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Estimation of Ramipril and Hydrochlorothiazide by First Order Derivative Method Using UV Spectrophotometer

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ABSTRACT

A new, simple, rapid and novel spectrophotometric method has been developed for estimation of Ramipril (RAM) and Hydrochlorothiazide (HCT) in bulk and combined pharmaceutical formulations using First order derivative method. It is a useful means of resolving the spectra and eliminating the interference. It involves conversion of normal spectrum to first and second or higher order spectra where the amplitude in the derivative spectra is proportional to the Conc. of analyte provided the Beer's law is obeyed.The successive development of zero crossing technique was accordingly due to the feature of derivative spectra to show singles with both positive and negative value. This technique exploits the single crossing through the abscissa axis, for given components. Zero crossing technique is particularly effective in the analysis of several complex mixtures, when peaks overlapping are present in the corresponding zero order spectra. λmax of RAM was found at 341nm and HCT at 229nm in ethanol respectively. Beer's law obeyed in concentration range of 0.1- 0.5 µg/ mL for RAM and 0.25- 1.25 µg/ mL for HCT respectively by the method. This method was validated for precision, reproducibility, linearity and accuracy as per ICH guidelines. The proposed method is recommended for routine analysis since this is rapid, simple, accurate, cost effective , also sensitive and specific. It involves neither heating nor use of any hazardous organic solvent for separation of the combination.

Keywords: Ramipril(RAM),Hydrochlorothiazide(HCT),First order Derivative method & UV spectrophotometer.

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INTRODUCTION

Ramipril,2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3-3-0]octane carboxylic acid (Figure 1), is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the rennin-angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin-I to the highly potent vasoconstrictor, angiotensin-II, and also reduces the degradation of bradykinin[1].

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-Sulphonamide 1, 1-dioxide (Figure.2), is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water[1]. Literature survey reveals few analytical methods for the determination of Ramipril in pharmaceutical preparations and biological fluids, viz.radioimmunoassay[2], spectrophotometry[3,4], potentiometry[5,6] GC,[7,8] and HPLC[9,10] and LCMS[11]. UV Spectroscopy[12-15], Ratio Spectra Derivative Spectrophotometry[16], HPLC[16, 17, 18] and HPTLC[19] methods are reported for simultaneous estimation of Hydrochlothiazide in combined dosage form.

Our objective of the study was to develop and validate a new UV spectrophotometric method for the routine estimation of Ramipril and Hydrochlorthizide in bulk and combined dosage form by using First order Derivative method.

MATERIALS AND METHODS:

Apparatus

The instrument used for the present study was a UV- VIS double beam spectrophotometer (model UV-1800, Shimadzu Limited) with 1cm matched pair quartz cell and Electronic weighing balance. Calibrated glass wares were used throughout the work.

Reagents and Materials:

The drug, RAM was procured as a gift sample from Lupin Lab., Goa and HCT from Matrix Laboratories Ltd., Hyderabad. The solvent system used for the entire analysis was Ethanol of AR grade, purchased from Merck Chemicals Limited, India and double distilled water was prepared in our laboratory.

Method Development:

Solubility Test: Solubility test for the drug RAM and HCT was performed by using various solvents. The solvents include Water, Methanol, Ethanol, 0.1 N Hydrochloric Acid, 0.1 N Sodium Hydroxide, Ethanol: Water (1:1) and Ethanol: Water (3:7). However, Ethanol was chosen as a solvent for developing the above method.

Determination of λ_{max}:

Preparation of Stock Solution:

Standard stock solution of RAM was prepared by dissolving 10mg of RAM in 10ml of ethanol to produce a concentration of 1000µg/ml. 1ml of this solution was taken and then diluted up to 10ml by using ethanol to produce a concentration of 100µg/ml. Again 1ml of this solution was taken and then diluted up to 10ml by using ethanol to produce a concentration of 10µg/ml. Further,1ml of this solution was taken and then diluted up to 10ml by using ethanol to produce a concentration of 1µg/ml. which is the standard stock solution.

