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RESEARCH ARTICLE……!!!

SIMULTANEOUS DETERMINATION AND VALIDATION OF PARACETAMOL, TRAMADOL AND SERRATIOPEPTIDASE BY DERIVATIVE SPECTROSCOPY METHODS IN BULK AND PHARMACEUTICAL FORMULATIONS

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KEYWORDS:

Paracetamol, Tramadol, Serratiopeptidase, Validation.

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ABSTRACT

In the present work two simple and sensitive UV- Spectrophotometric methods have been developed for the simultaneous estimation of Paracetamol, Tramadol and Serratiopeptidase in bulk drugs and pharmaceutical dosage forms by using distilled water as a solvent. Method A: First order derivative spectroscopy method is based on the measurement of absorbance at three selected wavelengths 226nm, 214nm and 263nm for the estimation of Paracetamol, Tramadol and Serratiopetidase respectively. Linearity range was found to be 5-25µg/ml, 1-5µg/ml and 0.5-2.5µg/ml with (r^2= 0.9993 % RSD = 0.307978-0.0.088228, r^2= 0.999, % RSD= 1.016395-0.50091 and r^2= 0.9994 % RSD = 0.583722-0.001966), for Paracetamol, Tramadol and Serratiopeptidase respectively. LOD for the above drugs was found to be 0.047506µg/ml, 0.011792µg/ml and 0.00905µg/ml respectively and LOQ of 0.143958µg/ml, 0.035734µg/ml and 0.027423µg/ml for Paracetamol, Tramadol and Serratiopeptidase respectively. Method B: Second order derivative spectroscopy method is based on the measurement of absorbance at three selected wavelengths 268nm, 233nm and 296nm for the estimation of Paracetamol, Tramadol and Serratiopetidase respectively. Linearity range was found to be 5-25µg/ml, 1-5µg/ml and 0.5-2.5µg/ml with (r^2= 0.9998 % RSD = 1.88926-1.073118, r^2= 0.9992, % RSD= 0.626115-0.136864 and r^2= 0.9997 % RSD = 1.013342-0.207479), for Paracetamol, Tramadol and Serratiopeptidase respectively. LOD for the above drugs was 0.50121µg/ml, 0.020443µg/ml and 0.016949µg/ml respectively and LOQ of 1.518817µg/ml, 0.06195µg/ml and 0.051626µg/ml for Paracetamol, Tramadol and Serratiopeptidase respectively.

INTRODUCTION:

Paracetamol (Fig.:1)[2] exerts analgesic and antipyretic effect like salicylates. It has weak activity on COX in the inflamed peripheral tissues, it does not produce GI irrition, acid-base imbalance, electrolyte disturbances, nor does it affect platelet activity. Chemically Paracetamol is N-(4-hydroxyphenyl) acetamide[5] The analgesic properties of Tramadol (Fig.:2)[2] can be attributed to norepinephrine and serotonin reuptake blockade in the CNS, which inhibits pain transmission in the spinal cord. Chemically Tramadol is (1R,2R)-2-[(dimethyl amino) methyl]-1-(3-methoxyphenyl) Cyclohexan-ol[4]

Serratiopeptidas[3] is an enzyme derived from the bacteria belonging to genus Serratia. Serratiopeptidase binds to α2-macroglobulin in the blood in a1:1 ratio. The mechanism of action of Serratiopeptidase appears to be hydrolysis of histamine, bradykinin and serotonin. Serratiopeptidase also has a proteolytic and fibrinolytic effect. Chemically Serratiopeptidase is Ca_7Zn^{+16} [6]

The combination of Paracetamol, Tramadol and Serratiopeptidase is prescribed for treating to relieve severe pain.

On literature survey, Paracetamol alone has been estimated and simultaneous estimation in combination with other drugs has been reported. Tramadol alone has been estimated and simultaneous estimation in combination with other drugs has been reported. Serratiopeptidase alone has been estimated and simultaneous estimation in combination with other drugs has been reported. It was found that no method has been reported for the simultaneous estimation of Paracetamol, Serratiopeptidase and Tramadol in combined dosage form and no method is available in the pharmacopoeias. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulations.

MATRIALS AND METHODS:

Instrument

For UV-Visible Spectroscopy methods, Shimadzu model 1800 double beam UV-Visible Spectrophotometer with spectral band width of 1 ± 0.2nm, wavelength accuracy of ± 0.3nm and a pair of quartz cuvettes having 1cm path length was used. Distilled water was used throughout the experimental work.

