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Development of validated analytical methods for the simultaneous determination of amlodipine, hydrochlorothiazide and telmisartan in tablet dosage form

Dhanya Maria Reji[1*], KJ Hanmanthachar[2], Susheel John Vargheese[3], Injamamul Haque[4]
[1, 2, 4] Sarada Vilas College of Pharmacy, Mysore, Karnataka, India
[3] College of pharmacy Sri Ramakrishna Institute of Paramedical Sciences Coimbatore, Tamil Nadu, India

Abstract
Amlodipine besylate is a long acting calcium channel blocker, hydrochlorothiazide is a calcium–sparing diuretic and telmisartan belongs to class of medicines called angiotensin II receptor antagonists. Combination of these drugs has been recently launched for the treatment of hypertension. Hence an attempt has been made to develop and validate an unsophisticated and accurate reverse phase high pressure liquid chromatography (RP-HPLC) method for the simultaneous estimation of Hydrochlorothiazide, Amlodipine and Telmisartan in tablet dosage form. In RP-HPLC method, the wavelength selected for the analysis was 238 nm for Hydrochlorothiazide, Amlodipine and Telmisartan. The solvent used was methanol. The mobile phases used in RP-HPLC were ammonium acetate buffer (adjusted to pH 5.5) and methanol (30:70, v/v). Column used in RP-HPLC was LichroCART @ 250-4Lichrosphere®100RP-18e (5µm) with flow rate of 1ml/min. Retention time (R_t) for hydrochlorothiazide, telmisartan and amlodipine was found to be 2.3 min, 5.3 min and 10.0 min respectively. The validation of the developed method was carried out for various parameters like linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, specificity and stability studies as per ICH guidelines.

Keywords: amlodipine, hydrochlorothiazide, telmisartan, RP-HPLC

Introduction
The most functional definition of analytical chemistry is that it is "the qualitative and quantitative characterization of matter". It means the identification of the chemical compounds or elements present in the sample. Modern analytical methods are extremely sensitive, providing precise and detailed information from small samples of materials. They are the most rapidly applied, and in general are rapidly amenable to automation.
HPLC is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. It is a convenient separation technique which can be used for wide variety of samples, with good resolving power, speed and nano molecular detection levels. This technique is based on the same modes of separation as that of classical chromatography i.e. adsorption, partition, ion exchange and gel permeation, but it differs from column chromatography in the fact the mobile phase is passed through the packed column under high pressure.

Materials & Methods
Amlodipine, hydrochlorothiazide and telmisartan (active pharmaceutical ingredient) was procured from STRIDES ARCOLAB Ltd., Bangalore, India. TELMA-AM H40 containing 5mg of amlodipine, 12.5mg of hydrochlorothiazide and 40mg of telmisartan. HPLC grade water was prepared by use of a Millipore Milli-Q Academic water purifier (Bangalore, India). Methanol HPLC grade, AR grade (Merck Pvt. Ltd., Mumbai). Ammonium acetate, GR grade (S.D fine chemicals Ltd., Mumbai).

Instruments Used
Shimadzu HPLC system with SPD-M10 A VP system PDA with 20 µl fixed volume manual injector and LC solutions chromatographic software, Shimadzu digital Electronic Balance BL – 220 H, Elico pH meter.
Development and validation of RP-HPLC method for the simultaneous determination of amlodipine, hydrochlorothiazide and telmisartan in tablet dosage form

Analysis of formulation
1) Preparation of Standard solution
Stock solution containing AMB, HDZ and TLM standards solution was prepared by transferring accurately 5 mg of AMB, 12.5 mg of HDZ and 40 mg of TLM API's to a 50 ml volumetric flask. Twenty millilitres of methanol was added initially to solubilise the drugs and the solution was diluted to volume with methanol and mixed well to get 100µg/ml of AMB, 250 µg/ml of HDZ and 800 µg/ml of TLM.

2) Preparation of sample solution
Five tablets, each containing 5 mg of AMB, 12.5 mg of HDZ, 40 mg of TLM were weighed and average was calculated. Weight equivalent to 5 mg of amlodipine was weighed, transferred to a 50 ml volumetric flask, added 20 ml methanol and sonicated for few minutes. This solution was filtered and suitable aliquots of formulation solutions were prepared.