Again, Standard stock solution of Hydrochlorothiazide was prepared by dissolving 10mg of Hydrochlorothiazide in 10ml of ethanol to produce a concentration of 1000µg/ml. 1ml of this solution was taken and then diluted up to 10ml by using ethanol to produce a concentration of 100µg/ml. Again, 1ml of this solution was taken and then diluted up to 10ml by using ethanol to produce a concentration of 10µg/ml. Further, 1ml of this stock solution was taken and then diluted up to 10ml by using ethanol to produce a concentration of 1µg/ml which is the standard stock solution.

Preparation of Working Standard Solution:

From the above stock solution of RAM, 2ml was pipetted into a 10ml volumetric flask and the volume was made up to the mark with ethanol to prepare a concentration of 0.2µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using ethanol as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 341nm(figure.1).

In case of hydrochlorothiazide, from the above stock solution of HCT, 5ml was pipetted into a 10ml volumetric flask and the volume was made up to the mark with ethanol to prepare a concentration of 0.5 µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using ethanol as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 229nm(figure.2).

Preparation of Calibration Curve of RAM:

1ml of the 1µg/ml solution was diluted to 10ml by using ethanol to produce 0.1µg/ml solution. 2ml, 3ml, 4ml and 5ml of 1µg/ml solution were diluted to 10ml using ethanol to produce 0.2µg/ml, 0.3µg/ml, 0.4µg/ml and 0.5µg/ml solutions respectively. Then the calibration curve was constructed by taking the above prepared solutions of different concentration ranging from 0.1 - 0.5µg/ml(fig.3,5). The curve showed linearity in the concentration range of 0.1 – 0.5µg/ml. The correlation coefficient (r^2) was found to be 0.993(table 1,3).

Preparation of Calibration Curve of Hydrochlorothiazide:

2.5ml of the 1µg/ml solution was diluted to 10ml by using ethanol to produce 0.25µg/ml solution. 5ml and 7.5ml of 1µg/ml solution were diluted to10ml using ethanol to produce 0.5µg/ml and 0.75µg/ml solutions respectively. For making the 1.25 µg/ml solution, 5ml of the 10 µg/ml solution was diluted to 10 ml using ethanol to produce 5µg/ml and from this again 5ml is diluted to 10 ml ethanol to produce 2.5µg/ml. Again 5m of the 2.5 µg/ml solution was diluted to 10 ml by using ethanol to produce 1.25µg/ml. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 0.25 - 1.25µg/ml. The curve showed linearity in the concentration range of 0.25 – 1.25µg/ml(figure .4,6).The correlation coefficient (r^2) was found to be 0.998(table 1,3).

Method Validation:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for RAM for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ)[22].

Linearity:

Various aliquots were prepared form the stock solution of RAM (1µg/ml) ranging from 0.1 – 0.5 µg/ml and HCT (10µg/ml) ranging from 0.25 - 1.25µg/ml. The samples were scanned in UV-VIS Spectrophotometer using ethanol as blank. It was found that the selected drug shows linearity between the 0.1-0.5µg/ml in case of RAM and between the 0.25 – 1.25 µg/ml for HCT (table 1,2).

Accuracy:

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation (RAMCOR- H5) was kept constant (10mg) and the amount of pure drug was varied i.e., 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table 4).

Precision:

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 0.2µg/ml were prepared in case of RAM and 9 different solutions of the same concentration that is 0.5 µg/ml were prepared

in case of HCT and analyzed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (table 5,6). In the interday variation study, solutions of same concentration 20µg/ml were prepared and analyzed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table 7).

Specificity:

10mg of both RAM and HCT were spiked with 50% (5mg), 100% (10mg), and 150% (15mg) of excipient mix and the samples were analyzed for % recovery of RAM & HCT (table 8).

Robustness:

Robustness of the method was determined by carrying out the analysis at two different temperatures i.e. at room temperature and at 18^0c. The respective absorbances were noted and the result was indicated by % RSD (table 9).

Ruggedness:

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD(table 9).

Limit of Detection (LOD):

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value (table 1).

Limit of Quantification:

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula:

LOQ = 3.3*LOD (table 1).