Chemicals

Standard Paracetamol, Tramadol and Serratiopeptidase were obtained as gift sample from micro labs, Bangalore.

METHODS

Preparation of standard solutions

Preparation of standard solution of Paracetamol (PAR)

100mg of Paracetamol was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 50 ml distilled water by ultra-sonication and volume was made upto the mark with distilled water to obtained finally concentration of 1000µg/ml (stock A). From the above stock A solution 10ml of aliquot was pipetted out to 100 ml volumetric flask and volume was made upto the mark with the distilled water to obtain a concentration of 100µg/ml (stock B). From the above stock B solution further dilutions were made to get concentration range from 5-25µg/ml for Paracetamol.

Preparation of standard solution of Tramadol (TMD)

100mg of Tramadol was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 50 ml distilled water by ultra-sonication and volume was made upto the mark with distilled water to obtained finally concentration of 1000µg/ml (stock A). From the above stock A solution 10ml of aliquot was pipetted out to 100 ml volumetric flask and volume was made up to the mark with the distilled water to obtain a concentration of 100µg/ml (stock B). From the above stock B solution further dilutions were made to get concentration range from 1-5µg/ml for Tramadol.

Preparation of standard solution of serratiopeptidase (SRT)

100mg of Serratiopeptidase was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 50 ml distilled water by ultra-sonication and volume was made up to the mark with distilled water to obtained finally concentration of 1000µg/ml (stock A). From the above stock A solution 10 mL of aliquot was pipetted out to 100 ml volumetric flask and volume was made upto the mark with the distilled water to obtain a concentration of 100µg/ml (stock B). From the above stock B solution further dilutions were made to get concentration range from 0.5-2.5µg/ml for Serratiopeptidase.

Preparation of sample solution

20 tablets of Paracetamol, Tramadol and Serratiopeptidase in combination were weighed and powdered. Tablet powder equivalent to 100 mg of Paracetamol was weighed accurately and dissolved in 70 mL of distilled water and sonicated for 15 min then filtered and the filtrate was diluted upto 100 mL with distilled water (stock A).

From the above stock A solution, 10 ml of aliquot was pipetted out in a 100 mL volumetric flask and the volume was made up to the mark with distilled water to obtain a concentration of 100µg/mL of each component (stock B).

From the stock B further dilutions were made to get the concentration of the drugs within the range.

Method A (First order derivative)

For the estimation of Paracetamol, Tramadol and Serratiopeptidase by first order derivative spectroscopy, zero crossing point for above drugs were obtained and the wavelengths were selected in such a way that at the zero crossing of one drug, the other drug should show substantial absorbance. From the first order derivative spectra of standard Paracetamol, Tramadol and Serratiopeptidase, zero crossing point of Paracetamol was found at 243nm, zero crossing point of Tramadol Hydrochloride was found at 215nm and zero crossing point of Serratiopeptidase was found at 275nm and wavelength selected for their estimation was 223nm for PAR, 214nm for TMD and 263nm for SRT.

Method B (Second order derivative)

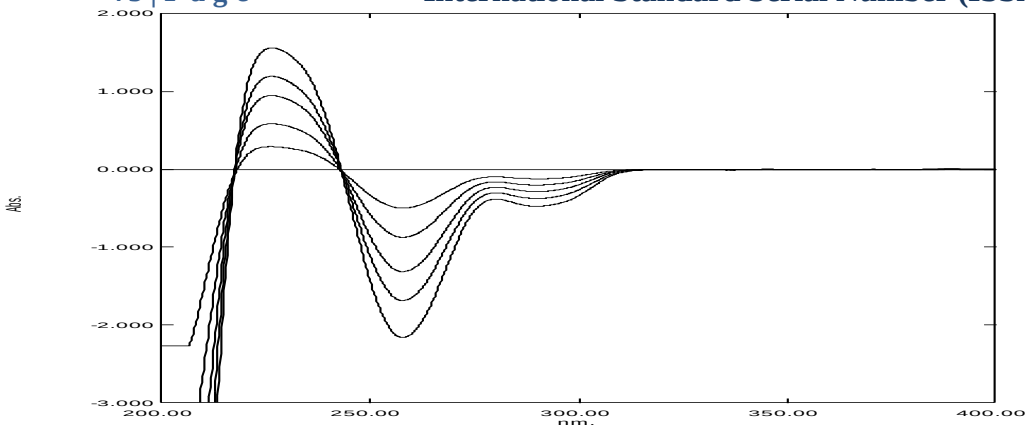
The most characteristic feature of a second-order derivative is a negative band with minimum at the same wavelength as the maximum on the zero-order band. It also shows two additional positive bands either side of the main band. Form the second order derivative spectra of standard PAC, TMD and SRT wavelength selected for their estimation was 268nm, 233nm and 296nm respectively.

