Section A-Research paper

METHOD DEVELOPMENT AND VALIDATION OF SOME ANTIHYPERTENSIVE DRUGS BY RP-HPLC: A COMPARETIVE STUDY

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ABSTRACT

An accurate, precise, specific, reliable, and simple reverse phase high performance liquid chromatography method was developed and it validated for simultaneous estimation of there (atenolol, metoprolol, propranolol) with hydrochlorothiazide in pharmaceutical dosage form. The formic acid (0.1%) in water and acetonitrile was used as mobile phase with flow rate of 1 mL min^{-1}. The detection carried out on colum Agilent HPLC 1200 series bridge C18 column (Xbridge C18, size: 250 mm x 4.60 mm, particle size 5 µm) at 228 nm was used. The method was found effective in the concentration range of 10 - 90%. This was applied for quantitative determination of atenolol, metoprolol, propranolol with hydrochlorothiazide in dosage form successfully. The retention times of hydrochlorothiazide, propranolol, metoprolol, and atenolol, were found to be 1.368, 4.123, 6.477, and 7.230 min, respectively. The present RP-HPLC method was validated and was used for the analysis of hydrochlorothiazide, metoprolol, atenolol, and propranolol in combined tablet dosage form (pharmaceutical formulations).

KEYWORDS: **H**ydrochlorothiazide, Antihypertensive Metoprolol, Atenolol, Propranolol; RP-HPLC; Method development, Validation.

INTRODUCTION

Antihypertensive is a group of drugs, which are used to treat the high blood pressure. This therapy is also used to prevent some complications arising due to high blood pressure, such as myocardial infarction and stroke. It has been suggested reduced blood pressure by 5 mm Hg can also decrease the risk of ischaemic heart disease by 21% and stroke by 34%. It also reduces the other complications of dementia, heart failure, and death. There are number of antihypertensive drugs which can lower down blood pressure by different mechanisms. The most commonly used drugs are ACE inhibitors, beta blockers (β- blockers) and thiazide diuretics, calcium channel blockers, angiotensin II

receptor antagonists (ARBs) and. β-Blockers are used to manage abnormal heart rhythms, and second heart attack (myocardial infarction). They are also generally used to treat hypertension, but they are not longer the first-line of treatment of most patients. Some β – blockers are atenolol, metoprolol, and propranolol.

Atenolol (RS)-2-[4-{2-Hydroxy-3(propane-2-ylamino) propoxy} phenyl] acetamide (Fig. 1(a)) is a β-blocker, which is primarily used to treat high blood pressure and heart associated chest pain. Only half the atenolol is absorbed via gastro-intestinal tract and most of this absorbed drug comes to the systemic circulation. Atenolol function as β-adrenergic to stimulate the heart beats more rapidly. It is also helpful in treating angina. It also be used in prophylactic treatment of migraine.[1]

Metoprolol (RS)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(propane-2-yl)amino] propan-2-ol (Fig. 1(b)) is a β - blocker (cardioselective) and generally used in treatment of angina pectoris, hypertension, myocardial infarction, arrhythmia, and heart failure. β_1 Adrenergic receptors have smaller activity against β_2 adrenergic receptors of the lungs and vascular smooth muscle. It is known that receptor selectivity decreases with higher doses.[2,3]

Propranolol (RS)-1-(1-Methylethylamino)-3-(1-naphthyloxy) propan-2-ol (Fig. 1(c)) is a non- selective β-adrenergic antagonist. It is also used in the management to hypertension, angina pectoris, pheochromocytoma, cardiac arrhythamias and myocardial infarction. It has different side effects such as nausea, mental depression, light headness, vomiting. It is continued for a long time then heart and arteries not work properly.[4]

Hydrochlorothiazide [6-Chloro-1,1dioxo-3,4-dihydro-2H-1,2,4-enzothiadiazine-7- sulfonamide] (HCTZ) (Fig. 1(d)) is diuretic and used to treat the high blood pressure and swelling. It is also used in diabetes insipidus, to reduce the risk of kidney stones and renal tubular acidosis. It is sometimes considered as a first-line treatment for the high blood pressure. The HCTZ is taken by mouth and may be combined with other blood pressure medicine as a single pill toincrease effectiveness.[6]

