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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE, AMLODIPINE BESYLATE AND LOSARTAN POTASSIUM IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

The present study describes a simple, accurate and precise stability indicating RP-HPLC technique for the simultaneous determination of hydrochlorothiazide, amlodipine besylate and losartan potassium in the tablet dosage form. The method involves an isocratic elution of the drug in a stationary phase of phenomenex ODS 2, C18 (150 mm × 4.6 mm, 5 µm) column using a mobile phase composition of methanol and 0.1% (v/v) orthophosphoric acid in the composition ratio of 65:35 v/v with a flow rate of 0.8 mL/min at 254 nm of detection. The injection volume is 20 µL. The method has been validated for specificity, linearity, range, precision, accuracy, limit of detection, limit of quantification and robustness. The retention times for hydrochlorothiazide, amlodipine besylate and losartan potassium are about 2.30, 3.52 and 5.09 min, respectively. Quantitative linearity was observed over the concentration range of 5.12 to 49.60 µg/mL for hydrochlorothiazide, 2.52 to 25.24 µg/mL for amlodipine besylate and 10.02 to 250.44 µg/mL for Losartan potassium, respectively. The regression equations of concentration for hydrochlorothiazide was found to be y = 207189.9x + 176236.2, it is y = 179464.9x + 2658.2 for amlodipine besylate and it is y = 47400x + 34309.7 for Losartan potassium where y is the peak area and x is the concentration of drug (µg/mL). All the validation parameters are within the acceptance range.

Key words: RP-HPLC, Isocratic, Hydrochlorothiazide, Amlodipine besylate, Losartan potassium.

INTRODUCTION

Hydrochlorothiazide is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide (Fig. 1a). Thiazides such as hydrochlorothiazide promote water

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loss from the body (diuretics). They inhibit Na^+/Cl^- reabsorption from the distal convoluted tubules in the kidneys and are used to treat hypertension.

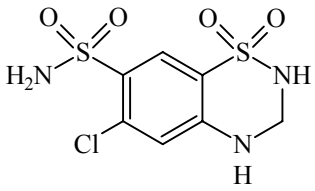

Fig. 1a: Structure of hydrochlorothiazide

Amlodipine besylate is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5dicarboxylate (Fig. 1b). Amlodipine is used to treat hypertension and chronic stable angina. It lowers blood pressure by relaxing the blood vessels so the heart does not have to pump as hard. It controls chest pain by increasing the supply of blood to the heart.

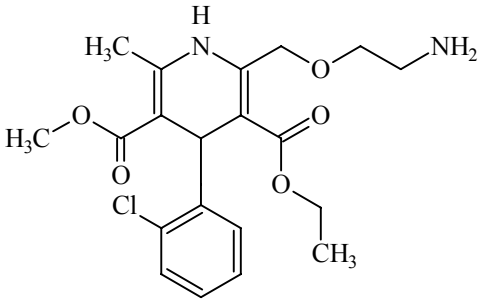

Fig-1b: Structure of Amlodipine besylate

Losartan Potassium chemical name is 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1, 1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, monopotassium salt (Fig. 1c). Losartan is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension. It may also be used as an alternative agent for the treatment of systolic dysfunction, myocardial infarction, coronary artery disease, and heart failure.

Literature survey revealed spectrophotometric methods[1,2] and RP- HPLC method[3,4], for the validation of hydrochlorothiazide in combination with other drugs. Amlodipine besylate in combination with other drugs is reported to be estimated by spectrophotometric method[5-9], and RP-HPLC method[10-13], absorption correction method[14], losartan potassium estimation in combination with other drugs in pharmaceutical dosage forms is reported by RP-HPLC method[15,16]. Simultaneous estimation of hydrochlorothiazide, amlodipine besylate

and Losartan potassium by RP-HPLC using Telmisartan as an internal standard was reported by[17].

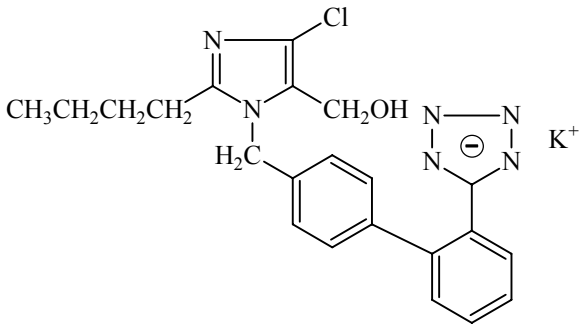

Fig. 1c: Structure of losartan potassium

Losartan potassium, amlodipine besylate and hydrochlorothiazide are available in the combination of 50 + 5 + 12.5 mg, 50 + 2.5 + 12.5 mg.