METHOD USED FOR THE ESTIMATION:

1st order Derivative Method (Derivative ZERO crossing spectroscopy method):

It is a useful means of resolving the spectra and eliminating the interference. It involves conversion of normal spectrum to first and second or higher order spectra where the amplitude in the derivative spectra is proportional to the Conc. of analyte provided the Beer's law obeyed.

The successive development of zero crossing technique was accordingly due to the feature of derivative spectra to show singles with both positive and negative value. This technique exploits the single crossing through the abscissa axis, for give components. Zero crossing technique is particularly effective in the analysis of several complex mixtures, when peaks overlapping are present in the corresponding zero order spectra.

However, suitable analytical signals are often placed on the peak shoulders or characterized by a too low stability of such signals is well known.

In the mixture of HCT & RAM, The 1st order derivative of RAM have peak at 341nm where the drug HCT have the value zero and in 229nm HCT have the peak where RAM show the value 0(fig7).

Assay of RAM and Hydrochlorothiazide tablet (RAMCOR H5):

A quantity of powder equivalent to 5mg of RAM and 12.5mg of hydrochlorothiazide was taken in a 10ml volumetric flask and it was dissolved and diluted up to the mark with ethanol. The resultant solution was ultrasonicated for 15 minutes. The solution was then filtered using Whatmann filter paper No.40. From the filtrate, appropriate dilutions were made in ethanol to obtain the desired Conc. (0.2µg/ml). This solution was then analyzed in UV and the result was indicated by % recovery given in table 1.

RESULTS AND DISCUSSION:

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries of the drug were obtained at each added Conc., indicating that the method was accurate.

Table 1: Summary of Validation

Parameter	Result	
	RAM	HCT
Linearity indicated by correlation coefficient	0.993	0.998
Precision indicated by %RSD	1.5%	0.37%
Accuracy indicated by % recovery	99.92%	99.93%
Specificity indicated by % recovery	99.53%	99.65%
Limit of Detection	0.05µg/ml	0.05 µg/ml
Limit of Quantification	0.165µg/ml	0.165 µg/ml
Range	0.1-0.5µg/ml	0.25-1.25 µg/ml
Linear regression equation	y =0.000x+.00002	y=0.019x+0.001
Robustness indicated by %RSD	0.16%	0.42%
Assay indicated by % recovery	99.95%	99.97%

The method was also found to be specific indicated by the % recoveries. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of Assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (99.95 & 99.97). Summary of validation parameters of proposed spectrophotometric method is shown in table 1.

VALIDATION:

Linearity:

Table 2: Linearity of RAM & HCT in Working Standard

Conc.(µg/ml) (RAM)	Absorbance (RAM)	Conc.(µg/ml) (HCT)	Absorbance (HCT)
0.1	0.25	0.00011	-0.0062
0.2	0.50	0.0002	-0.0106
0.3	0.75	0.00028	-0.0163
0.4	1.00	0.00036	-0.0212
0.5	1.25	0.00048	-0.257

Table 3: Optical characteristics

Parameters	RAM	HCT
Beer's Law limit (µg/mL)	0.1-0.5	0.25-1.25
Correlation coefficient	0.993	0.998
Regression equation (Y*) Equation	Y=0.000x+.00002	Y=0.019x+0.001
Slope (a)	0.000	0.019
Intercept (b)	0.00002	0.001

Accuracy:

Table 4: Accuracy readings of RAM & HCT

Observation / Results												
No.of preparations	Conc. (µg/lml) Formulation		Pure Drug		% Recovery		Statistical Results Mean		SD		%RSD	
Drug	RAM	HCT	RAM	HCT	RAM	HCT	RAM	HCT	RAM	HCT	RAM	HCT
S_1 : 80 %	5	12.5	4	10	100.1	100.2	99.92	99.93	0.27	0.23	0.27%	0.23%
S_2 : 80 %	5	12.5	4	10	100.06	99.8						
S_3 : 80 %	5	12.5	4	10	99.6	99.8						
S_4 : 100 %	5	12.5	5	12.5	99.7	99.9	99.93	100.06	0.32	0.15	0.32%	0.15%
S_5 : 100 %	5	12.5	5	12.5	99.8	100.2						
S_6 : 100 %	5	12.5	5	12.5	100.3	100.1						
S_7 : 120 %	5	12.5	6	15	100.2	100.3	100	99.93	0.43	0.32	0.43%	0.32%
S_8 : 120 %	5	12.5	6	15	100.3	99.7						
S_9 : 120 %	5	12.5	6	15	99.5	99.8						