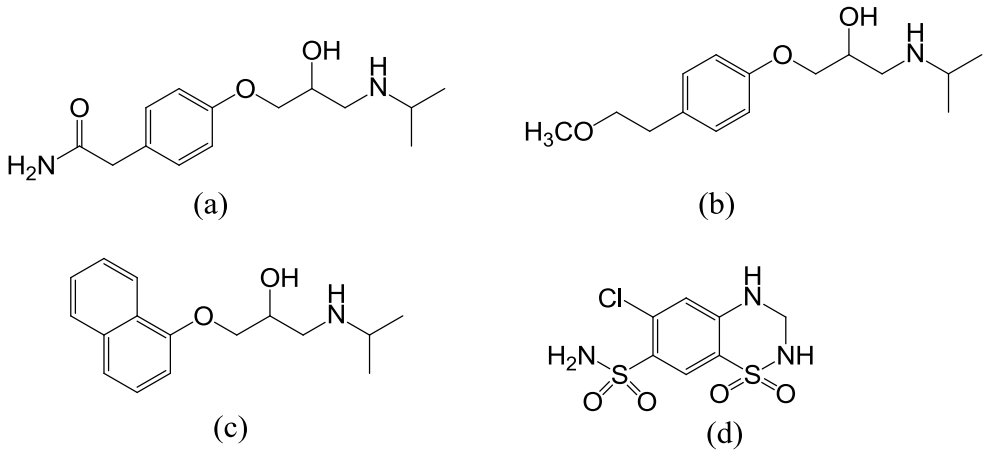


Figure 1: (a) Atenolol (b) Metoprolol (c) Propranolol (d) Hydrochlorothiazide

A combination of β-blockers with it blocks the β-adrenergic receptors in the sympathetic nervous system. As a result of heart rate, systolic pressure, and cardiac contractility is reduced. Diuretics decrease active reabsorption of sodium and Calcium ions by inhibiting the sodium/chloride co-transporter and can also increase loss of potassium ion. These thiazide diuretics also decrease the volume of blood by dieresis.

The present investigation was carried out to develop a simple, precise and accurate RP-HPLC method for determination of metoprolol, atenolol, and propranolol in combination with HCTZ.

MATERIALS AND METHODS

Instrumentation

HPLC separation of atenolol, metoprolol, propranolol and hydrochlorothiazide was carried out on Agilent 1260 infinity series equipped, PDA detector quaternary HPLC pump, column ovens (compatible with 250 mm length column) and an autosampler with partial loop volume injection system. The EZ Chrom software was used controlling system and data acquisition. HPLC separation of metoprolol, atenolol, propranolol and hydrochlorothaizide was observed using formic acid (0.1%) in water and formic acid (0.1%) in acetonitrile as mobile phase flow rate of 1 mL min^{-1} at 35°C. The X-bridge C18 column (250 mm × 4.6 mm, 5 μm) was used for this purpose.

Chemicals and Reagents

Metoprolol, atenolol, propranolol, (purity more than 98%) and hydrochlorothaizide (purity more than 98%) was from Torrent Pharmaceuticals (India). Methanol and acetonitrile (HPLC grade) were purchased from Finar Chemicals (India). Other chemicals were of analytical grade and from commercial sources.

Chromatographic calculations

The resolution factor (Rs) was determined from the software calculations using $Rs = 0.998\ (tR_2 - tR_1)/(w_1 + w_2)$, where tR is the retention time (min), w is the peak width measured at half height, and subscripts 1 and 2 represent the former and latter has eluted enantiomers, respectively. The retention factors for each enantiomers, k_1 and k_2, were calculated by the equation $k = (tR - t_0)/t_0$, where t_0 is the dead time under the experimental conditions, which is determined with 1, 3, 5-tributylbenzene. Selectivity factor (α) was obtained using equation $\alpha = k_2/k_1$. The theoretical plate number (N) was obtained from the software calculations.

