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Validated Chromatographical Methods for the Simultaneous Estimation of Antihypertensive Drugs in Multicomponent Formulations

N Delhiraj*[1], S Anbazhagan[2]

***Research Scholar,Department of Pharmacy, Aacharya Nagarjuna University, Guntur, Andhra Pradesh, India**

[1]Department of Pharmaceutical analysis, A.S.N Pharmacy College, Tenali, Andhra Pradesh, India

[2]Department of Pharmaceutical analysis, Karuna College of pharmacy, Iringuttoor, Thirumittacode, Kerala, India.

***Corres.author: pharmaraj1981@gmail.com**

Abstract: Two new, rapid, precise, accurate and specific chromatographic methods for the simultaneous determination of Amlodipine besylate, Telmisartan and Atorvastatin in combined pharmaceutical dosage forms. The first method based on reverse phase liquid chromatography by using Qualisil BDS C18 (250 x 4.6, 5µ) using mobile phase 0.02M Potasium dihydrogen phosphate and the pH was adjusted to 2.5 with orthophosphoric acid and acetonitrile (60:40) using a flow rate of 1ml/min, with a detection wavelength of 251nm. The second method involved silica gel 60F254 high performance thin layer chromatography and densitometric detection at 291nm using chloroform : methanol(90:10) as the mobile phase.
Keywords: Telmisartan;Atorvastatin;Amlodipine besylate;high performance liquid chromatography; high performance thin layer chromatography.

INTRODUCTION

Telmisartan,4’-[(1,4’-dimethyl-2'-propyl[2,6’-bi-1H-benzimidazol]-1'-yl)methyl][1,1’-biphenyl]-2-carboxylic acid. It works by blocking a substance in the body that causes blood vessels to tighten. As a result, it relaxes blood vessels. This lowers blood pressure and increases the supply of blood and oxygen to the heart [(1)]. Atorvastatin,(βR,dR)-2-(4-fluorophenyl)-β,d-dihydroxy-1-methylethyl)-3-phenyl-4[(phenylamino)carbonyl]-lH-pyrrole-1-tanoic acid, calcium salt, is a synthetic cholesterol-lowering agent[(2)].Amlodipine besylate is chemically 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro pyridine-3,5 dicarboxylate benzene sulphonate salt of amlodipine, which is a dihydropyridine calcium channel blocker is a calcium antagonist inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and

decreased after load [3].Literature review reveals that the analytival methods for Telmisartan,Amlodipine besylate and atorvastatin calcium alone or in combined dosage forms are UV(Nagaraj, et al. 2007[4],Bhatia N,et al. 2007[5],Kakde R B, et al. 2008[6], Nagavalli D, et al. 2011[7],Pournima Patil, et al. 2011[8], Rekha Gangola, et al. 2011[9],Vijaya Vichare, et al. 2011[10].)HPLC(Dhorda VJ,et al. 1999[11],Palled M S,et al. 2005[12], Wankhede SB,et al. 2007[13], Chaudhari BG,et al. 2007[14], Shah DA Bhatt, et al. 2007[15], Shah DA Bhatt, et al. 2007[16], Vaijanath G Dongre, et al. 2008[17], Chitlange SS, et al. 2008[18], Syed Shanaz Qutab, et al. 2009[19], Priyanka Patil R, et al. 2009[20], Safeer K, et al. 2010[21], Mustafa Celebier, et al. 2010[22].)RP-HPLC with fluorescence detection(Khedr A,et al. 2007[23].) ,HPTLC (Ilango K,et al. 1997[24], Agrekar AP, et al. 2000[25], Dhaneshwar SS, et al. 2007[26].).

EXPERIMENTAL

Chemicals

Telmisartan,Atorvastatin & amlodipine reference standards was supplied by M/s Microlabs limited, Bangalore**,**India. HPLC grade Acetonitrile, potassium dihydrogen phosphate triethylamine, orthophosphoric acid, chloroform, methanol was purchased from Merck (Mumbai, India). All chemicals were of analytical grade. Commercially available tablets (Telday-AV of Torrent, Gujarat, India), containing 40 mg Telmisartan and 10 mg Atorvastatin per tablet), (Stamlo-T of Dr.Reddys Labs, Andhra pradesh, India), containing 40 mg Telmisartan and 5mg Amlodipine per tablet were used for analysis. Stock solutions (1.0 mg mL-1) for RP-LC and HPTLC were prepared in methanol. 50 microlitter of the above stock solution was dissolved with 900 microlitter of methanol for HPTLC