The present work describes a validated stability indicating reverse phase HPLC method for simultaneous estimation of hydrochlorothiazide, amlodipine besylate and losartan potassium by RP-HPLC technique in tablets. The proposed method is validated as per ICH guidelines[18].

EXPERIMENTAL

Materials and methods

Reagents and chemicals

Orthophosphoric acid (AR grade, SD Fine chem limited), methanol (HPLC grade, Merck limited), Milli–Q water, losartan potassium (99.8% w/w procured from Emcure Pharmaceutical Ltd.), hydrochlorothiazide (99.8% w/w procured from Unichem Laboratories Ltd.) and amlodipine besylate (99.8% w/w procured from Sun Pharmaceutical Industries Ltd). All other chemicals are of the highest grade commercially available unless otherwise specified.

Instrumentation

The chromatographic system consisted of a Shimadzu class VP binary pump LC-10ATvp, SIL-10ADvp auto sampler, CTO-10Avp column temperature oven, SPD-10Avp UV-visible detector. All the components of the system are controlled using SCL-10Avp system controller. Data acquisition was done using LC solutions software.

The mobile phase consisted of 65:35 % (v/v) of Methanol and 0.1% orthophosphoric acid operated on isocratic mode. The flow rate is 0.8 mL/min. Chromatographic determination of hydrochlorothiazide, amlodipine besylate and losartan potassium was performed on phenomenex® C18 column (150 x 4.6 mm, 5 µm) with an injection volume of 20 µL. The wavelength of detection is 254 nm.

Preparation of standard solutions, calibration standards & quality control samples

Stock solutions of hydrochlorothiazide (10 mg/mL), amlodipine besylate (2.0 mg/mL) and losartan potassium (2.73 mg/mL) were prepared separately in a volumetric flask using methanol and labeled accordingly. Suitable dilutions were then prepared using 50:50% v/v methanol & Milli-Q water as diluent solution. For the linear calibration curve, seven non-zero standards were prepared using diluent solution in the concentration range of 5.12 to 49.60 µg/mL for Hydrochlorothiazide, 2.52 to 25.24 µg/mL for amlodipine besylate and 10.02 to 250.44 for losartan potassium. The calibration standard sample is then transferred into the auto sampler for analysis. Samples for specificity (Sample with hydrochlorothiazide alone, sample with amlodipine besylate alone, sample with Losartan potassium alone, Blank sample and sample containing all the three drugs) were also prepared accordingly.

For the preparation of quality control samples, a separate stock containing the concentration of 620 µg/mL for hydrochlorothiazide, 252.37 µg/mL for amlodipine besylate and 2504.38 µg/mL for Losartan potassium were prepared and labeled as quality control stocks. From these stocks, quality control samples containing hydrochlorothiazide, amlodipine besylate and losartan potassium were prepared at three concentration levels namely LQC, MQC, and HQC so as to obtain low, medium and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples.

Assay

The assay of tablets containing hydrochlorothiazide, amlodipine besylate and losartan potassium is done using the procedure given in Indian pharmacopoeia under tablets. The active ingredients in each of 10 dosage units is taken by random sampling and analyzed by the developed method. The tablets are said to be in compliance if the each individual content is 90-110% of the average content or labeled claim.

For the current assay, ten tablets were randomly taken and transferred separately into a 100 mL volumetric flask and dissolved in 20 mL methanol. The solution was then

ultrasonicated for 10 min and then made up to volume. Required amount of the solution is then taken and filtered through a 0.45 µ nylon membrane and diluted with diluent solution so that the resultant concentrations are within the calibration range of the developed method. The samples are then analyzed by using the validated method. The sample is then injected in triplicate.

Method validation

System suitability

A sample containing mixture of hydrochlorothiazide (approximate concentration of 24.80 µg/mL), amlodipine besylate (approximate concentration of 12.62 µg/mL) and losartan potassium (approximate concentration of 125.22 µg/mL) was used as system suitability sample. System suitability was assessed by six replicate analysis. A per cent coefficient of variation (% CV) less than 1% for retention times for the drugs is taken as the acceptance criterion.