VALIDATION OF THE METHOD:

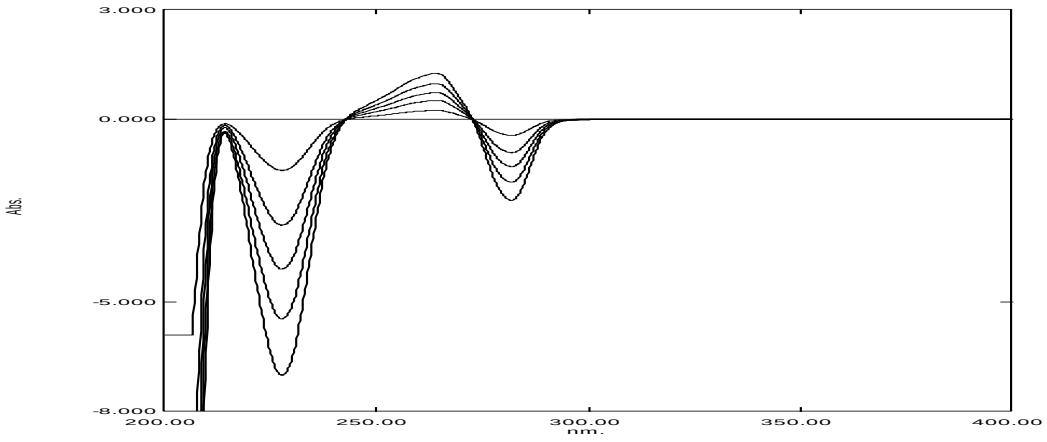
All the methods were validated according to ICH guidelines by carrying out analysis of six replicate samples of tablet. Recovery studies were carried out at three different levels i.e., 80%, 100%, 120% by adding the pure drug to previously analyzed tablet power sample. From the amount of the drug found, percentage recovery was calculated.

RESULT AND DISSCUSION:

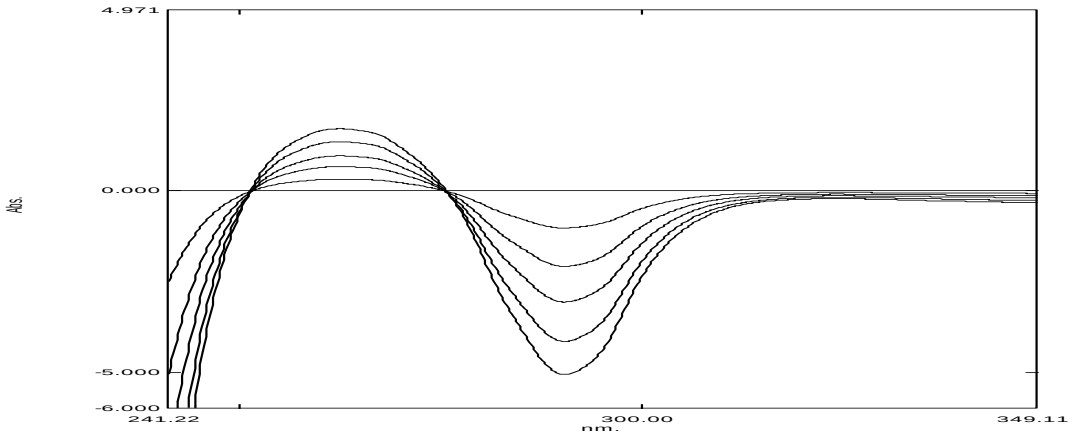
The selected drugs Paracetamol, Tramadol and Serratiopeptidase were estimated in Bulk and Formulation by using both first order derivatives and second order derivatives of UV spectrophotometric methods. The methods were validated for all validation parameters as per ICH guidelines. The linearity range in both methods for PARA, TMD and SRT was 5-25µg/ml, 1-5µg/ml and 0.5-2.5µg/ml respectively. The % RSD for intraday and inter-day precision was found to be less than 2%. The methods have been validated in assay of active pharmaceutical ingredients. The accuracy of the methods were validated by recovery studies and was found to be significant and under specification limits, with % recovery 99-100%. The assay results were found to be within the acceptable limits.