Apparatus

The determination was carried out on Agilent technologies 1220 series consisted of isocratic pump model G4286B liquid chromatographic system with 20µl loop manual injector was used. Samples were applied as 8 mm bands by means of a Camag Linomat V automatic samples applicator (Muttenz Switzerland) equipped with a 100 µL syringe. The distance between the bands was 11.4 mm. Silica gel 60 F_{254} HPTLC plates (20×10 cm, aluminium) were from Merck (Darmstadt, Germany). Densitometric scanning was performed at 270nm with a camag TLC scanner 3 equipped with camag Wincats software 1.42 using the deuterium light source and slit dimensions of 4.00 mm × 0.30 mm.

HIGH PRESSURE LIQUID CHROMATOGRAPHY

Chromatographic Conditions

The analytes were separated on Agilent, Qualisil BDS C18 column (250 mm X 4.6 i.d., 5 µm particle diameters, made in USA). Mobile phase consists of 0.02M potassium dihydrogen phosphate buffer pH 2.5 and Acetonitrile in the ratio of 60:40, with flow rate 1 ml min.-1 with isocratic elution and the UV- Variable wave length detector Model G1314 was set at 251 nm using data handling system EZChrom Elite Compact 3.3.2 SP2 software. HPLC grade methanol used as diluent. The column was conditioned for ≥ 30 min. All the determinations were performed at ambient temperature 25± 5 °C and the injection volume was 20 µl.

Preparation of Mobile Phase

A 20 millimolar phosphate buffer was prepared by dissolving 2.72 g of potassium dihydrogen orthophosphate in 1000 ml of water. To this 5 ml of triethyl amine was added and pH was adjusted to 2.5±0.05 with orthophosphoric acid. Above prepared buffer and Acetonitrile were mixed in the proportion of 60:40 v/v. The mobile phase so prepared was filtered through filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Calibration

For calibration purposes, a range of 10-80µg/ml for Telmisartan, 2-25µg/ml for amlodipine & 5-25µg/ml for Atorvastatin were prepared and 20 µL injections were carried out in triplicate.

Analysis of tablet formulations

Ten tablets were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 40mg of Telmisartan was transferred into a 100ml volumetric flask.100ml of diluent was added, sonicated for 30minutes with occasional stirring. Cool the solution to room temperature and dilute to the volume with diluent, filtered the solution through 0.45µm Teflon filter syringe. 1ml of the above filtered solution was transferred into a 10ml volumetric flask & dilute to the volume with diluent.

Recovery study

The accuracy of the proposed method was evaluated by the addition of a standard drug solution to a pre-analysed tablet sample solution at three different concentrations levels at 50,100 and 150% of linearity for both drugs.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Chromatographic conditions

Chromatography was performed on 20 × 10 cm aluminum HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck). Before use plates were washed with methanol and dried in an oven at 120°C for 20 min. ascending development of the plate with a migration distance of 50 mm was performed at 23± 2°C using chloroform: methanol (90:10 v/v) as the mobile phase and a Camag twin-trough chamber previously saturated with mobile phase for 20 min. the average development time was 5 minutes.

Calibration

Mixed working standard solutions equivalent to 4, 6, 8, 10, 12, 14, 16 µL were separately stopped on the TLC plate in order to obtain final concentrations at 400, 600,800,1000,1200,1400,1600 ng $spot^{-1}$ for both drugs respectively. The plates were developed in a 20 × 10 cm twin through chamber using 20 mL freshly prepared mobile phase.

Analysis of Tablet Formulation

The tablets were weighed, triturated and the average weight was calculated. A 0.1 mg/mL solution was prepared in methanol and filtered through Whatman filter paper no. 41. The above stock was diluted in the ratio of 1:00 with methanol which was used as the working standard solution. The 8µL solution was spotted on the HPTLC plate and the concentrations were calculated from the calibration graph.

Recovery study

The accuracy of the proposed method was evaluated by the addition of a standard drug solution at three different concentration levels at 50, 100, and 150% of linearity for both drugs.