Detection and quantification limits (Sensitivity)

Limits of detection (LOD) and limit of quantification (LOQ) (Fig. 2) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, with precision (%CV) and accuracy with (±) 20%.

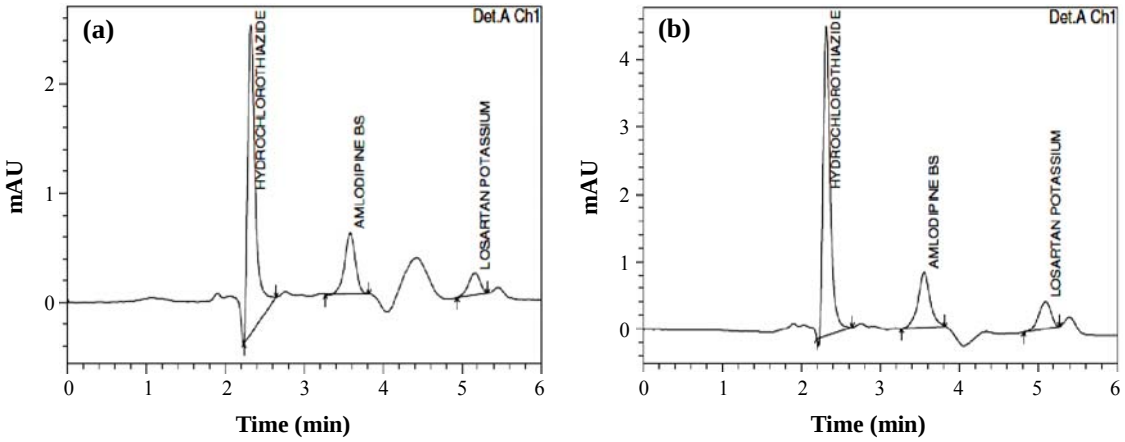

Fig. 2: Chromatogram for (a) LOD Sample and (b) LOQ Sample

Linearity (Calibration curve)

The Linearity of detector response to different concentrations of all the three drugs was studied with a series of working standard solutions prepared by diluting the stock solution. The standard plots were then constructed between concentration Vs. Peak area using six non-zero standards ranging from 5.12 to 49.60 µg/mL for hydrochlorothiazide, 2.52 to 25.24 µg/mL for Amlodipine besylate and 10.02 to 250.44 µg/mL for losartan potassium. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (Fig. 3).