3) Recording of the chromatogram
A steady base line was recorded with the fixed chromatographic conditions, standard and sample drug solutions were injected and chromatograms were recorded. Retention times were found to be 2.3, 5.3 and 10.1 minutes for hydrochlorothiazide, telmisartan and amlodipine, respectively. This was followed by injection of sample solution obtained from the formulation. Calibration curve was plotted using standard drug peak area versus

concentration of standard solutions. The results of formulation analysis are given.

Table1: Analysis of formulation

DRUGS	Amount of drug (mg/tablet)		% Label claim	% RSD*
	Labelled	Estimated		
AMB	5	4.4	93.0	1.18
HDZ	12.5	11.9	95.73	0.68
TLM	40	39.2	98.12	0.14

*RSD of six observations

Validation of RP-HPLC Method and Results

The validation of the developed method was carried out for various parameters like linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, specificity, stability studies as per ICH guidelines.

1. Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ values were determined by injecting lower concentrations of the drugs. The LOD value for AMB, HDZ and TLM were found to be 100, 0.01 and 1 ng/ml, respectively and their LOQ values were found to be 500, 0.1, and 5 ng/ml, respectively, fig. 34-39.

2. Linearity

a) Amlodipine

AMB was found to be linear in the concentration range of 1 -20µg/ml. Calibration curve was plotted using concentration (x) versus peak area (y). The slope, intercept, correlation co-efficient values were found to be 47679.8658, -6563.6358 and 0.9930, respectively. The regression equation is as follows

Peak area= (- 6563.6358) +47679.8658x concentration

b) Hydrochlorothiazide

HDZ was found to be linear in the concentration range of 2.5- 50 µg/ml. Calibration curve was plotted using concentration (x) versus peak area (y). The slope, intercept, correlation co-efficient values were found to be 10482.4458, 9194.9962 and 0.9991, respectively.

The regression equation is as follows

Peak area= (- 9194.9962) +10482.4458 x concentration

c) Telmisartan

TLM was found to be linear in the concentration range of 8-160 µg/ml. Calibration curve was plotted using concentration (x) versus peak area (y). The slope, intercept, correlation co-efficient values were found to be 20321.0927, 89476.8003 and 0.9997, respectively.

The regression equation is as follows

Peak area= (-89476.8003) + 20321.0927 x concentration

3. Precision

Precision of method was demonstrated by

a) Intraday precision
b) Inter day precision
c) Repeatability

a) Intraday precision

Intraday precision was studied by carrying out the analysis of the standard drugs at two different concentrations in the linearity range of drugs for three times on the same day and% RSD was calculated.

b) Inter day precision

Inter day precision was studied by carrying out the analysis of the standard drugs at two different concentrations in the linearity range of drugs for three days over a period of one week and% RSD was calculated.

c) Repeatability of injection

Standard drug solution was injected six times a day and its % RSD was calculated.

4. Accuracy

Recovery studies were done for determining the accuracy parameter. It was done by mixing known quantity of standard drug with the analysed sample formulation and the contents were reanalysed by the proposed method. Recovery studies were carried out at 80 and 100% levels. The percentage recovery and its % RSD were calculated.

5. Stability

The drug solutions were subjected to stability studies under room temperature and refrigerated conditions. The solution stored under room temperature was stable up to 19 hours in case of amlodipine and 22 hours in case of hydrochlorothiazide and telmisartan. Under refrigerated condition stability studies were carried out for 73 hours and it was found that all the three drugs were stable during this time period.

6. System suitability studies

The system suitability parameters like number of theoretical plates(N), resolution (Rs), tailing factor (Tf) etc. were studied.

7. Specificity

Conditions of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer, flow rate etc were changed. Although these changes were made, no additional peaks were found though there were some slight changes in retention times.

8. Peak purity tests

Peak purity test was done. The peak purity index of AMB, HDZ and TLM were found to be 0.9999, 0.9999 and 1.0000, respectively. Peak purity index values close to one proves peak purity of the drugs.

