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## Spectrophotometric and HPLC methods for simultaneous estimation of ramipril and hydrochlorothiazide from combined dosage forms

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### ABSTRACT

Two simple, accurate, economical and reproducible UV spectrophotometric and one HPLC method for simultaneous estimation of two-component drug mixture of ramipril and hydrochlorothiazide in combined tablet dosage form have been developed. The first developed method employs simultaneous equation method using mixed standards and 216.5nm and 273.0nm as wavelength maxima for estimation. The second method involves first derivative spectroscopy using 221.5nm and 283.0nm as zero crossing points for hydrochlorothiazide and ramipril respectively. For both spectrophotometric methods, 0.1N sodium hydroxide was used as solvent. Developed HPLC method is reverse-phase chromatographic method using C<sub>18</sub> column and methanol: water in ratio of 90:10 as mobile phase, at a flow rate 1.3ml/min. The separation was monitored by UV detection at 214nm. Paracetamol was used as internal standard for HPLC method. Linearity was observed in concentration range of 2-14µg/ml of ramipril and hydrochlorothiazide. Results of analysis were validated statistically and by recovery studies. © 2007 Trade Science Inc. - INDIA

### KEYWORDS

Ramipril;  
HPLC;  
UV.

### INTRODUCTION

Ramipril is a long acting angiotensin converting enzyme inhibitor used as an anti hypertensive drug, chemically it is (2S,3aS,6aS)-1-[(2S)-2-[[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl] octahydrocyclopenta [b] pyrrole-2-carboxylic acid. Official methods for the quantitative estimation of ramipril is, UV-spectrophotometric<sup>[1]</sup>, HPLC<sup>[2-3]</sup> and capillary electrophoresis<sup>[4]</sup> has been reported. Hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide. It is used as a diuretic official in U.S.P. and I.P. official methods for the estimation of hydrochlorothiazide is UV-spectrophotometric<sup>[5-7]</sup>, HPLC<sup>[8-10]</sup> and cap-

illary electrophoresis<sup>[11-12]</sup> has been reported. With the advancement in the field of analytical chemistry and software technology different methods have been developed for simultaneous estimation of mixtures of drugs. There are number of tablet formulations in the market, which contains these drugs (ramipril 2.5mg and hydrochlorothiazide 12.5mg). The objective of this investigation was to develop simultaneous spectrophotometric and HPLC methods for analysis of ramipril and hydrochlorothiazide from combined tablet dosage form. The developed methods were found to be simple, rapid, accurate, reproducible and economical. These methods can be used successfully for quality control testing of the drugs from combined tablet dosage form.

## MATERIALS AND METHODS

Standard drugs sample of ramipril from Cipla Ltd., Mumbai and hydrochlorothiazide from Ontop pharm. ltd. New Delhi was provided. Tablets of combined dosage form were procured from the local market. All other reagents used were of analytical grade for spectrophotometric methods and of HPLC grade for HPLC method. Shimadzu UV/VIS spectrophotometer 1700 pharماسpec with 1cm matched quartz cells was used for spectrophotometric methods. A gradient high-pressure liquid chromatograph (Shimadzu HPLC Class VP series) with LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10AVP and C18 LUNA(5micron 25cm×4.6mm) column from Pheno menex was used for separation and quantification.

### Standard solutions

Ramipril and hydrochlorothiazide(1mg/ml) were prepared in 0.1N NaOH for spectrophotometric method and in mobile phase for HPLC method. The standard solution of both the drugs was subsequently used to prepare working standard solution for spectrophotometric method in 0.1N NaOH and for HPLC method in the mobile phase. All solutions were kept in a refrigerator at 4°C and were stable for one week.

### Chromatographic conditions

For HPLC method, the mobile phase was methanol: water in ratio of 90:10. It was filtered by using a 0.45µm membrane filter and degassed in an ultrasonic bath before use. The samples were also filtered by using 0.45µm membrane filters. The flow rate was set at 1.3ml/min and UV detector at 214nm. The column was conditioned for 30min. All determinations were performed at ambient temperature 27±2°C and the injection volume was 20µl.

