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METHOD DEVELOPMENT AND VALIDATION OF SOME ANTIHYPERTENSIVE DRUGS WITH HYDROCHLOROTHIAZIDE BY RP-HPLC

Mohini A. Patel*, Dr. Pranav Pandya, Dr. Suresh Chanda Ameta, Dr. Mayank Mehta, Dr. Seema Kothari

Department of Chemistry, PAHER University, Udaipur (Raj.), India.

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***Corresponding Author**
Mohini A. Patel
Department of Chemistry, PAHER University, Udaipur (Raj.), India.

ABSTRACT

A simple, reliable, specific, accurate and precise reverse phase high performance liquid chromatography method was developed and validated for simultaneous estimation of atenolol, metoprolol, propranolol with hydrochlorothiazide in pharmaceutical dosage form. Chromatographic separation was carried out on Agilent HPLC 1200 series bridge C18 column (Xbridge C18, size: 250 mm x 4.60 mm, particle size 5 µm) with a mobile phase (0.1% formic acid in water and acetonitrile) with gradient mode of a flow rate of 1 mL/min. The detection was monitored at 228 nm. The method was found applicable in the concentration range of 10 to 90%. The proposed Method was successfully applied for quantitative determination of atenolol, metoprolol, propranolol with hydrochlorothiazide in dosage form. The retention times of Atenolol, metoprolol, propranolol and hydrochlorothiazide were found to be 7.230,6.477,4.123 and 1.368min respectively theoretical plate count as per ICH limits. The limit of detection is achieved on a lowest level for making a sensitive and robust method for simultaneous and assay analysis. The Rp-HPLC method was validated and was successfully employed for the analysis of pharmaceutical formulations containing Atenolol, metoprolol, propranolol and Hydrochlorothiazide in combined tablet dosage form.

KEYWORDS: Antihypertensive atenolol, metoprolol, propranolol, hydrochlorothiazide; RP-HPLC; Method development & validation.

INTRODUCTION

Antihypertensive is a group of drugs used to treat the high blood pressure. Antihypertensive therapy also used to prevent the complications arises due to high blood pressure, such as stroke and myocardial infarction. Evidence suggests that reduction of the blood pressure by 5 mm Hg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21% and reduce the complications of dementia, heart failure, and death from cardiovascular disease. There are many classes of antihypertensives drugs that's lower blood pressure by different mechanism. The most important and most widely used drugs are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs) and beta blockers (β-blockers). β-Blockers are a class of medications that are predominantly used to manage abnormal heart rhythms, and second heart attack (myocardial infarction). They are also widely used to treat hypertension, although they are not longer the first-line of treatment of most patients. They are Atenolol, metoprolol, and propranolol are β - blockers.

Atenolol (RS)-2-[4-{2-Hydroxy-3(propane-2-ylamino) propoxy} phenyl]acetamide (Fig. 1) is a β-blocker medication primarily used to treat high blood pressure and heart associated chest pain. Only about half of (50%) atenolol is absorbed via gastro-intestinal tract and most of absorbed drug comes to the systemic circulation. The pharmalogical effects as β-blocker and antihypertensive drug continues for at least 24 hours after oral dose of 50 mg or 100 mg. The important function of atenolol as β-adrenergic to stimulate the heart to beat more rapidly. Atenolol is also helpful in treating angina. It is used in management of hypertension, angina pectoris, and cardiac arrhythamiasand myocardial infarction. It also be used in prophylactic treatment of migraine.[1]

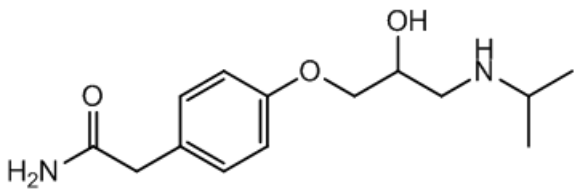

Figure 1: Structure of Atenolol.

Metoprolol (RS)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(propane-2-yl)amino]propan-2-ol (Fig. 2) is a cardioselective β - blocker used in the treatment of hypertension, angina pectoris, arrhythmia, myocardial infarction and heart failure. It is estimation an official in Indian pharmacopoeia and British pharmacopoeia. They are potentiometric method for describe IP and BP its estimation. RP - HPLC method for simultaneous determination of metoprolol with

hydrochlorothiazide are reported in literature for estimation of metoprolol in pharmaceutical dosage forms as well as in biological fluids. β_1 Adrenergic receptors are small activity against β_2 adrenergic receptors of the lungs and vascular smooth muscle. Receptor selectivity decreases with higher doses.[2,3]

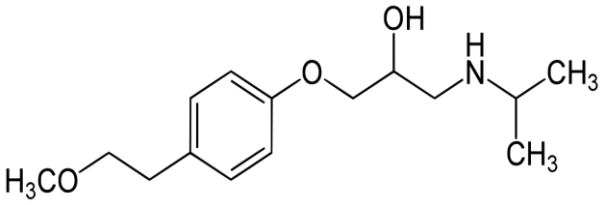

Figure 2: Structure of Metoprolol.

