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RESEARCH ARTICLE

HPLC method development for simultaneous estimation of hydrochlorothiazide, amlodipine besylate and telmisartan in tablet dosage form

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ABSTRACT

A reverse phase high performance liquid chromatographic assay method has been developed and validated for simultaneous estimation of hydrochlorothiazide, amlodipine besylate and telmisartan in tablet dosage form. Stationary phase was 150 mm x 4.6 mm, 5 µm C-18 column, from Novapak, mobile phase was degassed mixture of 50mM ammonium acetate buffer pH 4.5, and acetonitrile in the ratio of 65:35 and flow rate at 1.2 ml/min, at ambient condition with detector setting at 235nm. The retention times of hydrochlorothiazide, amlodipine and telmisartan are 3.0 min, 4.0 min and 5.1 min, respectively. Above method was validated as per ICH guidelines. Specificity was confirmed by comparing the placebo chromatogram with that of standard. Linearity of the method was achieved from 80 per cent to 120 per cent of test concentration. The precision of the method is carried out by six different test preparations and the % relative standard deviation was calculated. The accuracy of the method extracted in triplicate at three concentration levels, *i.e.* 50 per cent, 100 per cent and 150 per cent of test concentration and recovery calculated. Robustness was performed by changing flow rate, mobile phase ratio. Above validated method can be recommended for simultaneous analysis of these drugs in tablets.

Key words : Hydrochlorothiazide, Amlodipine besylate, Telmisartan, High performance liquid chromatography

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INTRODUCTION

Hydrochlorothiazide (Budavari, 2001) is 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.It is white or almost white, odourless crystalline powder, freely soluble in dimethylformamide,very slightly soluble in water and alcohol; insoluble in chloroform, in ether, and in dilute mineral acids. Hydrochlorothiazide dissolves in dilute solutions of alkali hydroxides. Amlodipine besylate (Sweetmau, 2005) is 3-ethyl 5-methyl 2-[(2-amino- ethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate. It occurs as white or almost white powder. Amlodipine besylate is slightly soluble in water and in isopropyl alcohol; sparingly soluble in dehydrated alcohol; freely soluble in methyl alcohol. Telmisartan (Budavari, 2001) is potassium4'-[(1,7'-dimethyl-2'-propyl-1*H*,3'*H*-2,5'-bibenzimidazol-3'-yl)-methyl]biphenyl-2-carboxylate . It is white to off-white crystalline powder that is insoluble in water, sparingly soluble in strong acid, methylene dichloride, methanol, ethanol, ethyl acetate and acetone, it is soluble in chloroform, and in strong base.

Keeping cost and time with technical requirement as main aspect, method has been developed. Profound search from data and literature available, it reveal that several methods have been reported including colorimetric determination, LC-MS, ultraviolet spectrophotometry, high performance liquid chromatography for the analysis of hydrochlorothiazide, amlodipine besylate and telmisartan either alone or in

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combinations with others (Verghes and Rair, 2011; Nalwade *et al.*, 2011; Thomas *et al.*, 2010) . So keeping the grounds for the study, method has been developed and validated as per ICH guidelines (ICH,Q2B, 1996).

MATERIALS AND METHODS

HPLC (Shimadzu) equipped with pump model LC-10Atvp and SPD-10Avp UV detector, was used for analysis. The chromatographic separation was performed using Novapak C-18 (150 mm x 4.6 mm, 5 µm) column. All chemicals and reagents used was of analytical grade and water used was of HPLC grade. A pH meter (mettler toledo), a sonicator was used in this study. Standards and sample tablets of hydrochlorothiazide (12.5 mg), amlodipine besylate (5 mg) and telmisartan (40 mg) were procured from celogen pharma, Mumbai. Chromatographic separation were executed after preparing mobile phase with buffer (ammonium acetate buffer pH 4.5: acetonitrile (65:35), the flow rate was 1.2 ml/min at 235 nm. Run time, injection volume were 12 min., 10 µl, respectively. The retention times of hydrochlorothiazide, amlodipine and telmisartan were 3.0 min, 4.0 min and 5.1 min, respectively.