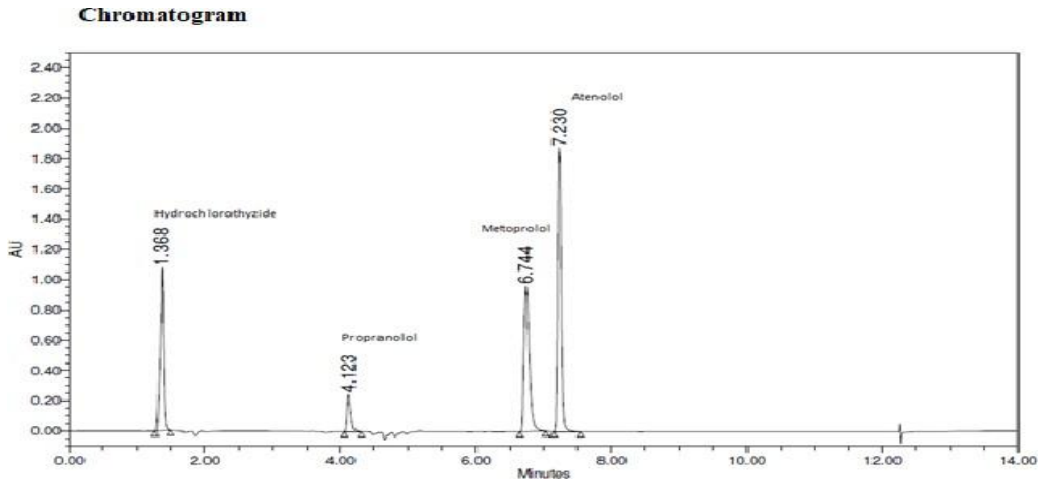


Figure 5: Chromatogram of all spiked drugs

Accuracy

Accuracy is the explanation between actual results obtained and accepted true value (reference value). Accuracy recovery studies are normally evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed.

Precision

Precision was found on intra-day and inter-day basis. The intra-day precision means use of that analytical method within a laboratory over a short time by same operator with same equipment. While inter-day precision is the estimation of variations in analysis, when a method is used within a laboratory on different days by same operator.

Application of the Method to Dosage Form

The proposed method was applied for the determination of the HCTZ drug in its pharmaceutical dosage with Three Different Antihypertensive Drugs using XbridgeC18 column. The Recovery calculated for metoprolol, atenolol, propranolol and hydrochlorothiazide were 98.5, 101.4, 100.7 and 99.50%, respectively. Which indicated that the proposed method can successfully applied for the estimation of hydrochlorothiazide in pharmaceutical dosage form.

RESULTS AND DISCUSSION

Method Validation

This analytical method was also validated before before determining HCTZ in dosage sample by evaluating of precision, linearity and limit of detectia (LOD) and limit of qualificalia (LOQ) using columns as follows.

Method development

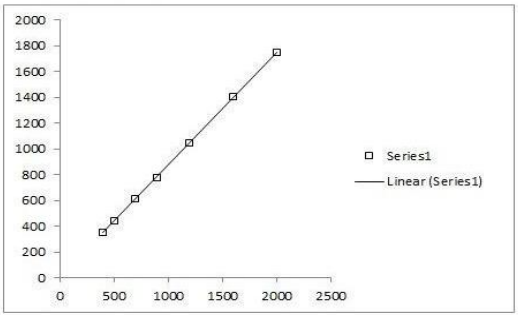
Various mixtures of formic acid (0.1%) in water and acetonitrile were tried to get mobile phase for the analysis of the selected drug combination, in of symmetric peak of 228 nm in short run time. Injection volume was selected as 5 µg/mL giving a good peak area. The EZ chrome Xbridge C18 was used as column which gives symmetrical good peak shape. The flow rate was fixed at 1.0 mL/min. It was observed that chromatogram gave a peak of metoprolol, atenolol, propranolol and hydrochlorothiazide at retention time 6.744, 7.230, 4.123 and 1.368 min, respectively.

Linearity of the Calibration Curve

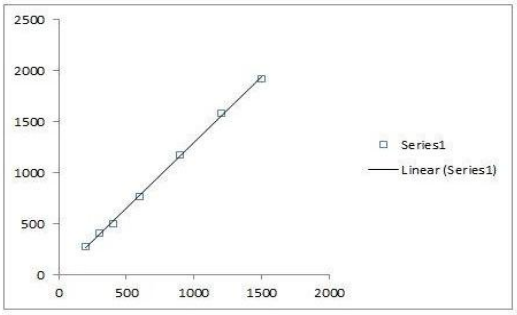
Linearity of the calibration curve was found in concentrations ranges of metoprolol, atenolol, propranolol and hydrochlorothiazide 4.0 to 20, 2.0–15, 10.0 to70 and 3.0 to 15 µg/mL of HCTZ, respectively. The regrassion coefficient of was in

acceptable range ≥ 0.9978, which indicated good linearity over these ranges. The literality plots are given in Fig. 6.