Propranolol (RS)-1-(1-Methylethylamino)-3-(1-naphthyloxy) propan-2-ol (Fig. 3) is a non-selective β-adrenergic antagonist with not intrinsic sympathomimetic activity. It is used in the management to hypertension, pheochromocytoma, angina pectoris, myocardial infarction and cardiac arrhythamias. It is also used to prevent migraine headaches and to further heart problems in those with angina. However, it has different side effects such as mental depression, nausea, vomiting, light headness. Atenolol, metoprolol with hydrochlorothiazide (HCTZ) may lower blood pressure and heart rate. This can cause dizziness or feeling like pass out weakness, fainting, fast and irregular heartbeats or loss of blood glucose control. Propranolol with HCTZ is used to treat the high blood pressure. The high blood pressure adds to the workload of the heart and arteries. It is continues for a long time are heart and arteries not work properly.[4]

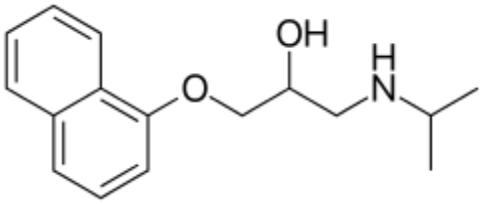

Figure 3: Structure of Propranolol.

Hydrochlorothiazide [6-Chloro-1,1dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide] (HCTZ or HCT) is a diuretic medication often used to treat the high blood pressure and swelling due to fluid buildup. They are other uses include diabetes insipidus, to decrease the risk of kidney stones and renal tubular acidosis and in those with a high calcium level in the urine. For the high blood pressure and it is sometimes considered as a first-line treatment. Chlortalidone is more effective with a similar rate of affected. HCTZ is taken by

mouth and may be combined with anyother blood pressure medications as a single pill to increase effectiveness.[6]

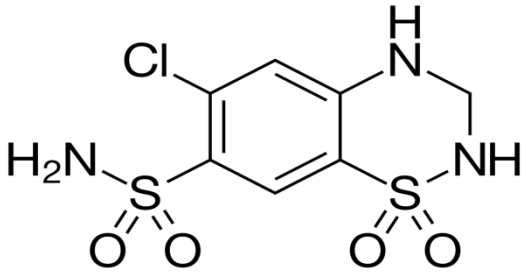

Figure 4: Structure of Hydrochlorothiazide.

Combination of β-blockerswith HCTZ acts by blocking the β-adrenergic receptors in the sympathetic nervous system and which results in a reduction of systolic pressure, heart rate, cardiac contractility. β - Blockers are routinely used to treat hypertension and to reduce the risk of cardiovascular disease and stroke. Thiazide diuretics decrease active reabsorption of Na and Ca ions by inhibiting the sodium/chloride co-transporter in the distal convoluted tubule. They also increase potassium ion loss. Thiazide diuretics decrease the blood volume by diuresis and are also used in the treatment of hypertension. The combination of a β - blockers with thiazide diuretic results in additive antihypertensive effect as compared to using either agent alone.

The aim of the present investigation was to develop a simple, precise and accurate RP-HPLC method for determination of atenolol, metoprolol, and propranolol in combination with HCTZ drugs. The method was validated is compliance with ICH guidelines.

MATERIALS AND METHODS

Instrumentation

HPLC separations of atenolol, metoprolol, propranolol and hydrochlorothiazide were performed on a Agilent 1260 infinity series equipped with an quaternary HPLC pump, an autosampler with partial loop volume injection system, column ovens (compatible with 250 mm length column) and a PDA detector. The EZ Chrom software was used for system control and data acquisition. HPLC separation of atenolol, metoprolol, propranolol and hydrochlorothaizide was carried out with 0.1% formic acid in water and 0.1% formic acid in acetonitrile with gradient elution and the flow rate of 1 mL/min at 35°C. The X-bridge C18 column (250 mm × 4.6 mm, 5 µm) was used.

Chemicals and Reagents

Atenolol, propranolol, metoprolol (purity > 98%) and hydrochlorothaizide (purity > 98%) was provided from Torrent Pharmaceuticals (Ahmedabad, India). Methanol and acetonitrile (HPLC grade) were purchased from Finar Chemicals (Ahmedabad, India). They are other chemicals were of analytical grade and bought from commercial sources.

Chromatographic calculations

The resolution factor (Rs) is 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 the latter has eluted enantiomers, respectively. The retention factors for each enantiomers, k_1 and k_2, are 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-tri-butylbenzene. Selectivity factor (α) is obtained according to the equation $\alpha = k_2/k_1$. In addition, theoretical plate number (N) is obtained from the software calculations.

