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Research Paper

ANALYTICAL METHOD DEVELOPMENTFOR THE SIMULTANEOUS DETERMINATION OF CHLORTHALIDONE AND METOPROLOL SUCCINATE

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To obtain effective separation of the eluted drugs, the HPLC settings were optimized in this study. Initially, various compositions of mobile phase were used in this approach. Peak metrics (TP, TF), run time, and Rs were used to choose the mobile phase.ACN and 0.1 percent TEA (pH 4.5 regulated using 1 percent OPA) in 90:10 percent v/v flowing at 1mL/min. The optimal detection wavelength was reported to be 224nm, and gave a greater detection of drug samples. This method was validated on the report of precision, accuracy, specificity, linearity. In all the cases the method was stable with acceptance criteria followed by ICH guidelines. Mobile phase used in this method was very commonly available and it is sufficient for the quantification analysis of CLT and MPS either in single dosage or in combination form. These drugs were separated in less than 6.4 min with low tailing factor and good resolution without any interference of excipients.

Keywords: Chlorthalidone, Metoprolol Succinate, High Performance Liquid Chromatography, Method Development.

INTRODUCTION

Chlorthalidone is a medication primarily used to treat high blood pressure (hypertension) and fluid retention (edema) associated with conditions like heart failure or kidney disease.[1]It is a thiazide-like diuretic, which means it helps the body get rid of excess salt and water by increasing urine production. This action helps lower blood pressure and reduces swelling.

Chlorthalidone works by inhibiting sodium reabsorption in the kidneys, which decreases blood volume and reduces pressure on blood vessel walls. It's often preferred over other diuretics due to its longer duration of action.[2-3]

Chlorthalidone is a thiazide-like diuretic, structurally related to thiazide diuretics but with some differences. Its chemical name is 2-chloro-5-(1-hydroxy-3-oxo-1,2,3,4-tetrahydro pyrimidin-4-yl)-1,3-benzothiazole.

Chlorthalidone works primarily as a diuretic, and its mechanism of action involves inhibition of sodium and chloride reabsorption in the kidneys, specifically in the distal convoluted tubules.[4]

Metoprolol succinate is a beta-blocker commonly prescribed for the treatment of high blood pressure (hypertension), heart failure, angina (chest pain), and to prevent heart attacks. It is the extended-release (ER) formulation of metoprolol, which allows for once-daily dosing.[5]The drug consists of a propanolamine backbone (typical of beta-blockers), an ethoxy group, and a succinate

salt, which is used to provide the extended-release form. The succinate portion of the compound helps slow the absorption and release of metoprolol in the body. Metoprolol works by selectively blocking the beta-1 adrenergic receptors in the heart. These receptors are part of the sympathetic nervous system and are primarily responsible for regulating heart rate, contractility, and conduction.[6-7]

Hence it is planned to develop a new, simple, precise, accurate, and stability indicting method for the simultaneous estimation of Chlorthalidone and Metoprolol succinate. Accordingly the authors made several attempts to optimize the conditions and validated for documenting the capabilities of the proposed method.

EXPERIMENTAL

Chemicals and Reagents:

The reference standard samples of Chlorthalidone and Metoprolol Succinate were obtained as gift samples from Ind Swift Ltd, India.

Double distilled water was used. AR grade solvents were used during the whole study. HPLC grade Acetonitrile and Methanol(Merck) was used for the mobile phase. sodiumdihydrogen phosphate were of also AR Grade (Merck).

Instrument:

The chromatographic separation was carried out on an LC – 10 – ATVP HPLC system (Shimadzu class VP).

Selection of wavelength

When using a UV detector in HPLC, the method's selectivity is dependent on theright wavelength selection. The wavelength of the drug's response must be chosen carefully, because both medicines had UV spectrums that were comparable to each other,the 224 nm wavelength was chosen for this study,

Initial operating conditions

Stationary phase : Thermo Scientific BDS C18 column (250 × 4.6 mm i.d, 5)

Flow rate : 1mL/minute

Operating temperature : Room temperature

Selected wavelength : 224 nm

Selection of mobile phase

Chromatographic conditions were optimised by varying solvent selectivity (solvent type), solvent strength, buffer strength and pH, flow rate etc. Various mobile phases assessed for selecting the appropriate mobile phase are given in Table 1.