### Procedures

#### Calibration for spectrophotometric method

Aliquots of standard solution equivalent to(4-16µg/ml) ramipril for simultaneous equation method and derivative spectrophotometric method, similarly aliquots of standard solution equivalent to(8-26µg/ml) hydrochlorothiazide were transferred into 10ml volumetric flasks separately. The volume was completed to the

mark with 0.1N NaOH. Absorbance of the aliquots was measured at 216.5nm and 273nm of both the drugs at both the wavelength for simultaneous equation method and for derivative spectrophotometric method 221.5nm and 283nm. The calibration curve was plotted and the regression equation was recorded.

#### Calibration for HPLC method

Aliquots of standard solution(1mg/ml) equivalent to(2-14µg/ml) of ramipril and hydrochlorothiazide and 10µg/ml of paracetamol as internal standard were transferred into 10ml volumetric flasks and the volume was completed to the mark with the mobile phase. Triplicate 20µl injections were made of each concentration. Mean retention time for ramipril was found to be 5.267min, for hydrochlorothiazide 4.413min, and for paracetamol 5.972min. The average peak areas were calculated and plotted versus concentrations, linear relationship was obtained and the regression equation was recorded.

## METHOD DEVELOPMENT

### Method 1-Spectrophotometric method

#### Based on simultaneous equation method

Overlain spectra(Figure 1) of standard solutions of ramipril and hydrochlorothiazide were scanned. Ramipril shows absorption maxima at 216.5nm and hydrochlorothiazide shows at 273nm. The calibration

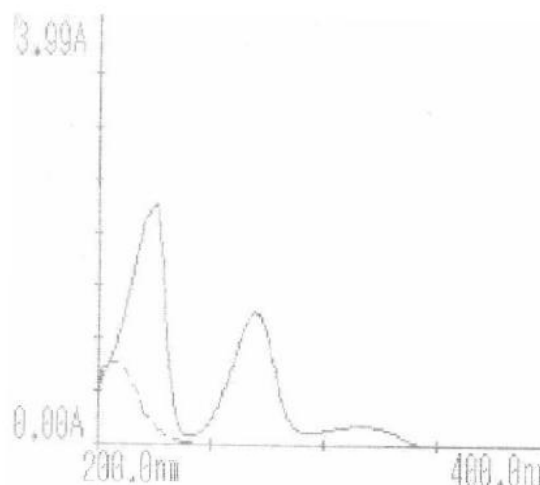


Figure 1 : Overlain spectra of ramipril and hydrochlorothiazide (-----)ramipril, (-) hydrochlorothiazide

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curves for ramipril and hydrochlorothiazide were prepared in the concentration range of 4-16 $\mu$ g/ml and 8-26 $\mu$ g/ml for both the drugs respectively. The absorptivity coefficients were determined for both the drugs at both the wavelengths and following equations were made.

$$A_1 = 360.90C_{\text{rami}} + 756.47C_{\text{hydro}} \dots\dots \text{(at } \lambda_{216.5}) \quad (1)$$

$$A_2 = 488.16C_{\text{hydro}} \dots\dots \text{(at } \lambda_{273}) \quad (2)$$

$A_1$  and  $A_2$  are absorbances at 216.5nm and 273nm respectively and  $C_{\text{rami}}$  and  $C_{\text{hydro}}$  are concentrations of ramipril and hydrochlorothiazide respectively. The concentrations of both the drugs in the mixture were determined by eqs. (1) and (2)

### Analysis of commercial formulation

Twenty tablets were accurately weighed and average weight per tablet determined. Tablets were grounded to fine powder, and weighed tablet powder equivalent to 2.5mg ramipril and 12.5mg hydrochlorothiazide was transferred to 100ml volumetric flask. The powder was extracted four times with 20ml portion of methanol. The extract was collected in 100ml volumetric flask after filtration through Whatman filter paper no.41. The filter paper was washed with 20ml methanol, and the washing was added to the filtrate. The methanol was evaporated on water bath and the residue was dissolved in 75ml of 0.1N NaOH and again filtered through Whatman filter paper no.41. The filter paper was washed with 20ml of 0.1N NaOH and the washing was added to the filtrate. The volume of filtrate was made to 100ml mark with 0.1N NaOH. In 10ml volumetric flask, 1.0ml of filtrate was taken and diluted to the mark with 0.1N NaOH. The sample solution was scanned over the range of 400nm to 200nm in spectrum mode and concentration of each component was estimated by analysis of spectral data of sample solution with respect to that of mixed standards by the instrument. Results of analysis are reported in (TABLE 3).