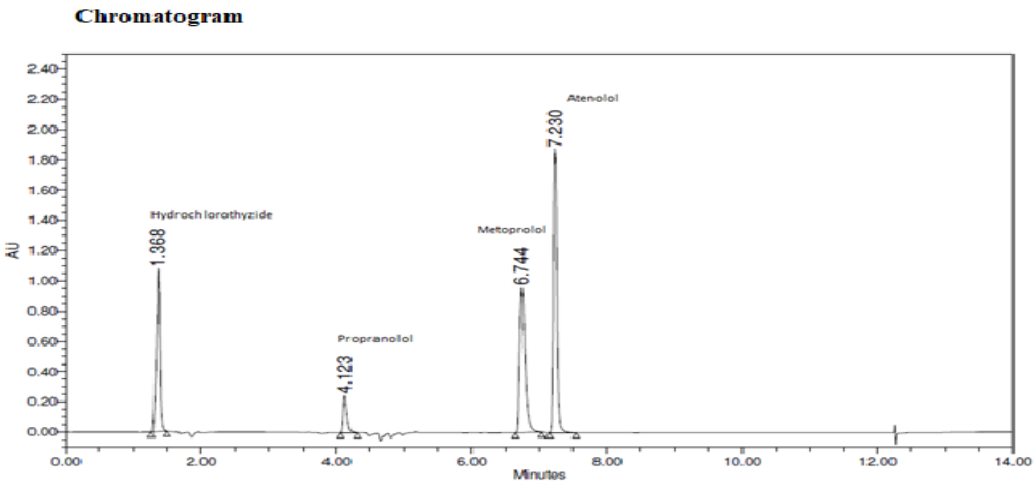

Figure 5: Chromatogram of all spiked drugs.

Accuracy

Accuracy is the explanation between the accepted true value or a reference value and the actual result obtained. Accuracy recovery studies were usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed.

Precision

Precision is find to be intra-day and inter-day study. The intra‑day precision refers to the use of analytical method within a laboratory over a short time of using the same operator with the

same equipment. The inter-day Precision include that estimation of variations in analysis when a method is used within a laboratory on different days, by same analysts.

Application of the Method to Dosage Form

The proposed method was applied successfully for the determination of the HCTZ drug in its pharmaceutical dosage. This application was done using XbridgeC18 column. The Recovery calculated was equal to Atenolol, metoprolol, propranolol and hydrochlorothiazide were 101.4%, 98.5%, 100.7% and 99.50% indicating that the proposed method is applicable for the determination of Hydrochlorothiazide in pharmaceutical dosage form.

RESULTS AND DISCUSSION

Method Validation

Validation of the analytical method prior to determining HCTZ in dosage sample was done by examining parameters of precision, linearity and LOD & LOQ using columns as follows.

Method development

For getting mobile phase for the analysis of the selected drug combination, various mixtures of 0.1% formic acid in water and acetonitrile in a composition of gradient resulted in symmetric peak of 228 nm in short run time. Injection volume was selected to be 5 µg/mL which gave a good peak area. The column used for study was EZ chrome Xbridge C18 chosen good peak shape of symmetrical. Temperature was found to be suitable for drug solution. The flow rate was fixed at 1.0 mL/min because of good peak area. Chromatogram showed a peak of atenolol, metoprolol, propranolol and hydrochlorothiazide at retention time 7.230, 6.744, 4.123 and 1.368 min, respectively.

Linearity of the Calibration Curve

Linearity of the calibration curve was determined ranges of concentrations of Atenolol, metoprolol, propranolol and hydrochlorothiazide were found to be 2.0–15 µg/Ml, 4.0 to 20 µg/mL, 10.0 to70 µg/mL and 3.0 to 15 µg/mL of HCTZ respectively. The values of coefficient of determination were found to be acceptable ≥ 0.9978 showing good linearity over the ranges studied of the assay method.

