A NEW RP-HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE, THEOPHYLLINE AND FUROSEMIDE .

Sawant Ramesh L., Bharat Anjali V *., Tanpure Kallyani D., Jadhav Kallyani A.

Pad. Dr. Vitthalrao Vikhe Patil Foundation's College Of Pharmacy,
Viladghat, Post MIDC, Ahmednagar, Maharashatra.
Email- anjalibharat055@gmail.com

ABSTRACT

The objective of the current study was to develop a simple, precise and accurate isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of salbutamol sulphate, theophylline and furosemide in synthetic mixture. Isocratic RP-HPLC separation was achieved on an Hibar^R 250 × 4.6 mm HPLC column Purosphens^R STAR RP-18, using a mobile phase of methanol : water (50:50, v/v) at a flow rate of 1.0 mL/min. Good sensitivity for all analysts was observed with UV detection at wavelength of 274 nm. The method result in excellent separation with good resolution between the three analysts. The retention times of salbutamol sulphate , theophylline and furosemide was found to be 10.04, 4.55and 4.58 mins.The method was used successfully for the simultaneous determination of salbutamol sulphate, theophylline and furosemide in synthetic mixture.

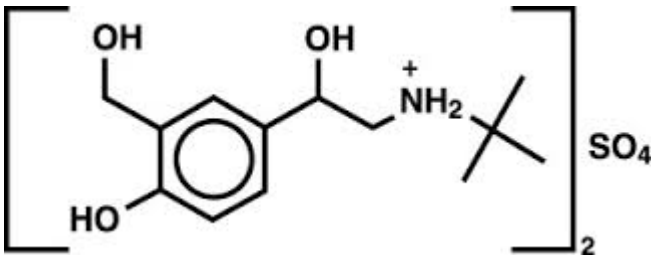
Keywords- furosemide , isocratic, methanol, RP-HPLC, salbutamol sulphate, theophylline.

INTRODUCTION

Salbutamol sulphate:

IUPAC Name: 4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol,Molecular formula:$C_{13}H_{21}NO_3$, Molecular weight: 239.311 g/mol, Description:It is a white powder, crystalline powder Solubility: freely soluble in water, practically insoluble or very slightly soluble in alcohol and in methylene Category: Anti-asthmatic drug[(2)].

Salbutamol sulphate is a β2-adrenergic receptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease[(2,3)]. Selective β2-adrenoceptor stimulant that causes the relaxation of the smooth muscles through the increase of the intracellular cyclic adenosine monophosphate (cAMP) due to this, bronchial and uterine muscles get relaxed, the peripheral vessels are dilated and heart rate increases[(3)]. Activation of the β-2 adreno-receptors opens ATPase channels and drives potassium from the extra cellular to the intracellular space. This both decreases extracellular hyperkalaemia and increases intracellular potassium, so decreasing the chance of arrhythmia[(4)].

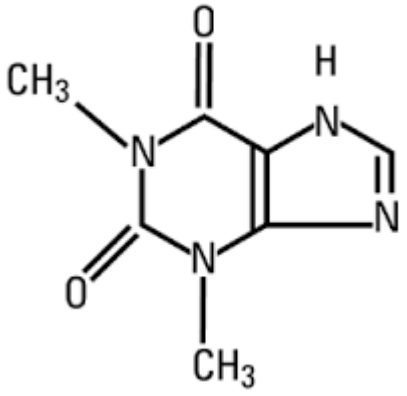


Theophylline:

IUPAC Name: 1,3-Dimethyl-7H-purine-2,6-dione, Molecular formula: $C_7H_8N_4O_2$, Molecular weight: 180.164 g/mol, Description: It is a white powder, Solubility: Freely soluble in methanol, sparingly soluble in water, Category: Anti-asthmatic drug[(6)].

Theophylline is the drug of choice for the treatment of asthma and chronic obstructive pulmonary disease (Current Index of Medical Specialties 2010; The Indian Pharmacopoeia 2007). Theophylline (THE) is competitive nonselective phosphodiesterase inhibitor. Which raises intracellular cAMP, activates PKA, inhibits

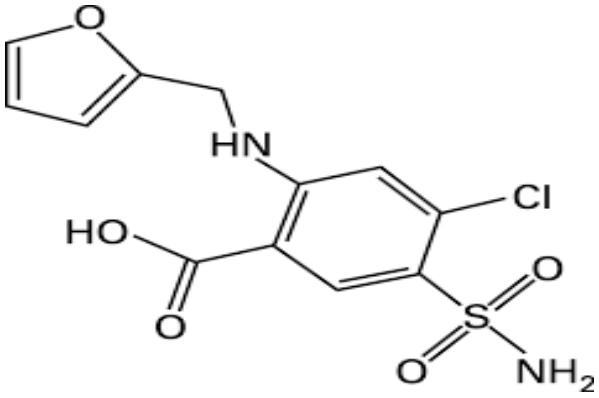
TNF-alpha and inhibits leukotriene synthesis, and reduces inflammation and innate immunity. Nonselective adenosine receptor antagonist, antagonizing A1, A2, and A3 receptors almost equally, which explains many of its cardiac effects. THE has been shown to inhibit TGF-beta-mediated conversion of pulmonary fibroblasts into myofibroblasts in COPD and asthma via cAMP-PKA pathway and suppresses COL1 mRNA, which codes for the protein collagen[7,8].



