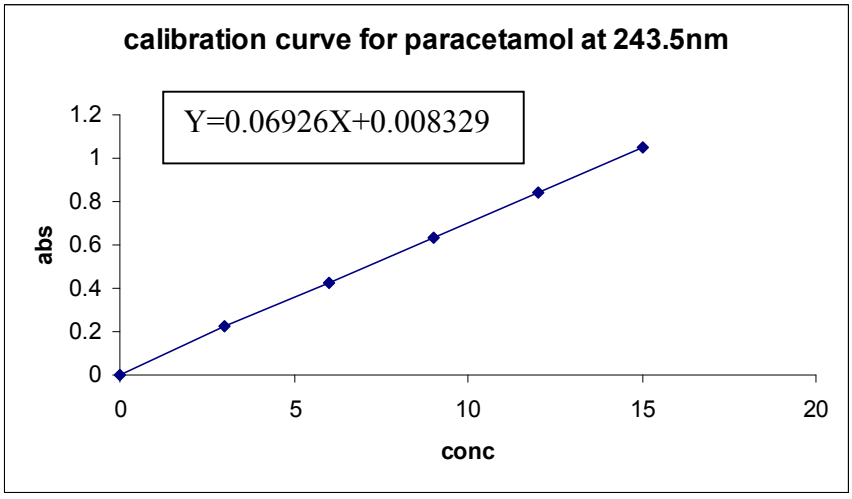


Fig. 2: Calibration curve of Paracetamol in distilled water



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References

1. Anonymous, The Martindale Extra Pharmacopoeia, 1985, EP 31, 1742-1744.
2. Abdellatef H.E., Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation, Journal of Pharmaceutical and Biomedical Analysis, 2002, 29, 835-842.
3. Puranik M., Hirudkar A., Wadher S.J., and Yeole P.G., Development and validation of spectrophotometric methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in tablet dosage form, Indian J. Phar. Sci., 2006, 737-739.
4. Abdellatef H.E., El-Henawee M.M., El- Sayed H.M. and Ayad M.M., Spectrophotometric and spectrofluorimetric methods for analysis of tramadol, acebutolol and dothiepin in pharmaceutical preparations, Spectrochimica Acta A Molecular and Biomolecular Spectroscopy, 2006, 65(5),1087-1092
5. Negro S., Salama A., Sánchez Y., Azuara M.L. and Barcia E., Compatibility and stability of tramadol and dexamethasone in solution and its use in terminally ill patients, Journal of Clinical Pharmacology and Therapeutics, 2007,32(5), 441-444.
6. Tao Q., Stone D.J., Borenstein M.R., Jean-Bart V., Codd E.E., Coogan T.P., Desai-Krieger D., Liao S. and Raffa R.B., Gas chromatographic method using 425 nitrogen-phosphorus detection for the measurement of tramadol and its Odesmethyl metabolite in plasma and brain tissue of mice and rats, Journal of Chromatography B: Biomedical Science Applications, 2001, 763, 165-171.
7. Moore C., Marinetti L., Coulter C. and Crompton K., Analysis of pain management drugs, specifically fentanyl, in hair, Application to forensic specimens, Forensic Science International, 2008, 176(1), 47-50.
8. Li J. and Ju H., Simultaneous determination of ethamsylate, tramadol and lidocaine in human urine by capillary electrophoresis with electro chemiluminescence detection, Electrophoresis, 2006,27(17), 3467-3474.
9. Krzek J. and Starek M., Quality assessment for tramadol in pharmaceutical preparations with thin layer chromatography and densitometry, Biomedical Chromatography, 2004, 18(8),589-599.
10. Ahrens B., Blankenhorn D. and Spangenberg, B., Advanced fibre optical scanning in thin-layer chromatography for drug identification, Journal of Chromatography B Analytical Technology in Biomedical and Life Sciences, 2002, 772, 11-18.
11. Venkateshwarlu K., Reddy Y.N., Srisailam K., Rajkumar V. and Pai M.G., Determination of tramadol in capsules by high performance thin layer chromatography – densitometry, Current Trends in Biotechnology and Pharmacy, 2008, 2 (3), 421 -425.
12. International Conference on Hormonization, Guidance for Industry In; Q2B Validation of Analytical Procedures: Methedology. 1996: 2.
13. Srinivasan K.K., Shirwaikar A., Joseph A., Jacob S. and Prabu S.L., Simultaneous estimation of aceclofenac and paracetamol in solid dosage form by ultraviolet spectrophotometry, Indian Drugs, 2006, 43(2), 141 – 145.
14. Mahaparale P.R., Sangshetti J.N. and Kuchekar B.S., Simultaneous spectroscopic estimation of aceclofenac and paracetamol in tablet dosage form, Indian J. Pharm. Sci., 2007, 289-292.
15. Nikam A.D., Pawar S.S.and Gandhi S.V., Estimation of aceclofenac and paracetamol in tablet formulation by ratio-spectra derivative spectrophotometry, Indian J. Pharm. Sci., 2008, 635-638.
16. British Pharmacopoeia, Vol. – I, Her Majesty's Stationary office: London, 2002, 35 – 37.

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