Overlay of first order derivative spectrom of PAC at 226nm



Overlay of first order derivative spectrom of PAC at 226nm



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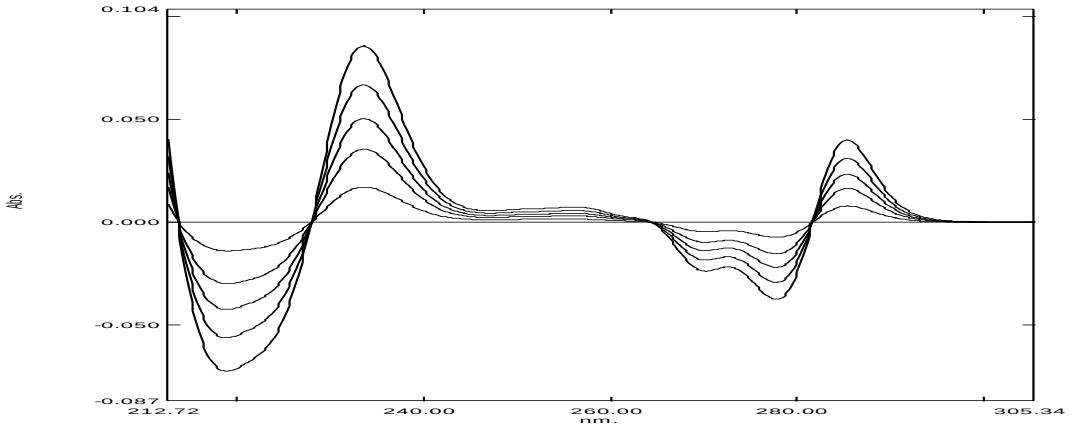
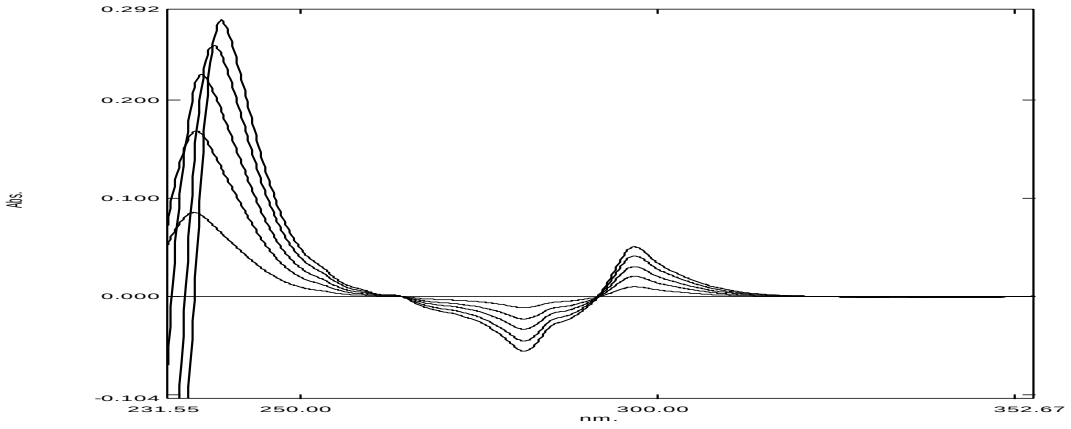
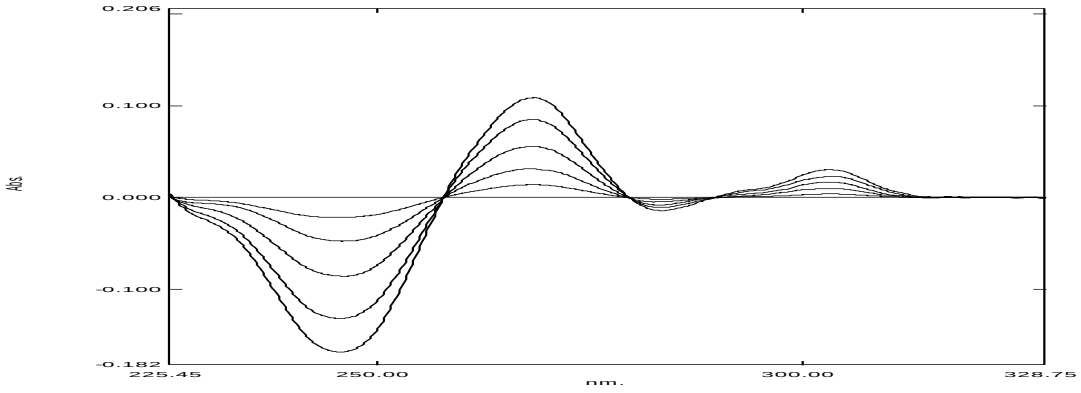