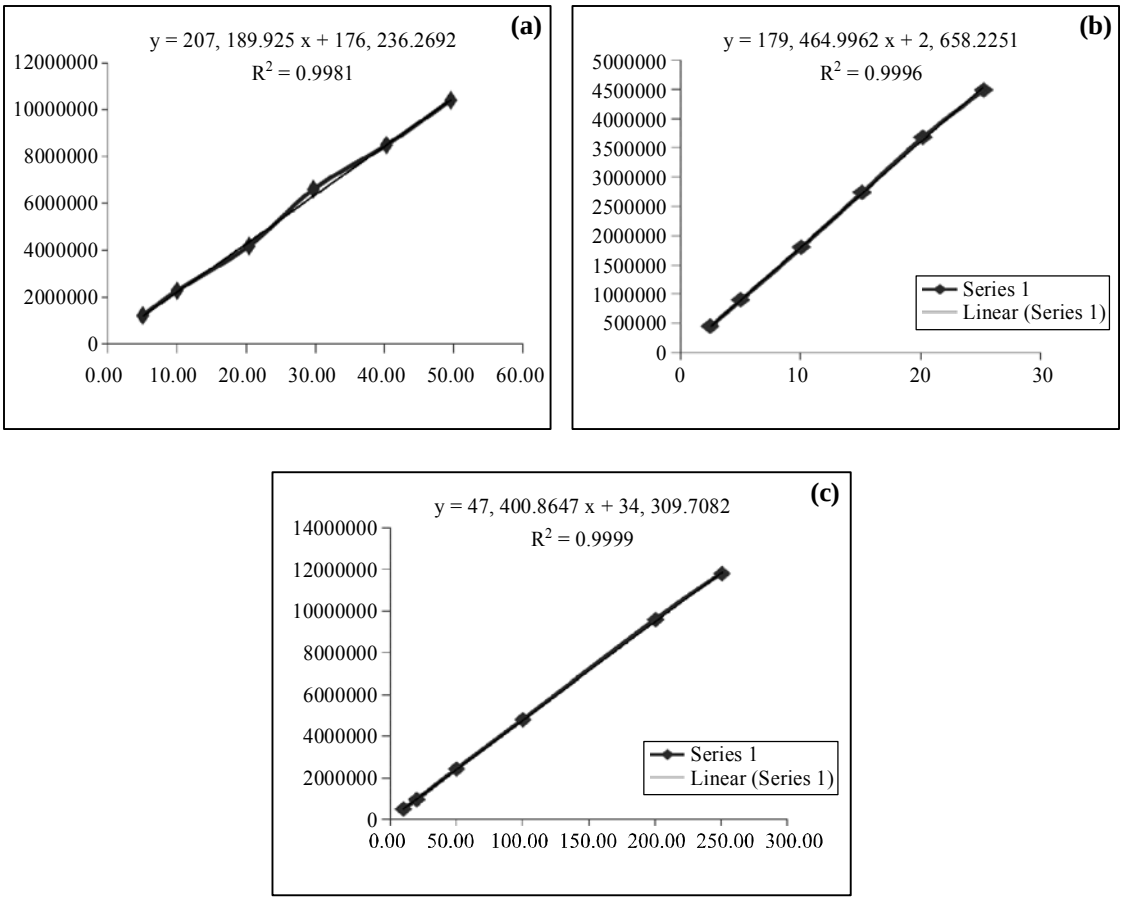

Fig. 3: Linear calibration curve of (a) Hydrochlorothiazide, (b) Amlodipine besylate and (c) Losartan potassium

Accuracy and precision

According to the ICH guidelines, repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e. three concentrations and three replicates of each concentration). Precision was studied to find out intra and inter day variations of the proposed method at three different levels. The %CV values less than 2% indicate that the method was precise.

Specificity

For demonstration of the specificity, 4 samples namely blank sample, sample containing hydrochlorothiazide alone, sample containing amlodipine besylate alone, losartan potassium alone and the sample containing the mixture of hydrochlorothiazide, amlodipine besylate and losartan potassium were prepared separately. Specificity of the method was determined by comparing results of all the samples (Fig. 4). The developed method is said to be specific if the % interference calculated as peak area (if any) at the retention time of each of the analytes in the blank sample is less than 20% of peak area at the corresponding retention times of each of the drugs in the lowest calibration standard. Sample specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

Stability

The stability of the drug is determined by placing the MQC samples for the short term stability at room temperature up to 12 hrs and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hrs was studied and established.

Stress degradation studies

For stress degradation analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100 µL of 0.1 N HCl (Acid stress), 0.1 N NaOH (alkaline stress), 5% v/v hydrogen peroxide (oxidative stress), for 24 hrs. Samples for photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples. The analysis is performed in triplicate.

RESULTS AND DISCUSSION

Method development and validation

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like hypersil-BDS-C18, symmetry C18, Ymc-pack C18, Ymc-pack pro, spherisorb C18, phenomenex C18 have been tried with different buffer salts such as ammonium formate, orthophosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran is done. However less tailing and high theoretical plates are obtained with Phenomenex ODS2 C18, (150 x 4.6 mm), 5 micron. The final mobile phase composition consisted of (65:35v/v) of methanol and 0.1% orthophosphoric acid on isocratic mode. The flow rate of the method is 0.8 mL/min. Calibration standards were prepared in diluents solution containing 50:50% v/v of methanol and Milli-Q water. The wavelength of detection is 254 nm. The column temperature is maintained at 25^{o}C. At the reported flow rate, peak shape was excellent; however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape. Hence 0.8 mL/min was optimized flow rate, decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. To evaluate the feasibility of the experiment under regular lab conditions, the assessment of the stability of hydrochlorothiazide**,** amlodipine besylate and losartan potassium under room temperature and under normal light conditions is done.

Method validation

System suitability

The % CV of the peak area for all the three drugs is within the acceptable criteria (Table 1). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 5390 ± 74.42 for hydrochlorothiazide, 7198.67 ± 58.86 for amlodipine besylate and 10216.17 ± 130.37 for losartan potassium. The USP tailing factor was 1.15 ± 0.0 for hydrochlorothiazide, 1.14 ± 0.02 for amlodipine besylate while that of Losartan potassium is 0.90 ± 0.01.