### Method 2-First order derivative spectroscopy

From first derivative spectra of ramipril and hydrochlorothiazide in 0.1N NaOH zero crossing points, 221.5nm and 283nm were selected for simultaneous estimation of two drugs (figures 2 and 3). Accurately weighed pure drug sample of ramipril and hydrochlorothiazide were dissolved in 0.1 N NaOH and diluted with same so as to give several dilutions in the range of

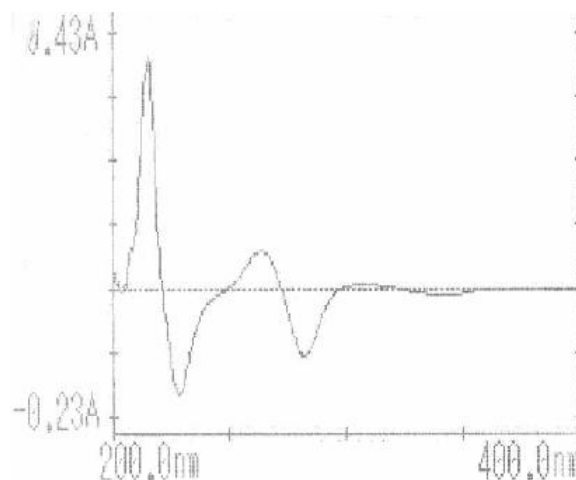


Figure 2 : First order derivative spectra of hydrochlorothiazide

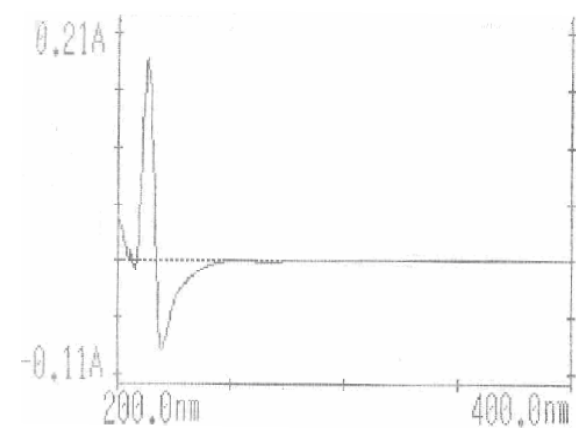


Figure 3 : First order derivative spectra of ramipril

4-16 $\mu$ g/ml of ramipril and 8-26 $\mu$ g/ml of hydrochlorothiazide. The absorbances of these dilutions were recorded in first derivative mode at 221.5nm for estimation of ramipril, and 283nm for estimation of hydrochlorothiazide. Respective calibration curves were prepared. Absorbance was measured of marketed formulations at respective selected zero crossing points, and concentration of two drugs, using respective calibration curve, was determined. Validation studies gave satisfactory results.

### Analysis of commercial formulation

Tablet sample was prepared in similar manner as for method 1. Absorbance of final dilution of sample was recorded at 221.5nm and 283nm from first derivative spectra of sample and amount of two drugs calculated using respective calibration curve. Results of analysis are reported in (TABLE 3).

TABLE 1 : Validation report of spectrophotometric and hplc methods for the determination of ramipril and hydrochlorothiazide

Parameters	Simultaneous equation method		Derivative spectrophotometric method		HPLC method	
	Ramipril	Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril	Hydrochlorothiazide
Linearity range	4-16µg/ml	8-26µg/ml	4-16µg/ml	8-26µg/ml	2-14µg/ml	2-14µg/ml
Regression equation						
Slope	0.0283	0.0513	0.0021	0.0035	23.37	63.515
S.D. of slope	0.0049	0.0069	0.0014	0.0069	4.127	2.085
Intercept	0.0629	0.0444	0.0044	0.0038	5.181	179.46
S.D. of intercept	0.0051	0.0081	0.0032	0.0071	2.061	3.412
Correlation coefficient	0.9967	0.9946	0.9935	0.9985	0.9907	0.9919
S.D. of correlation coeff.	0.0012	0.0022	0.0008	0.0016	0.0038	0.0041
Precision±RSD%						
Intra day <sup>a</sup>	99.69±0.52	99.12±0.38	100.11±0.74	99.92±0.48	99.81±0.65	99.67±0.71
Inter day <sup>a</sup>	99.87±0.98	99.57±0.54	99.52±1.23	99.87±0.52	99.37±0.12	100.06±0.32
Accuracy (mean) <sup>b</sup> ±S.E.	99.91±1.23	99.89±0.99	99.77±0.94	99.74±0.44	99.86±0.54	99.39±0.41