Furosemide:

IUPAC Name: 4-Chloro-N-furfuryl-5-sulphamoyl anthranilic acid, Molecular formula: $C_{12}H_{11}ClN_2O_5S$, Molecular weight: 330.75 g/mol, Description: It is a white or slightly yellow crystalline powder, Solubility: Freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol and practically insoluble in water, Category: Diuretic drug[16].

Like other loop diuretics, furosemide acts by inhibiting $NKCC_2$, the luminal Na-K-2Cl symporter in the thick ascending limb of the loop of henle[17]. The action on the distal tubules is independent of any inhibitory effect on carbonic anhydrase or aldosterone, . It also abolishes the corticomedullary osmotic gradient and blocks negative, as well as positive, free water clearance. It is a potent diuretic that inhibits the active reabsorption of chloride in the diluting segment of the loop of henle, thus preventing the reabsorption of sodium, which passively follows chloride. Additionally, FUR is a non-competitive subtype specific blocker of GABA-A receptors[18,19].



Asthma has been linked to cardiovascular diseases (CVDs) and related risk factors such as hypertension in adults. It is unclear whether the relationship between asthma and hypertension found among adults is also observed.

Literature survey reveals that the methods like UV- Spectrophotmetry and HPLC were reported for the estimation of SAL , THE, and FUR, individually and in combination with other drugs. Where as no RP-HPLC method has been reported for their simultaneous estimation. Hence, it is necessary to develop a simple, rapid, and accurate RP-HPLC method for the determination of SAL , THE, and FUR in synthetic mixture. These drugs are official in IP, BP and USP. This paper describes the development of reliable, simple reversed phase HPLC method, using UV detection, for the simultaneous estimation of SAL , THE, and FUR in synthetic mixture.

EXPERIMENTAL

Instrument:

HPLC system (PU2080HPLC2000, JASCO, Power requirement: 230V, 50Hz) with Jasco PU-2080 Plus (intelligent HPLC Pump), Jasco UV-2075 Plus Intelligent UV/Vis detector wit column was employed. BROWIN CHROMATOGRAPHY SOFTWARE was used for data acquisition and processing.

Analytical column:

Metformin hydrochloride and amlodipine besylate was analyzed by reverse phase-HPLC nalysis using HiQ sil C18 HS size 4.6 mm inner diameter 250 mm length, No. OH500218.

Chemical and Reagents: All analytical grade reagents were used.

Chromatographic Conditions:

A mixture of Methanol and Water in the ratio of (50:50 v/v) was used as as mobile phase and pH 3.20±0.05 adjusted with ortho-phosphoric acid. It was filtered through 0.45 µ membrane filter. The flow rate used was 1 ml/min. The detection was carried out at 272 nm. The injected volume was 20 µl. Run time used was 10 min.

Preparation of mobile phase

A mobile phase was prepared by mixing, methanol and water in the ratio of 50:50, v/v. The mobile phase was filtered using 0.45µ nylon filters (Millipore, USA) and was degassed by sonication before use.

Preparation of standard stock solution

Standard stock solutions for each drugs were prepared separately by dissolving 25 mg of drugs in mobile phase up to 25 mL. The volumetric flasks having 10 mL of mobile phase with the drugs were shaken ,sonicated for 5 min and finally volume was made up to get a concentration of 1000 µg.mL.. standard drugs solutions were filtered through a 0.4 µm membrane filter.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 10-60 µg/ml for SAL, THE, and FUR respectively.

Selection of detection wavelength

The ultraviolet spectra of SAL, THE, and FUR showed λ max at 225 nm, 272 nm and 275 nm respectively. Therefore the detection wavelength was selected as 272 nm where three drugs shows significant absorbance and hence this λ max was selected for further studies.

RESULT AND DISCUSSION

Method development and optimization

Method development process was carried out by examining conditions like flow rate (0.8 mL.min-1, 1.0 mL.min-1 and 1.2 mL.min-1). A flow rate of 1 ml/min gave an optimal signal to noise ratio with a reasonable separation time. mobile phase compositions like methanol: water and ratios (50:50, 60:40 and 70:30, v/v) were used. the drugs SAL, THE, and FUR were found showing a significant UV absorbance at 272 nm in methanol: water (50:50, v/v), so this wavelength was chosen for UV detection. By use of a C18 column it was found the mobile phase consisting of methanol: water (50:50, v/v) provided well defined peak shape with good resolution. The retention times for SAL, THE and FUR was found to be 10.30, 2.16 and 3.75 min respectively. The representative chromatograms of pure drug and combined drug product are shown in Figure 1,2,3 and 4 respectively.