Detection and quantification limits (Sensitivity)

Fig. 2 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive, which can be determined from the data obtained from the (Table 2).

Table 1: System suitability for hydrochlorothiazide

Sample ID	Peak retention time	Peak area	Theoretical plates	Tailing factor
Hydrochlorothiazide				
1	2.32	5839337	5548	1.15
2	2.29	5836404	5372	1.15
3	2.30	6040148	5326	1.15
4	2.30	5979892	5355	1.15
5	2.29	5954441	5340	1.15
6	2.30	5798056	5401	1.15
Mean	2.30	5908046.33	5390.33	1.15
STDEV	0.011	96660.83	74.42	0.00
%CV	0.48	1.64	1.38	0.00
Amlodipine besylate				
1	3.55	1067927	7211	1.10
2	3.51	1026437	7251	1.15
3	3.52	1048460	7087	1.13
4	3.52	1059352	7160	1.14
5	3.51	1044886	7253	1.15
6	3.52	1025123	7230	1.14
Mean	3.52	1045364.17	7198.67	1.14
STDEV	0.015	17219.15	58.86	0.02
%CV	0.42	1.65	0.82	1.50
Losartan potassium				
1	5.11	2581567	10414	0.90
2	5.07	2555063	10367	0.92
3	5.09	2656296	10212	0.91

Cont…

Losartan potassium				
Sample ID	**Peak retention time**	**Peak area**	**Theoretical plates**	**Tailing factor**
4	5.09	2717955	10088	0.89
5	5.08	2709304	10119	0.89
6	5.09	2616586	10097	0.88
Mean	5.09	2639461.83	10216.17	0.90
STDEV	0.01	66814.33	130.37	0.01
%CV	0.26	2.53	1.28	1.50

Table 2: Sensitivity of hydrochlorothiazide, amlodipine besylate and losartan potassium

S. No.	**LOD Hydrochlorothiazide**		**LOQ Hydrochlorothiazide**	
	Retention time	**Peak area**	**Retention time**	**Peak area**
1	2.32	55335	2.31	28689
2	2.32	54730	2.31	28817
3	2.31	54779	2.32	28734
Mean	**2.317**	**54948.0**	**2.313**	**28746.7**
ST DEV	**0.01**	**336.05**	**0.01**	**64.93**
% CV	**0.25**	**0.61**	**0.25**	**0.23**

S. No.	**LOD Amlodipine besylate**		**LOQ Amlodipine besylate**	
	Retention time	**Peak area**	**Retention time**	**Peak area**
1	3.60	12514	3.55	8517
2	3.60	12429	3.55	8411
3	3.58	12487	3.57	8246
Mean	**3.593**	**12476.7**	**3.557**	**8391.3**
ST DEV	**0.01**	**43.43**	**0.01**	**136.57**
% CV	**0.32**	**0.35**	**0.32**	**1.63**

Cont…

S. No.	LOD Losartan potassium		LOQ Losartan potassium	
	Retention time	**Peak area**	**Retention time**	**Peak area**
1	5.18	19525	5.09	4295
2	5.18	19148	5.11	4255
3	5.21	19150	5.14	4206
Mean	**5.190**	**19274.3**	**5.113**	**4252.0**
ST DEV	**0.02**	**217.09**	**0.03**	**44.58**
% CV	**0.33**	**1.13**	**0.49**	**1.05**

Linearity

The linearity was demonstrated in triplicate. The results of the best fit line ($y = mx + c$) for the triplicate analysis is given in Table 3.

Table 3: **Results of best-fit line for triplicate analysis for hydrochlorothiazide, amlodipine besylate and losartan potassium**

Hydrochlorothiazide			
Curve	**Slope**	**Intercept**	**r^2**
1	207190	176236	0.998
2	207198	176237	0.999
3	207193	176240	0.997
Mean	207193.6	176237.6	0.998
Amlodipine besylate			
1	179464	2658	0.999
2	179465	2660	0.998
3	179451	2670	0.996
Mean	179460	2662.6	0.997
Losartan potassium			
1	47400	34309	0.999
2	47410	34311	0.998
3	47408	34320	0.999
Mean	47406	34313.3	0.998

The accuracy of the calibration standards was evaluated from the back calculated concentrations (Table 4). All the standards were found to be within the range of 94-104%.