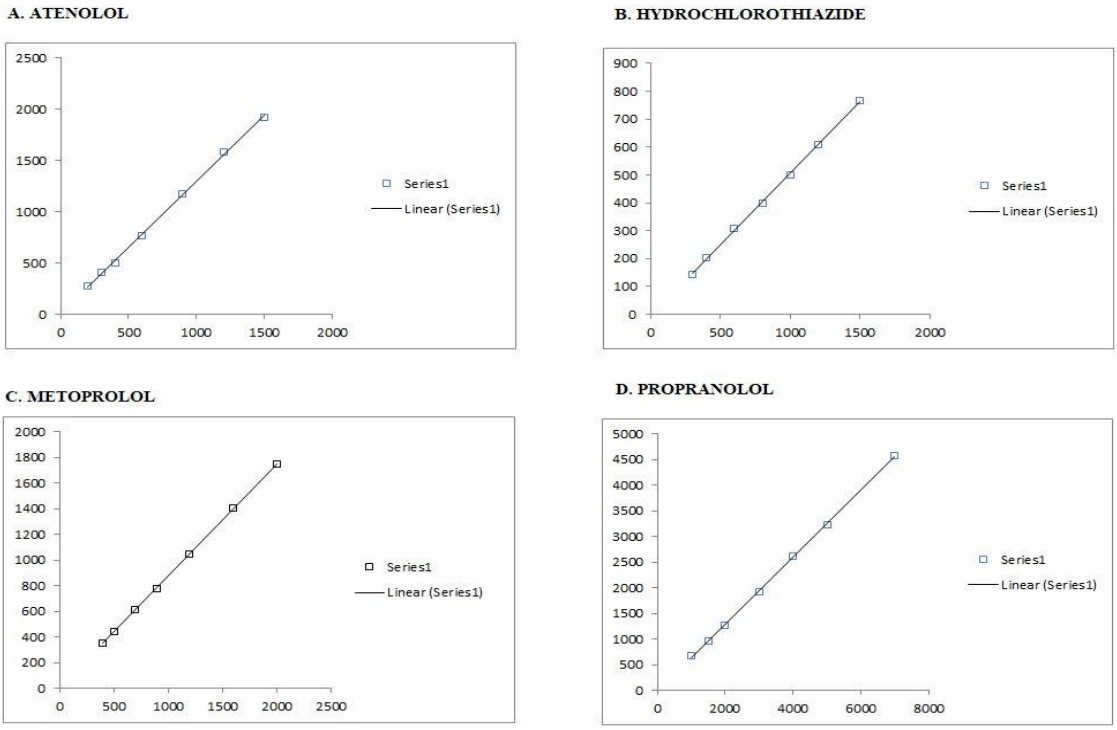

Figure 6: Linearity plot of atenolol, metoprolol, propranolol and hydrochlorothyzide.

LOD and LOQ Determination: The LOD and LOQ were determined for the analytical method using column by the statistical data of calibration curve of the concentration. The results obtained showed values found to be above drugs.

Table 1: Values of LOD and LOQ.

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

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

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

Temperature: 35° C; detection wavelength: 228 nm; flow rate: 1.0 mL/min.

Table 3: Intra-day and inter-day accuracy and precision data for all analytes in neat samples.

Conc.(µg/mL)	Intra-day (*n* = 5)			Inter-day (*n* = 5)		
	Meanconc. Found (µg/mL)	Precision (% CV)	Accuracy (%)	Mean conc. found (µg/mL)	Precision (% CV)	Accuracy (%)
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
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
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
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

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

Assay of tablet formulation

In order to evaluate the content of pharmaceutical formulations, Tenoretic, Lopressor and Inderide, tablets of each were separately weighed and ground to fine powder. An amount equivalent 150 mg ATE/1000 mg HCTZ for Tenoretic, 5 mg METO/500 mg HCTZ for Lopressor and 5 mg PRO/500 mg HCTZ for Inderide were transferred into separate volumetric flasks containing 250 mL distilled water. The solutions were sonicated for 15 min and then diluted with methanol. After filtration, a working solution having concentration of 1000 µg/mL ATE and 1000 µg/mL HCTZ for Tenoretic, 1000 µg/mL METO and 1000 µg/mL HCTZ for Lopressor and 1000 µg/mL PRO and 1000 µg/mL HCTZ for Inderide, respectively were prepared by diluting the stock solution with methanol and was applied to the column in 100 replicates. Peak areas were measured at 228 nm and the amount of each drug present in the tablet 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					
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
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
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

Evolution of the Precision of Analytical Method

The method was checked by replicate injections of 5 µg/mL of the solution five times on the same day as intraday precision study of HCTZ using columns. % RSD values were found to be Atenolol, Metoprolol, Propranolol and hydrochlorothiazide were1.30,2.11,0.75 and 0.293 on the Xbridge C18column, respectively, indicating a good precision of the HPLC method.

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

Drug	Amount of drug taken (µg/mL)	Amount of pure drug added (µg/mL)	Total amount found (µg/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
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
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
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	Atenolol-7.230 min
	Metoprolol-6.744 min
	Propranolol-4.123 min
	Hydrocholorothiazide-1.368 min

CONCLUSIONS

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method for simultaneous estimation of the drugs in used multi component formulations. Single HPLC method has been reported so far for simultaneous estimation of atenolol, metoprolol, propranolol and hydrochlorothiazide in combined dosage forms. The proposed high performance liquid chromatographic method has accuracy, precision, linearity, LOD and LOQ. The measured signals were shown to be precise, accurate and linear. Morever the solvent consumption along with analytical run time is 17 min. Therefore, this HPLC method can be used a routine sample analysis.

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