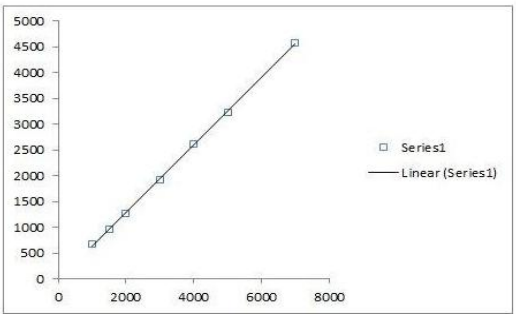
A. METOPROLOL



B. ATENOLOL



C. PROPRANOLOL



D. HYDROCHLOROTHYZIDE

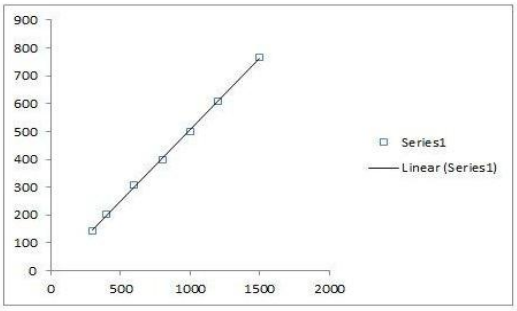


Figure 6: Linearity plot of metoprolol, atenolol, propranolol and hydrochlorothyzide

Determination of LOD and LOQ:

The LOD and LOQ were evaluated for the analytical method using a column by the statistical data of calibration curve of the concentration. The results obtained are reported in Table 1.

Table 1: Values of LOD and LOQ

Parameter	Metoprolol	Atenolol	Propranolol	Hydrochlorothiazide
LOD	53.50	54.19	136.46	77.13
LOQ	160.68	162.75	409.80	231.64

The effect of concentration of mobile phase on retention time and resolution of the analyte was also observed. The results are reported in Table 2.

Table 2: Effect of mobile phase composition on the retention time and resolution of the analytes on C18 column.

Mobile phase	**Formic acid (0.1%) in water & Formic acid (0.1%) in acetonitrile**
Analyte	Retention time (min)
Metoprolol	6.477
Atenolol	7.230
Propranolol	4.123
Hydrochlorothaizide	1.368

Detection wavelength: 228 nm; Temperature: 35° C; flow rate: 1.0 mL/min. Accuracy are precision data on intra-day and inter-day basis was also evaluated. The results are reported in Table 3.

Table 3: Intra-day and inter-day accuracy and precision data

Conc. (g/mL)	**Intra-day (*n* = 5)**			**Inter-day (*n* = 5)**		
	Meanconc. Found (g/mL)	**Precision (% CV)**	**Accuracy (%)**	**Mean conc. found (g/mL)**	**Precision (% CV)**	**Accuracy (%)**
Metoprolol						
600	603.148	1.284	100.524	603.06	1.273	100.51
1000	1000.546	0.613	100.054	1002.462	0.725	100.24
1800	1803.6	0.588	100.2	1803.346	0.600	100.185
Atenolol						
350	350.732	1.310	100.209	351.248	1.748	100.356
750	748.288	0.987	99.771	749.82	0.829	99.977
1300	1298.166	0.557	99.858	1297.81	0.507	99.831
Propranolol						
1750	1751.07	0.458	100.061	1750.2	0.450	100.011
3500	3501.79	0.420	100.051	3502.56	0.460	100.07
6000	5999.94	0.194	99.999	5999.79	0.229	99.996
Hydrochlorothaizide						

500	500.77	1.519	100.154	499.81	1.599	99.962
750	747.08	0.917	99.611	747.48	1.265	99.664
1300	1298.64	0.571	99.895	1299.37	0.586	99.951

CV: Coefficient of correlation; *n*: Number of replicates

Various tables were analyzed by this developed method and results are reported in Table 4.

Assay of tablet formulation

In order to evaluate the content of pharmaceutical formulations, tablets of different samples (Lopressor, Tenoretic, and Inderide,) were separately weighed and ground to fine powder. An amount equivalent 5 mg METO/500 mg HCTZ for Lopressor 150 mg ATE/1000 mg HCTZ for Tenoretic, and 5 mg PRO/500 mg HCTZ for Inderide were transferred into separate volumetric flasks in 250 mL distilled water. Then, solutions were sonicated for 15 min and diluted with methanol. A working solution after filtration, with concentration of 1000 µg/mL METO and 1000 µg/mL HCTZ for Lopressor, 1000 µg/mL ATE and 1000 µg/mL HCTZ for Tenoretic, and 1000 µg/mL PRO and 1000 µg/mL HCTZ for Inderide, were prepared by diluting this stock solution with methanol. It was then applied to the column in 100 replicates. Peak areas were measured at 228 nm and the amount of drug present in the tablet in each care was estimated from their regression equations.