Precision:

Table 5: Precision results showing Repeatability of RAM & HCT

Conc. (µg/ml)		Absorbance		Statistical analysis	
RAM	HCT	RAM	HCT	RAM	HCT

0.2	0.5	0.0002	0.0108		
0.2	0.5	0.00018	0.0106		
0.2	0.5	0.00021	0.0105		
0.2	0.5	0.0002	0.0107	Mean =0.000203	Mean =0.01064
0.2	0.5	0.00022	0.0106	SD = 0.000003	SD = 0.00004
0.2	0.5	0.00023	0.0108	%RSD =1.5%	%RSD =0.37%
0.2	0.5	0.0002	0.0105		
0.2	0.5	0.00021	0.0106		
0.2	0.5	0.0002	0.0107		
0.2	0.5	0.00018	0.0106		

Table.6: Intra-assay Precision

Conc. (µg/ml)		**Abs 1**		**Abs 2**		**Abs 3**		**Avg %RSD**	
RAM	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**
0.2	0.5	0.00017	0.0106	0.00021	0.0108	0.00021	0.0101		
0.2	0.5	0.00019	0.0106	0.00019	0.0108	0.00021	0.0101		
0.2	0.5	0.00017	0.0106	0.00021	0.0108	0.00021	0.0101		
0.2	0.5	0.0002	0.0106	0.00021	0.0108	0.00018	0.0103		
0.2	0.5	0.0002	0.0108	0.00018	0.0105	0.00018	0.0109		
0.2	0.5	0.00022	0.0108	0.00017	0.0105	0.0002	0.0109		
0.2	0.5	0.00023	0.0108	0.00022	0.0103	0.0002	0.0109		
0.2	0.5	0.0002	0.0108	0.0002	0.0104	0.00021	0.0108		
0.2	0.5	0.0002	0.0106	0.00022	0.0103	0.00018	0.0108		
%RSD		1.5%	0.75%	0.5%	0.28%	1.5%	0.56%	1.16%	0.53%

Table. 7:Inter assay precision

Conc.		**Day 1**		**Day 2**		**Day 3**		**% RSD**	
RAM	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**
0.2	0.5	0.00021	0.0107	0.00021	0.0107	0.00019	0.0105	1.65%	0.31%

Table 8: Test for Specificity showing no effect of excipient.

Sl. No.	**Excipient Conc.%**	**Input (mg)**		**Recovered (mg)**		**Recovered (%)**		**Mean Recovered (%)**		**S.D.**		**% R.S.D.**	
		RAM	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**
1	100%	5	12.5	4.9	12.55	98%	100.4%	99.53%	99.65%	1.33	0.65	1.33%	0.65%
2	50%	5	12.5	5.01	12.42	100.2%	99.36%						
3	150%	5	12.5	5.02	12.4	100.4%	99.2%						

Table . 9: Ruggedness of method for Ramipril & Hydrochorthiazide

Room Temp.						**Temp. 18^{0}C**			
Conc. (µg/ml)		**Abs.**		**Statistical Analysis (%RSD)**		**Abs.**		**Statistical Analysis(%RSD)**	
RAM	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**	**RAM**	**HCT**
0.2	0.5	0.00021	0.0108			0.00021	0.0104	0.16%	0.63%
0.2	0.5	0.0002	0.0105	0.16%	0.31%	0.00019	0.0104		
0.2	0.5	0.00018	0.0106			0.00019	0.0108		

Limit of Detection (LOD)

The LOD for RAM & HCT was found to be 0.05µg/ml.

Limit of Quantification (LOQ):

The LOQ for RAM & HCT was found to be 0.165µg/ml.