(a) Average of n=9. (b) Average of n=6

### Method 3-High-performance liquid chromatographic method

The developed HPLC method was applied to the determination of ramipril and hydrochlorothiazide in combined dosage form. To optimize HPLC assay parameters, the mobile phase composition and pH were studied. A satisfactory separation was obtained with a mobile phase of methanol: water in ratio of 90:10 using C<sub>18</sub> column at ambient temperature. The analysis was carried out by isocratic elution with flow rate 1.3ml/min<sup>-1</sup> and detection at 214nm (Figure 4). A linear range of 2-14µg/ml of both the drugs was obtained as show in TABLE 1. The system suitability tests of HPLC method were evaluated TABLE 2.

### Procedure for analysis of formulations

Twenty tablets of the formulation were weighed and the average weight per tablet was calculated. Twenty tablets were crushed and ground to a fine powder. Pow-

TABLE 2 : Results of system suitability tests of HPLC method

Parameters	Value		Comments
	Ramipril	Hydrochlorothiazide	
Retention time	5.267±0.029	4.413±0.038	±Standard deviation
Injection repeatability	0.71%	0.38%	RSD of 6 injections
K	1.743	1.298	Capacity factor
Selectivity factor	0.744	0.744	Selectivity factor
Theoretical plate (N)	2656	4030	Column efficiency plate/column
HETP	0.0094	0.0062	Height equivalent theoretical plate

der equivalent to 2.5mg of ramipril and 12.5mg hydrochlorothiazide was weighed and transferred to a 100ml volumetric flask containing about 75ml mobile phase. To this volumetric flask, accurately weighed 10mg of pure standard paracetamol was added. The powder mixture was dissolved in the mobile phase with the aid of ultrasonication. The solution was filtered through

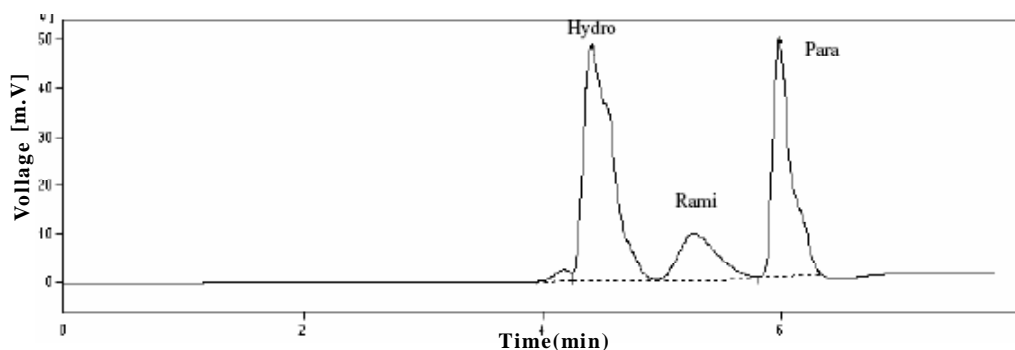


Figure 4 : HPLC chromatogram of ramipril and hydrochlorothiazide



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**TABLE 3 : Determination of ramipril and hydrochlorothiazide in marketed formulations**

Sample no.	Simultaneous equation method		Derivative spectrophotometric method		HPLC method	
	rami	hydro	rami	hydro	rami	hydro
1	100.0	100.36	100.0	100.0	100.29	99.94
2	103.2	99.96	98.0	100.2	99.98	100.91
3	97.0	100.44	99.0	99.64	101.08	99.52
4	100.0	100.36	98.0	100.0	99.12	100.09
5	104.6	100.04	100.8	99.88	99.26	99.68
6	101.6	100.12	98.0	100.0	99.54	100.08
Mean	101.067	100.21	98.96	99.95	99.87	100.03
RSD	2.659	0.198	1.202	0.184	0.421	0.289
S.D.	2.688	0.287	1.215	0.287	0.367	0.309