Table 1: Different Mobile phase conditions

Mobile Phase condition	Observation
Methanol : Water (70:30)	Broad Peak for Metoprolol
Methanol: 0.1% triethyl amine buffer pH 4 (70:30)	Tailing Splited peaks
Methanol:0.1% tri ethylamine buffer pH 4 (80:20)	Tailing Splited Broad peaks
Methanol: Acetonitrile (50:50)	Tailing Fronting for Chlorthalidone
Acetonitrile: 0.1% tri ethyl amine pH 4.5 1ml/min (80:20)	Fronting with good Resolution
Acetonitrile: 0.1% tri ethylamine pH 4.5 1ml/min (90:10)	Symmetrical peaks with good Resolution

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Optimization of separation conditions

➢ **Effect of strength of tri ethylamine**

In the 70:30 v/v acetonitrile: trimethylamine ratio, several trimethylamine ionic strengths such as 0.1 percent, 1 percent, 0.5 percent, etc. were tested at pH 4. 0.1 percent triethylamine was found to have excellent peak characteristics. As a result, a concentration of 0.1 percent tri ethylamine was determined to be optimal.

Table 2: Effect of strength of tri ethylamine

Strength (%v/v)	Observation
0.1	Good
0.5	Tailing
1	Tailing

➢ **Effect of ratio of mobile phase**

Several alternative acetate/tri ethylamine ratios, such as 80:20:90:10 v/v, were tested in the mobile phase system. There was good separation between the peaks, so the ratio of 90:10 percent was chosen as the best one.

Table 3: Effect of ratio of mobile phase

Acetonitrile: 0.1% tri ethylamine (v/v)	Observation
80:20	Fronting and Broad peaks
90:10	Good Symmetrical peaks

➢ **Effect of Ph**

Keeping mobile phase composition at 90:10 %v/v, the chromatograms were captured using varied pH such as 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0etc, adjusted using 1% Orthophosphoric acid. It was determined that the peak forms of both medicines at pH 4.5 were satisfactory, and they were selected for further investigation.

Table 4: Effect of pH Conditions

pH	Observation
3.0	Fronting Tailing andSplited peaks
3.5	Tailing andSplited peaks
4.0	Broad peaks
4.5	Good Symmetrical peaks
5.0	Tailing
6.0	Unsymmetrical peaks

RESULTS AND DISCUSSION

On the basis of above analytical method development trials following optimized chromatographic conditions were found suitable for separation of drugs.

Mobile Phase: Acetonitrile: 0.1% TEA (TEA pH 4.5, maintained using OPA)

Column : Thermo Scientific BDS C18 column (250 × 4.6mm,5 µm)

Solvent Ratio: 90:10%v/v

Flow Rate : 1.0mL/Min

Sample Temperature: 25^0C

Column Temperature: 25^0C

Volume : 20µL

Mode of operation : Isocratic elution

Detector : 224

Retention Time (RT): Chlorthalidone : 2.6 min

Metoprolol Succinate : 6.4 min

Procedure

20 µl of standard solution was fed into the column, then peak areas LAM, TDF and DOR were noted, and the percent assay was

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Table 5: Drugs with their RT, Area, TP, TF

Drug	RT	AREA	USP PLATE COUNT	USP TAILING
Lamivudine	2.531	2623699	6532	1.30
Tenofovir	3.530	3511298	7676	6.81
Doravirine	5.691	1690785	8702	10.41

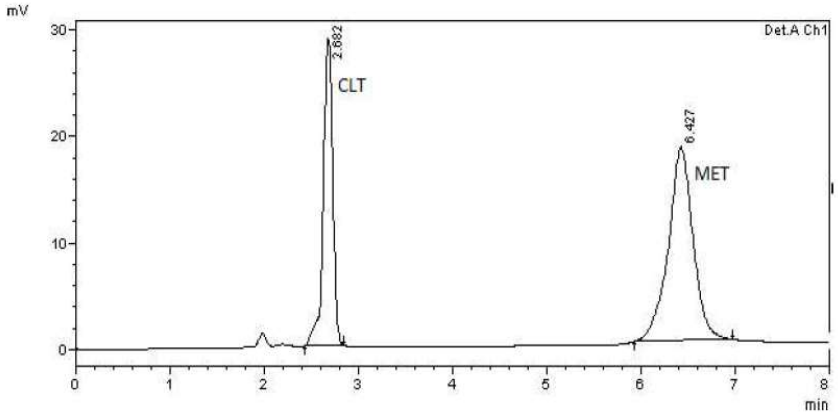

Fig. 1: Chromatogram for optimized method

computed using the formula.

CONCLUSION:

To obtain effective separation of the eluted drugs, the HPLC settings were optimized in this study. Initially, various compositions of mobile phase were used in this approach. Peak metrics (TP, TF), run time, and Rs were used to choose the mobile phase.ACN and 0.1 percent TEA (pH 4.5 regulated using 1 percent OPA) in 90:10 percent v/v flowing at 1mL/min. The optimal detection wavelength was reported to be 224nm, and gave a greater detection of drug samples.

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Conflict of Interest

The authors declare that they have no conflict of interest