Overlay of first order derivative spectrom of PAC at 226nm



Overlay of first order derivative spectrom of PAC at 226nm



Overlay of first order derivative spectrom of PAC at 226nm

Table 1: Summary of Validation Parameters by Developed Methods.

parameter	Method A			Method B		
	PAC	**TMD**	**SRT**	**PAC**	**TMD**	**SRT**
Wavelength (nm)	226	214	263	268	233	218
Linearity Range (µg/ml)	5-25	1-5	0.5-2.5	5-25	1-5	0.5-2.5
Regression equation (y = a + bc)	y = 0.0625x - 0.0167	y = 0.0735x + 0.006	y = 0.6822x – 0.0137	y = 0.0054x + 0.0001	y = 0.1693x - 0.0022	y = 0.2042x + 0.001
Slope (b)	0.0625	0.0735	0.6822	0.0054	0.1693	0.2042
Intercept (a)	0.0167	0.006	0.0137	0.0001	0.0022	0.001
Correlation Coefficient (r^2)	0.9993	0.999	0.9991	0.9998	0.9992	0.9997
LOD (µg/ml)	0.047506	0.011792	0.00905	0.50121	0.020443	0.016949
LOQ (µg/ml)	0.143958	0.035734	0.027423	1.518817	0.06195	0.051626

Table 2: Statistical Validation Data for Accuracy Determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Method A			Method B		
				Total amount recovered (µg)	% Recovery	RSD	Total amount recovered (µg)	% Recovery	RSD
80%	PAC	15	8.2	18.17	99.835	0.1384	18.16	99.78	0.0550
	TMD	3	8	3.75	101.351	1.0213	3.75	101.35	1.1780
	SRT	1.5	12.15	1.91	100.525	0.6081	1.89	99.47	0.5291
100%	PAC	15	8.2	17.99	99.94	0.1158	17.99	99.94	0.1112
	TMD	3	8	1.005	105.5	0.9341	10.05	100.5	0.7395
	SRT	1.5	12.15	1.99	99.5	0.7663	1.99	99.5	0.7663
120%	PAC	15	8.2	22.17	100.09	0.1804	22.17	100.09	0.0902
	TMD	3	8	4.63	100.652	0.7645	4.63	100.65	0.5751
	SRT	1.5	12.15	3.78	99.166	01.3375	2.38	99.16	0.2422

Table 3: Statistical Validation Data for Intra-day Precision.

Components	Method A			Method B		
	PAC	TMD	SRT	PAC	TMD	SRT
Mean	99.81	99.83	99.23	99.87	99.33	99.14
Standard Deviation	0.0779	0.5477	0.6716	0.1424	0.5962	0.8521
Relative Standard Deviation	0.6780	0.5483	0.6768	0.1426	0.6002	0.8594
Standard Error	0.0313	0.2236	0.2740	0.0581	0.2434	0.3478

Table 4: Statistical Validation Data for Inter-day Precision.

Components	Method A			Method B		
	PAC	TMD	SRT	PAC	TMD	SRT
Mean	100.21	99.97	99.8	99.92	99.80	99.14
Standard Deviation	0.0918	1.1107	0.9373	0.1148	1.2611	0.9692
Relative Standard Deviation	0.09169	1.1110	0.9392	0.1149	1.2635	0.9170
Standard Error	0.0375	0.4534	0.3826	0.0468	0.5148	0.3711

CONCULSION:

The developed first order derivative and second order derivative methods were found to be simple, precise, specific and accurate. Hence the above methods can be used for simultaneous estimation of Paracetamol, Tramadol and Serratiopeptidase. Both the methods were validated as per ICH guidelines.

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