RESULTS AND DISCUSSION

High Pressure Liquid Chromatography

A satisfactory separation was obtained (Telmisartan Rt 7.68, Amlodipine besylate Rt 4.86 and Atorvastatin Rt 5.91) when using Qualisil BDS (250 x 4.6, 5µ) column using mobile phase 0.02M Potasium di hydrogen phosphate and the pH was adjusted to 2.5 with orthophosphoric acid and acetonitrile (60:40) with a flow rate of 1ml/min with a detection wavelength of 251nm for both the compounds with a injection volume of 20µl. The chromatograms depicted in figure 1&2. A calibration curve was made and concentration examined within the detection range of 10-80µg/ml for Telmisartan, 2-25µg/ml for amlodipine & 5-25µg/ml for Atorvastatin and correlation coefficient was found to be 0.9992, 0.9996 & 0.9998 for both the compounds respectively. The assay values obtained by proposed method and recovery experiment values obtained were performed by adding a fixed amount of drug to preanalysed formulation summarized in Table 2. The stability of sample was checked by forced degradation in different conditions and the studies indicate that any other impurity is not merging

with the main peak The analyte solution was stable up to 24hrs.A method was developed for the determination of Amlodipine besylate, Telmisartan and Atorvastatin in tablets which is rapid, stable & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

Figure 1: HPLC Sample chromatogram for Telmisartan and Atorvastatin

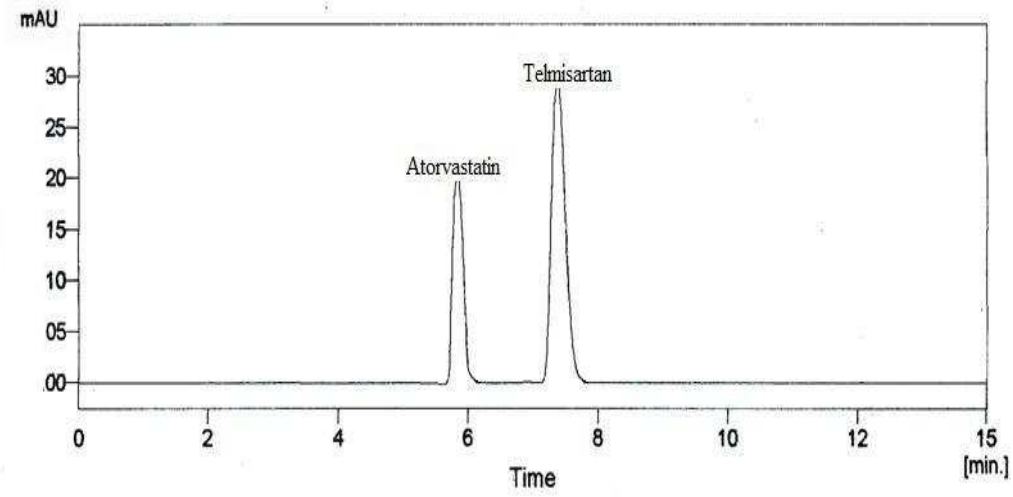
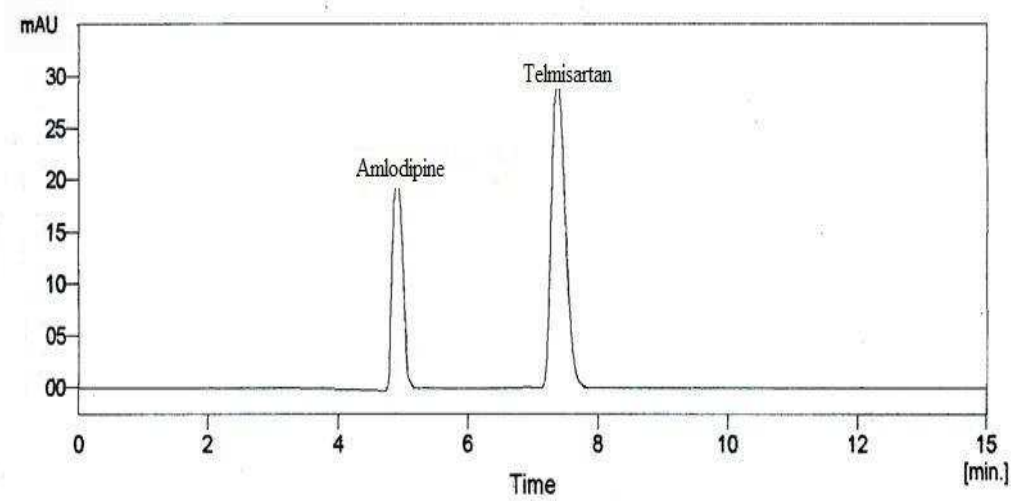