Table 2: System suitability studies

Drug	Resolution (Rs)	Number of theoretical plates (N)	Tailing Factor (Tf)
AMB	5.430	894.31	1.37
HDZ		2367.20	1.41
TLM	11.572	3523.74	1.12

Chromatogram of Formulation

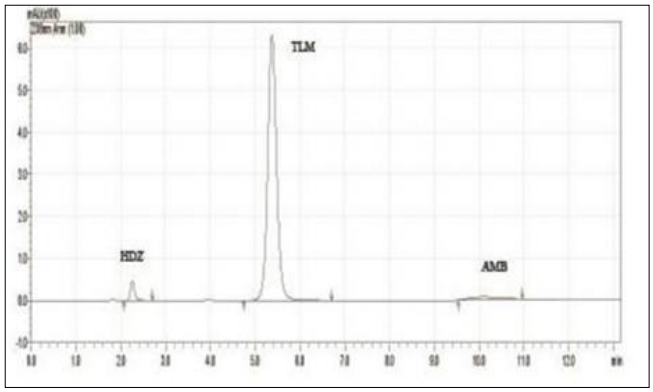

Fig 1: AMB- 12µg/ml, HDZ-30 µg/ml, TLM- 96 µg/ml

Standard Chromatograms

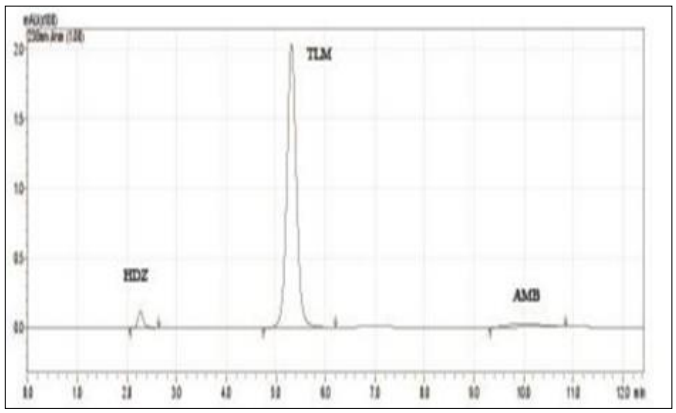

Fig 2: AMB-1µg/ml, HDZ-2.5 µg/ml, TLM- 8 µg/ml

Table2: Summary of analysis of amlodipine, hydrochlorothiazide and telmisartan by HPLC

HPLC				
DRUGS	**Linearity**	**Recovery**		**Formulation (% Label claim)**
		80%	**100%**	
AMB	1-20 (ug/ml) r = (0.9930)	92.8	95.6	93.0 (%RSD-1.18)
HDZ	2.5-50 (ug/ml) r = (0.9991)	93.2	97.5	95.73(%RSD-0.68)
TLM	8-16 (ug/ml) r = (0.9997)	97.29	99.8	98.12(%RSD-0.14)

Conclusion

An attempt has made to develop simple and economic validated RP-HPLC methods for the simultaneous determination of amlodipine, hydrochlorothiazide and telmisartan in tablet dosage forms. In RP-HPLC method, optimizations of different chromatographic parameters like selection of chromatographic method, detection wavelength, selection of mobile phase, ionic strength of mobile phase, mobile phase ratio, flow rate etc. were done. A wavelength of 238nm was selected for the study. A mobile phase system consisting of ammonium acetate buffer (adjusted to pH 5.5) and methanol (30:70,v/v), was employed for the determination of amlodipine, hydrochlorothiazide and telmisartan. With this system, symmetrical peaks with good separation (hydrochlorothiazide- Rt=2.3 min, telmisartan- Rt= 5.3 min, amlodipine- Rt= 10.0 min) were obtained at a flow rate of 1ml/min. The method was validated as per ICH guidelines. Calibration curve was plotted using concentration (x) versus peak area (y). AMB, HDZ and TLM were found to be linear in the concentration range of 1 to 20, 2.5 to 50 and 8 to 160 µg/ml, respectively. LOD values for AMB, HDZ and TLM were found to be 100, 0.01 and 1 ng/ml, respectively and their LOQ values were found to be 500, 0.1 and 5 ng/ml, respectively. Precision of the developed method was studied under inter day, intraday and repeatability studies. A low relative standard deviation value shows that the developed method is precise. Stability studies were carried for the standard solutions, amlodipine was found be stable upto 19 hours and hydrochlorothiazide and telmisartan were found to be stable for about 22 hours under room temperature. Under refrigeration condition the stability studies were carried out for about 73 hours and it was found that all the three drugs were stable during this time period. Recovery studies were carried out at 80 and 100% levels. System suitability parameters like number of theoretical plates (N), resolution (Rs) and tailing factor (Tf) were studied. The validated RP-HPLC method was applied to the simultaneous estimation of amlodipine, hydrochlorothiazide and telmisartan in tablet dosage form.

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