Standard and sample solution preparation:

Standard stock solutions of hydrochlorothiazide, amlodipine and telmisartan in methanol with concentration of 0.28 mg/ml, 0.25 mg/ml and 0.44 mg/ml, respectively were prepared from which standard solution of hydrochlorothiazide, amlodipine and telmisartan in mobile phase with concentration of 0.01 mg/ml, 0.005 mg/ml and 0.08 mg/ml, respectively were prepared. Weighed and crushed twenty tablets in mortar-pestle. Weight equivalent to one tablet were dissolved in methanol and sonicated at controlled temperature to get concentration of 0.28 mg/ml, 0.25 mg/ml and 0.44 mg/ml of hydrochlorothiazide, amlodipine and telmisartan, respectively. Sample solution was filtered with 0.45 µ filter. From above, test solution was prepared in mobile phase with concentration of 0.01 mg/ml, 0.005 mg/ml and 0.08 mg/ml of hydrochlorothiazide, amlodipine and telmisartan, respectively.

Validation of the method:

Specificity and placebo interference of the method was checked by running placebo and standard solution. Linearity was established from 80 per cent to 120 per cent of test concentration, correlation coefficient was calculated. Six separate test preparations were injected and per cent relative standard deviation was studied to execute precision. Accuracy was performed by recovery of spiked samples from 50 per cent to 150 per cent of test concentration. Robustness was performed by changing flow rate, mobile phase ratio.

RESULTS AND DISCUSSION

Standard solution were injected, per cent relative standard deviation of area of peak obtained for six consecutive injections was 0.5 per cent for hydrochlorothiazide, 1.0 per cent for amlodipine and 0.9 per cent for telmisartan, which confirm suitability of the system.

Sample solution for assay were injected twice and mean peak area were obtained which were comparable with that of mean peak area of standard.

Chromatograms obtained were compared with blank chromatogram which clearly reveals that there was no interference from placebo at retention time of hydrochlorothiazide, amlodipine besylate and telmisartan.

Calibration curves for hydrochlorothiazide, amlodipine besylate and telmisartan were linear over the concentration range from 0.010 mg/ml to 0.015 mg/ml, 0.004 mg/ml to 0.006 mg/ml, and 0.064 mg/ml to 0.096 mg/ml, respectively. The results are presented in Table 1 and showed a good correlation between the peak area of analytes and concentration with $r > 0.9998$.

The precision of the assay method was evaluated with respect to Intra-assay precision and intermediate precision. Repeatability with respect to method was evaluated by carrying out six independent assays of test samples. The percentage of RSD of six assay values was calculated in same day, whereas intermediate and inter day precision was calculated by performing six independent assay by second analyst on another day (Table 2). % Relative standard deviation obtained in each case clearly depicted the precision of the method.

Accuracy was determined by spiking pure drugs into solution with a concentration range from 50 per cent to 150 per cent of test preparation. Thereafter per cent recovery was obtained which proved accuracy of the method.

Robustness of the method was studied by deliberate change of parameters like organic solvent composition, flow rate, pH of buffer, thereafter six replicates injection of standards were chromatographed and per cent RSD with respect to response of individual peaks, retention time were studied. Per

Table 1 : Linearity results from M80% to 120% of test concentration

	Hydrochlorothiazide	Amlodipine	Telmisartan
r	0.9999	0.9997	0.9998

r=Correlation coefficient

Table 2 : Per cent RSD values for precision

%RSD	Hydrochlorothiazide	Amlodipine	Telmisartan
1st Day 1st analyst	0.9	1.1	0.8
2nd Day 2nd analyst	1.0	1.2	0.9

cent RSD values obtained shows that there was no significant difference between developed and deliberate changed method.

If the formulation is in capsule, the method is to be checked once again especially for specificity and placebo interference, but comprehensive study will make picture clear.

Conclusion:

The HPLC assay method described here is simple, precise and accurate for quantitation of hydrochlorothiazide, amlodipine besylate and telmisartan in tablet dosage. The main advantages of the method were sensitivity, simplicity and rapidity. Results of assay obtained for test samples and validation parameters proved that, method can be recommended for simultaneous estimation of hydrochlorothiazide, amlodipine besylate and telmisartan in tablet dosage form.

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