Figure 2: HPLC Sample chromatogram for Telmisartan and Amlodipine



High Performance Thin Layer Chromatography

Maximum separation was achieved (Atorvastatin Rf 0.24, Telmisartan Rf 0.64) and minimum tailing were obtained when using a mobile phase composition of chloroform: methanol (90:10 v/v) respectively.The chromatogram shown in figure 3. Table 1 shows that correlation coefficients were 0.998 for TML and 0.998 for ATOR. The LOD values were 100 ng spot -1and 50 ng spot -1, while LOQ values were 300 ng spot -1and 150 ng spot -1for both Atorvastatin and Telmisartan respectively. The proposed method was used for the determination of both drugs in tablets and results are also shown in Table 2. Good recoveries and standard deviations were observed.

Table 1: Optical and system suitability parameters

Parameter	HPLC			HPTLC	
	Tlm	**Ator**	**Amlo**	**Tlm**	**Ator**
Linearity range	10-80µg/ml	5-25µg/ml	2-25µg/ml	400-1600 ng/ml	400-1200 ng/ml
Regression equation					
Slope	45373	40434	54316	3.36	4.861
Intercept	22921	14784	14784	3291	1107
Coefficient of correlation	0.9992	0.9996	0.9998	0.998	0.998
Limit of detection (LOD)	2µg/ml	1µg/ml	0.05µg/ml	50 ng/ml	100 ng/ml
Limit of quantitation (LOQ)	7µg/ml	3µg/ml	1µg/ml	300 ng/ml	150 ng/ml
System suitability					
Tailing factor	1.75	1.22	1.68		
No. of theoretical plates	2313	4976	3161		

Figure 3: HPTLC Sample chromatogram for Telmisartan and Atorvastatin

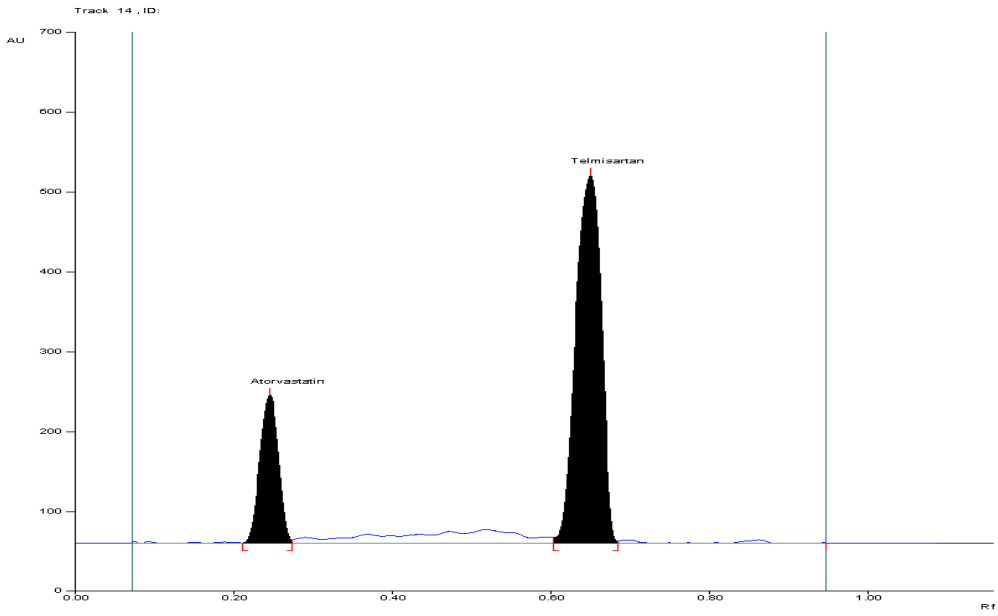


Table 2: Assay and Recovery studies

Method	Brand name	Compound	% Assay	% recovery
HPLC	Telday-AV	Telmisartan	100.24	99.97
		Atorvastatin	101.70	100.90
	Stamlo-T	Telmisartan	95.80	100.35
		Amlodipine	99.94	103.14
HPTLC	Telday-AV	Telmisartan	99.90	100.51
		Atorvastatin	99.18	99.63

CONCLUSION

A method was developed for the determination of tablets which is simple, quick, reliable, inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

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