Table 4: Analysis of pharmaceuticals using the develop RP-HPLC method.

Formulation	**Name of drug**	**Claimed value (mg)**	**Found values (mg ± SD)**	**Assay (%)**		**Spiked amount (µg/mL)**	**Amount recovered (µg/mL)**	**Recovery (%)**	**Precision (% CV)**
				HPLC method					
Lopressor	Metoprolol	5	4.97 ± 0.07	4.96 ± 0.06 t = 2.16; F = 3.64	99.20	2.0	1.97	98.5	2.01
	Hydrochlorothaizide	500	496.9 ± 8.04	498.6 ± 5.04 t = 0.75; F = 1.56	99.72	20.0	19.95	99.7	1.19

Tenoretic	Atenolol	150	149.1 ± 2.68	148.7 ± 2.94 t = 1.69; F = 4.64	99.13	8.0	8.11	101.4	1.85
	Hydrochlorothaizide	1000	999.5 ± 13.5	1004.5 ± 11.5 t = 1.85; F = 3.39	100.45	20.0	19.86	99.3	1.70
Inderide	Propranolol	5	4.95 ± 0.07	4.94 ± 0.08 t = 2.05; F = 3.12	98.43	4.0	4.03	100.7	2.15
	Hydrochlorothaizide	500	498.8 ± 10.39	501.8 ± 6.39 t = 0.98; F = 1.37	100.36	20.0	19.91	99.6	1.26

SD: Standard deviation; CV: Coefficient of correlation

Precision of Analytical Method

The proposed method was tested by replicate injections of 5 µg/mL of the solution five times on the same day as an intraday precision study of HCTZ using columns. The % RSD values were found to be metoprolol, atenolol, propranolol and hydrochlorothiazide were 2.11, 1.30, 0.75 and 0.293 on the Xbridge C18column, respectively, which indicated a good precision of the HPLC method. The results of recovery (accuracy) of the drug are reported in Table 5.

Table 5: Recovery (accuracy) of the drugs in dosage forms by standard addition technique.

Drug	Amount of drug taken (g/mL)	Amount of pure drug added (g/mL)	Total amount found (g/mL ± SD)
Hydrochlorothiazide	8.0	6.4	14.4 ± 1.131
	8.0	8.2	16.2 ± 0.141
	8.0	9.6	17.6± 1.131
Metoprolol	4.0	3.2	7.2 ± 0.565
	4.0	4.1	8.1± 0.070

	4.0	4.8	8.8 ± 0.565
Atenolol	6.0	4.2	10.2± 1.272
	6.0	6.4	12.4 ± 0.28
	6.0	8.3	14.3 ± 1.626
Propranolol	2.0	1.6	3.6 ± 0.282
	2.0	2.2	4.2 ± 0.141
	2.0	42.4	4.4 ± 0.282

SD: Standard deviation; *n*: Number of replicates

Table 6: Optimized chromatographic conditions and system suitability parameters

Parameters	**Chromatographic condition**
Instrument	Agilent 1260 infinite series HPLC pump
Column	EZ chrome X bridge C18(150 x 4.6, 5µ)
Detector	PDA
Mobile phase	0.1% Formic acid in water & 0.1% Formic acid in acetonitrile
Flow rate	1 mL/min
Detection wavelength	228 nm
Run time	17 min
Temperature	35^{o} C
Injection volume	5 µg/ml
Retention time	Metoprolol-6.744 min
	Atenolol-7.230 min
	Propranolol-4.123 min
	Hydrocholorothiazide-1.368 min

CONCLUSIONS

A RP- HPLC method for simultaneous estimation of the drugs in used multi component formulations was developed which was simple, precise, accurate, and suitable. Thus, a single HPLC method has been reported for simultaneous estimation of metoprolol, atenolol, propranolol and hydrochlorothiazide in combined dosage forms.

This proposed method is high performance liquid chromatographic method with reasonably good, accuracy, precision, linearity, LOQ and LOD. The obtained signals were be accurate, precise, and linear. The solvent consumption along with analytical run time is 17 min. Hence, this HPLC method can be used a routine sample analysis.

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