Table 4a: Linearity and range for hydrochlorothiazide

Sample ID	Concentration (microgram/mL)	Retention time	Peak area	Back calc. concentration	% accuracy
Blank	Blank	**NA**	**0**	**NA**	**NA**
CC - 01	5.12	2.31	1235023	5.11	99.91
CC - 02	10.08	2.31	2298893	10.24	101.69
CC - 03	20.46	2.31	4199686	19.42	94.91
CC - 04	29.76	2.30	6604728	31.03	104.26
CC - 05	40.30	2.30	8496728	40.16	99.65
CC - 06	49.60	2.33	10401027	49.35	99.50
CC - 07	Blank	NA	0	**NA**	**NA**

Table 4b: Linearity and range for amlodipine besylate

Sample ID	Concentration (microgram/mL)	Retention time	Peak area	Back calc. concentration	% Accuracy
Blank	Blank	**NA**	**0**	NA	**NA**
CC - 01	2.52	3.58	450384	2.49	99.00
CC - 02	5.05	3.56	901608	5.01	99.19
CC - 03	10.09	3.57	1800593	10.02	99.29
CC - 04	15.14	3.55	2737108	15.24	100.64
CC - 05	20.19	3.54	3679700	20.49	101.48
CC - 06	25.24	3.57	4486103	24.98	98.98
CC - 07	Blank	NA	0	**NA**	**NA**

Table 4c: Linearity and range for losartan potassium

Sample ID	Concentration (microgram/mL)	Retention time	Peak area	Back calc. concentration	% Accuracy
Blank	Blank	**NA**	**0**	NA	**NA**
CC - 01	10.02	5.16	494444	9.71	96.88
CC - 02	20.04	5.16	949765	19.31	96.37
CC - 03	50.09	5.16	2428375	50.51	100.83
CC - 04	100.18	5.16	4807051	100.69	100.51
CC - 05	200.35	5.16	9609331	202.00	100.82
CC - 06	250.44	5.19	11832526	248.90	99.39
CC - 07	Blank	NA	0	**NA**	**NA**

Accuracy and precision

Accuracy and precision calculated for the QC samples during the intra- and inter-day run are given in the Table 5. The intra-day (day-1) and inter-day accuracy for hydrochlorothiazide ranged from 99.50-103.44%, the value for amlodipine besylate is 98.26-104.86% while that of losartan potassium ranged from 98.07-103.88%. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

Table 5: Results of inter and intra-day accuracy & precision for hydrochlorothiazide, amlodipine besylate and losartan potassium

Hydrochlorothiazide	Nominal concentration (µg/mL)		
	12.40 (LQC)	**24.80 (MQC)**	**37.20 (HQC)**
Day 1 (Intra day)			
Mean (n = 6)	99.50	102.46	103.28
STDEV	1.34	0.66	0.23
% CV	1.35	0.64	0.22
Day 2			
Mean (n = 6)	101.02	103.02	103.38
STDEV	1.32	0.56	0.26
% CV	1.30	0.54	0.25

Day 3			
Mean (n = 6)	99.50	101.74	103.44
STDEV	1.43	0.68	0.28
% CV	1.43	0.66	0.27
Amlodipine besylate	**Nominal concentration (µg/mL)**		
	6.31 (LQC)	**12.62 (MQC)**	**18.93 (HQC)**
Day 1 (Intra day)			
Mean (n = 6)	102.64	98.37	100.07
STDEV	1.40	0.25	1.92
% CV	1.36	0.25	1.92
Day 2			
Mean (n = 6)	104.86	98.26	100.66
STDEV	1.32	0.26	1.80
% CV	1.25	0.26	1.78
Day 3			
Mean (n = 6)	103.48	99.38	101.62
STDEV	1.36	0.28	1.65
% CV	1.31	0.28	1.62
Losartan potassium	**Nominal concentration (µg/mL)**		
	62.61 (LQC)	**125.22 (MQC)**	**212.87 (HQC)**
Day 1 (Intra day)			
Mean (n = 6)	103.79	102.97	98.07
STDEV	0.38	0.32	0.97
% CV	0.37	0.31	0.99
Day 2			
Mean (n = 6)	103.88	102.83	98.70
STDEV	0.40	0.38	0.98
% CV	0.38	0.36	0.99