rami-Ramipril, hydro-Hydrochlorothiazide

**TABLE 4 : Compilation of results of drug recovery study**

Sample no.	% Recovery*					
	Simultaneous equation method		Derivative spectrophotometric method		HPLC method	
	rami	hydro	rami	hydro	rami	hydro
1	99.73	99.94	99.68	100.0	99.84	99.47
2	98.0	100.05	97.77	100.0	99.97	100.89
3	100.13	99.94	100.31	99.77	100.26	99.99
4	100.53	99.84	99.68	99.70	99.59	100.03
5	98.0	100.05	100.31	99.77	101.36	99.41
6	99.73	99.94	99.68	100.07	99.12	99.63
Mean	99.35	99.96	99.57	99.88	100.02	99.90
RSD	1.096	0.079	0.939	0.155	0.084	0.068
S.D.	1.089	0.039	0.621	0.069	0.037	0.149

\*Average of six determinants, rami-Ramipril, hydro-Hydrochlorothiazide

Whatman filter paper no. 41 into another 100ml volumetric flask. The filter paper was washed with mobile phase and washings were added to the filtrate. Volume of filtrate was made up to the mark with the mobile phase. To another 10 ml volumetric flask, 1.0ml of this solution was transferred and the volume was made up to the mark with the mobile phase. This solution was filtered through a 0.2 $\mu$  membrane filter.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 $\mu$ l fixed sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated five times and the peak areas were recorded. A representative chromatogram has been given in (Figure 4). The peak area ratios of each of the drugs to the internal standard were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves. The results of

analysis are presented in TABLE 3.

## RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of ramipril and hydrochlorothiazide in combined tablet dosage form were found to be simple, accurate, rapid and economical. The values of coefficient of variance were satisfactorily low and recovery was close to 100%, indicating reproducibility of the methods. First method involving simultaneous equation method is specific to instrument having software for provision of such determination. Selection of proper sampling wavelengths and concentration of components in mixed standard is critical. Since calculations are done by the instrument itself, chances of manual error are nil; furthermore, the method is quite rapid.

Second method involving first order derivative ultraviolet spectroscopy requires recording spectrophotometer with data processing mode. Proper selection of zero crossing point in derivative spectra completely eliminates the interference of unwanted component, and thus, concentration of two components can be easily calculated without prior separation of components from combined dosage form.

Third developed method for simultaneous estimation of two drugs from combined dosage form is reverse phase chromatographic method utilizing C<sub>18</sub> column and methanol: water as mobile phase. Detection of eluent was carried out using UV detector. The method was developed using paracetamol as internal standard. The run time per sample is just 8min. The excipients in the formulation did not interfere in the accurate estimation of ramipril and hydrochlorothiazide.

Since none of the methods is reported for simultaneous estimation of ramipril and hydrochlorothiazide from combined dosage form, these developed methods can be used for routine analysis of two components without prior separation.

## METHODS VALIDATION

### Linearity/Range

Aliquots of different dilutions were prepared for the linearity test. Each solution was measured (or injected)

three times and linear regression analysis of ramipril and hydrochlorothiazide was driven (TABLE 1).

### Precision

The precision of the methods were assessed by determining RSD values of intra-day and inter-day analysis (n=9) of ramipril and hydrochlorothiazide standard solutions over 3 days (TABLE 1).

### Accuracy

The calculated t-test and F-test are not exceeding their theoretical values at  $p=0.05$ , indicating that there is no significant difference between each method.

### Standard addition technique

The proposed methods were applied for the analysis of the drug in pharmaceutical dosage form (TABLE 3). The validity of the methods was assessed by applying the standard addition technique at different level (viz; 50%, 100% and 150%). The results (TABLE 4) indicate no interference from tablets excipients such as calcium carbonate, hydroxypropyl cellulose, aluminium magnesium silicate, povidone, sodium starch glycolate, saccharin sodium and magnesium stearate.

## CONCLUSION

The presented work describes validated spectrophotometric and HPLC methods for the assay of ramipril and hydrochlorothiazide in pharmaceutical dosage form and bulk drug. The suggested methods are simple, selective, and accurate can be used for the routine quality control analysis of the cited drug either in bulk or in dosage form without any interference from common excipients. The spectrophotometric method is rapid with low cost for both identification and quantification.

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