The unit of Conc. in each calibration curve is µg/ml.

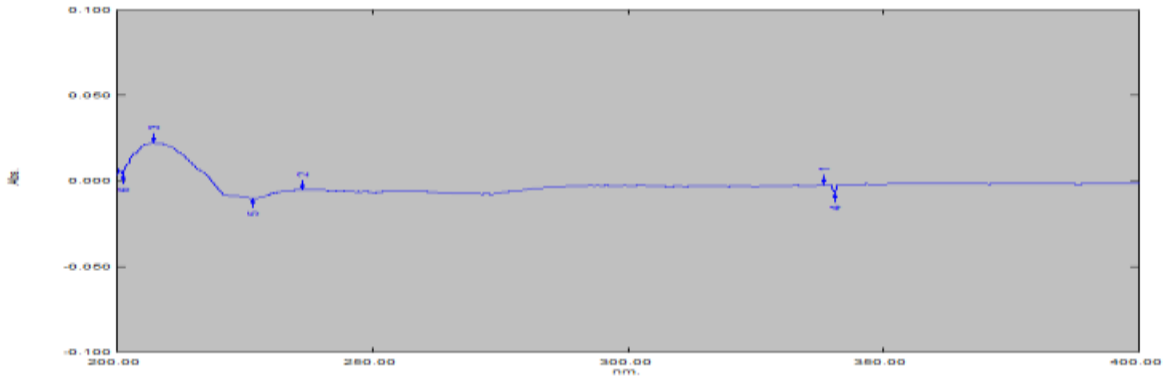

Figure .1 Determination of λ_{max} of RAM:

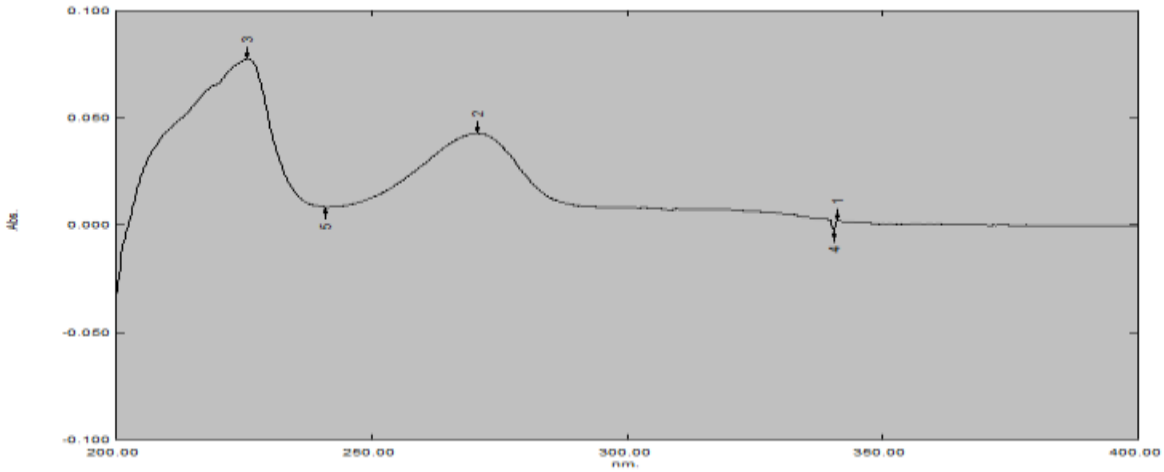

Figure .2 Determination of λ_{max} Hydrochlorothiazide:

Preparation of Calibration Curve:

Conc.	Abs.
0.1	0.00011
0.2	0.0002
0.3	0.00028
0.4	0.00036
0.5	0.00048

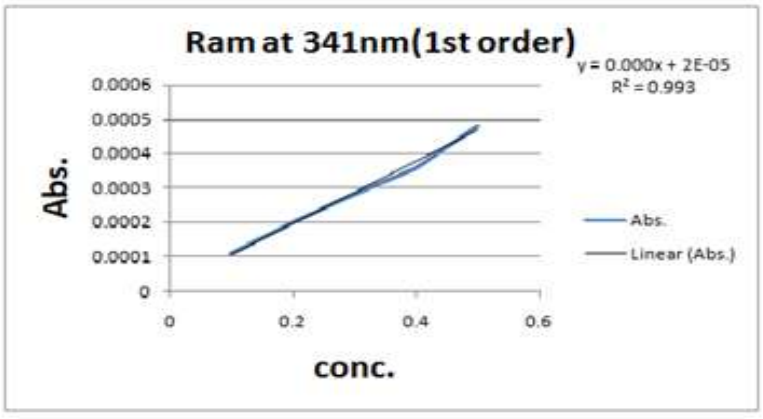

Figure 3 Calibration Curve of RAM at 1st order derivative 341nm

Conc.	Abs
0.25	-0.0062
0.5	-0.0106
0.75	-0.0163
1	-0.0212
1.25	-0.0257

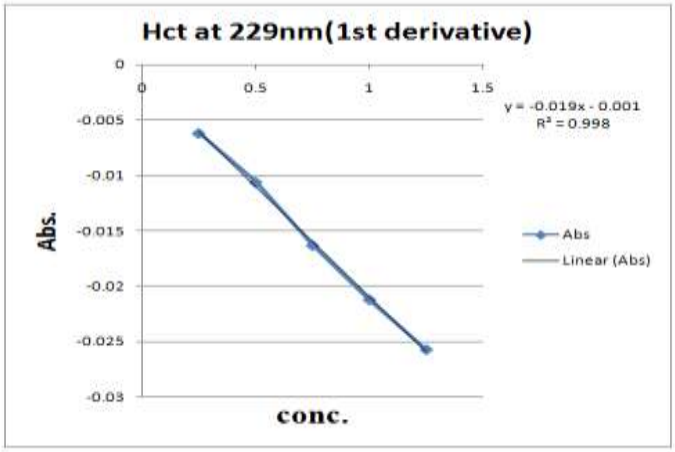

Figure 4 Calibration Curve of Hydrochlorothiazide at 1st order derivative

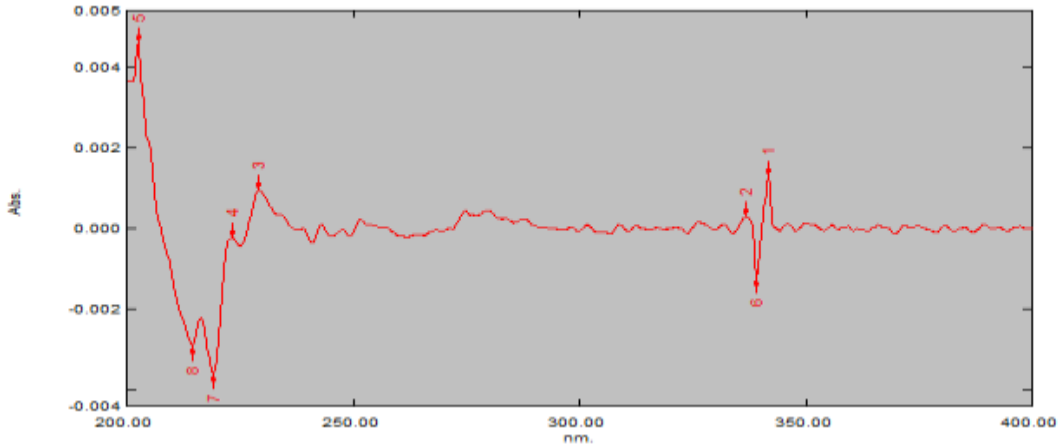

Figure . 5 Graph of Ram 0.2 µg/ml

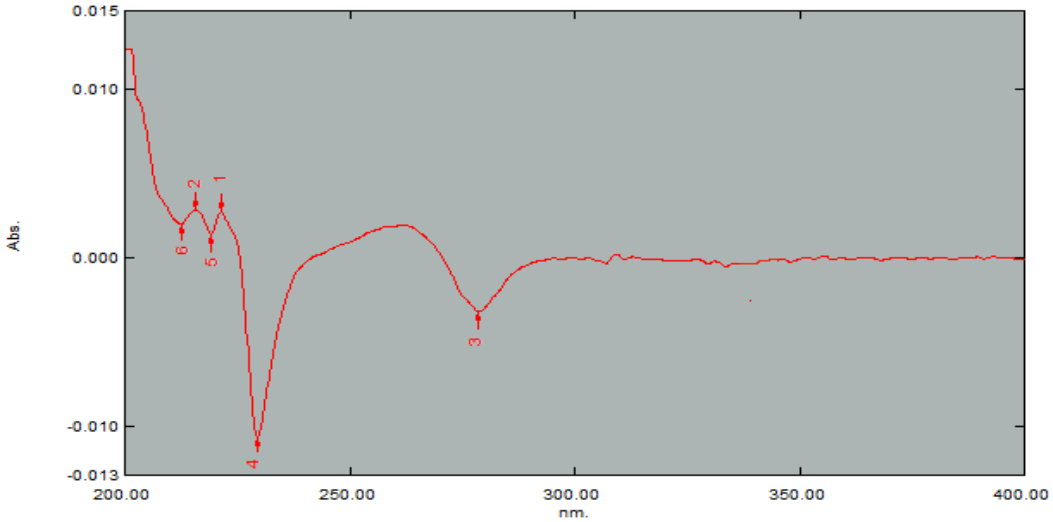

Figure .6 Graph of Hydrochlorthiazide 0.5 µg/ml

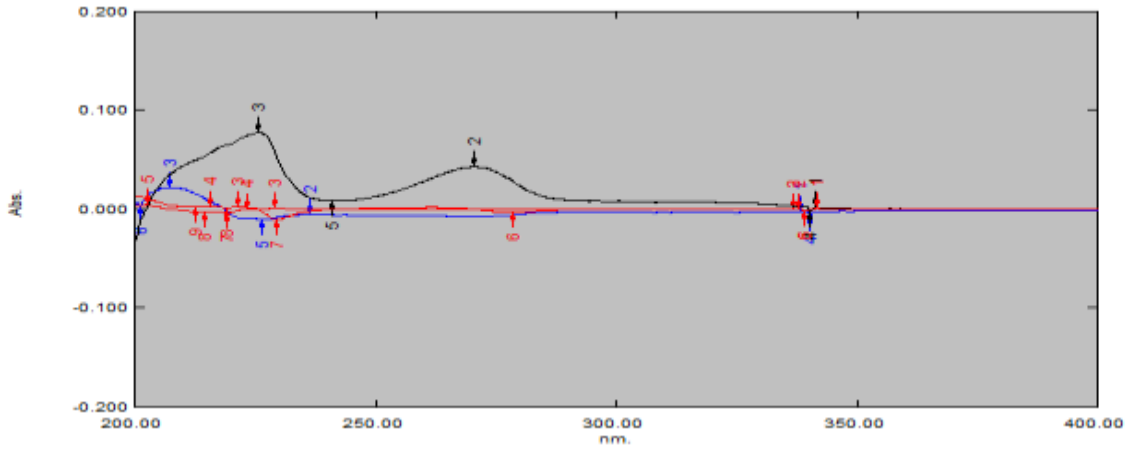

Figure .7 GRAPH OF RAM (0.2 µg/ml) & HYDROCHLORTHIAZIDE (0.5 µg/ml) OVERLAY(1ST ORDER & ZERO ORDER)

Table 10

S.No	Parameter	Acceptable limit	Remark
1.	Accuracy	% recovery 100±2%	Passed
2.	Specificity	% recovery 100±2%	Passed
3.	Precision	RSD<2	Passed
4.	Linearity	R^2: NLT 0.98	Passed
5.	Robustness	RSD<2	Passed
6.	Assay	%recovery 100±2%	Passed

CONCLUSION:

It involves neither heating nor use of any hazardous organic solvent for separation of the combination. This method was validated for precision, reproducibility, linearity and accuracy as per ICH guidelines. All the above parameters lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the routine estimation of Ramipril and Hydrochlorthiazide in bulk and pharmaceutical formulation.

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