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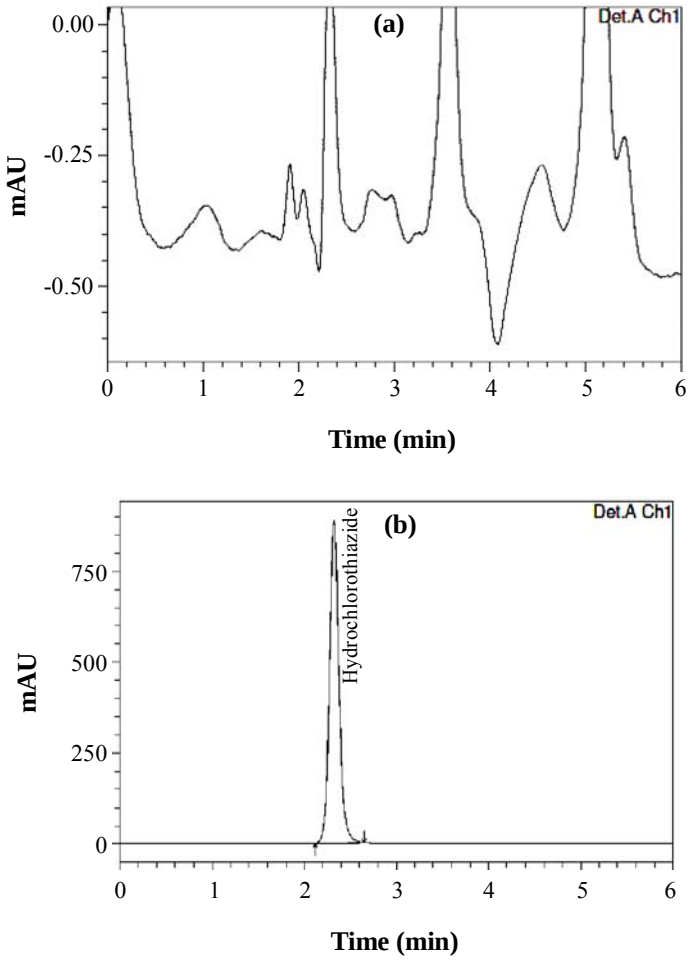
Day 3			
Mean (n = 6)	103.38	101.75	98.70
STDEV	0.44	0.40	1.20
% CV	0.42	0.39	1.21

Specificity

Specificity was determined by comparison of the blank chromatogram with that of the Standard chromatogram (Fig. 4).



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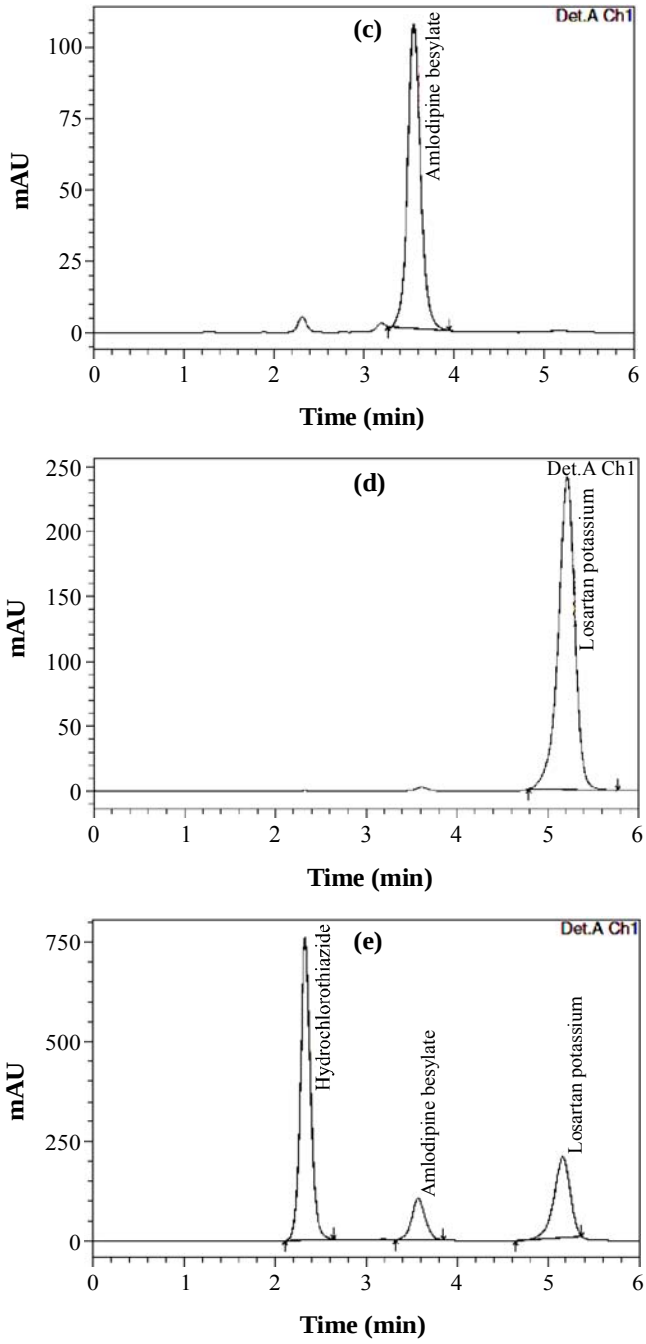

Fig. 4: Comparison of (a) Blank chromatogram, (b) Hydrochlorothiazide alone, (c) Amlodipine besylate alone (d) Losartan potassium alone and (e) Sample containing hydrochlorothiazide, amlodipine besylate and Losartan potassium

Room temperature stability

Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n = 6). The room temperature stability was found to be 97.94% for hydrochlorothiazide; it is 101.11% for amlodipine besylate and 98.37 % for losartan potassium.

Stress degradation

Stress studies revealed that hydrochlorothiazide is not susceptible to degradation under acid, oxidative stress, light (UV) stress conditions. However, in alkaline conditions (0.1 N NaOH), the drug was unstable and the degradation peak eluted later accompanied with a drastic peak distortion and increased tailing. Except for alkaline conditions, the drug content was within 98.04-98.84% for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Stress studies on amlodipine besylate indicated the stability of drug under acid, oxidative stress and light (UV) conditions. However, in alkaline conditions (0.1 N NaOH), the drug was unstable and the degradation peak eluted later accompanied with a peak distortion and increased tailing. For all the other stress conditions, the amlodipine besylate content was within 95.19-99.11% indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Stress studies on losartan potassium indicated the stability of drug under acid, oxidative stress and light (UV). However, in alkaline conditions (0.1 N NaOH), the drug was unstable and the degradation peak eluted later. For all the other stress conditions, the Losartan potassium content was within 96.96-98.23% indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Robustness study

Robustness is the measure of the method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. In order to evaluate the robustness of the method, the experimental conditions were deliberately altered. The impact of factors like the flow-rate (0.8 ± 0.1 mL/min), and the effect of mobile-phase composition (± 5%) on the chromatographic parameters such as retention time, theoretical plates, and tailing factor were studied**.** It was observed that there was no significant variation in chromatograms, due to the variation of mobile phase composition or flow rate variation and this demonstrates that the proposed method was robust in nature.

Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of hydrochlorothiazide, amlodipine besylate and losartan potassium. Also the method is validated for different parameters; hence it has been applied for the simultaneous estimation in pharmaceutical dosage forms. The amount of hydrochlorothiazide, amlodipine besylate and losartan potassium in the commercial tablet dosage form is within the pharmacopoeial specifications. None of the tablets' ingredients interfered with the analyte peak. The spectrum of hydrochlorothiazide, amlodipine besylate and losartan potassium in the extracted tablet was matching with that of the standard compounds indicating the purity of the compounds in the tablets.

CONCLUSION

The method gave accurate and precise results in the concentration range of 5.02 to 49.60 µg/mL for hydrochlorothiazide, 2.52 to 25.24 µg/mL for amlodipine besylate and 10.02 to 250.44 µg/mL for losartan potassium. The mobile phase composition consists of 65:35% v/v of methanol and 0.1% orthophosphoric acid at the flow rate of 0.8 mL/min. The retention time of hydrochlorothiazide is 2.30 ± 0.01 min, amlodipine besylate is 3.52 ± 0.01 min and that of losartan potassium is 5.09 ± 0.01. The column is phenomenex ODS 2, (150 x 4.6 mm), C18 column with the particle size of 5 µm. A rapid sensitive and specific method for the simultaneous estimation of hydrochlorothiazide, amlodipine besylate and losartan potassium in the pharmaceutical tablet formulations has been developed and validated.

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