CONCLUSION

The proposed HPLC method was found to be simple, accurate, precise, reproducible, rugged, robust linear, rapid and economical and can be used in quantitative analysis of the drug in synthetic mixture. RP-HPLC method can be employed successfully for the simultaneous determination of SAL, THE and FUR in combination.

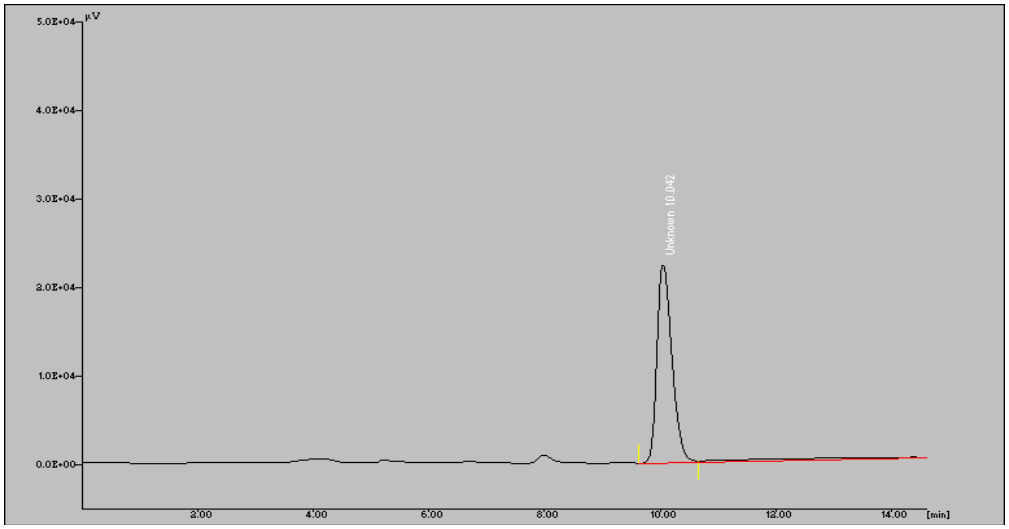


Figure 1. Representative chromatogram for salbutamol sulphate (retention time = 10.042)

Table 1: Chromatographic data for salbutamol sulphate

Name	RT	Area [µV.Sec]	Resolution	Plates	Capacity	Asymmetry
Salbutamol sulphate	10.042	374351.770	0.00	8682.42	1204.00	1.32

Total Area of Peak= 374351.770[µV.Sec]

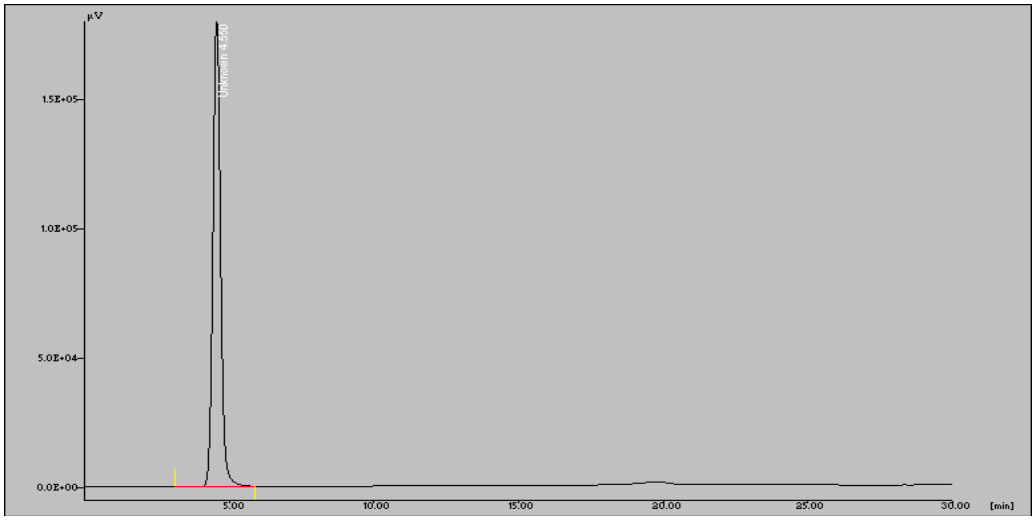


Figure 2. Representative chromatogram for theophylline (retention time = 4.550)

Table 2: Chromatographic data for Theophylline

Name	RT	Area[µV.Sec]	Resolution	Plates	Capacity	Asymmetry
Theophylline	4.550	2871388.000	0.00	2152.06	545.00	1.10

Total Area of Peak = 2871388.000 [µV.Sec]

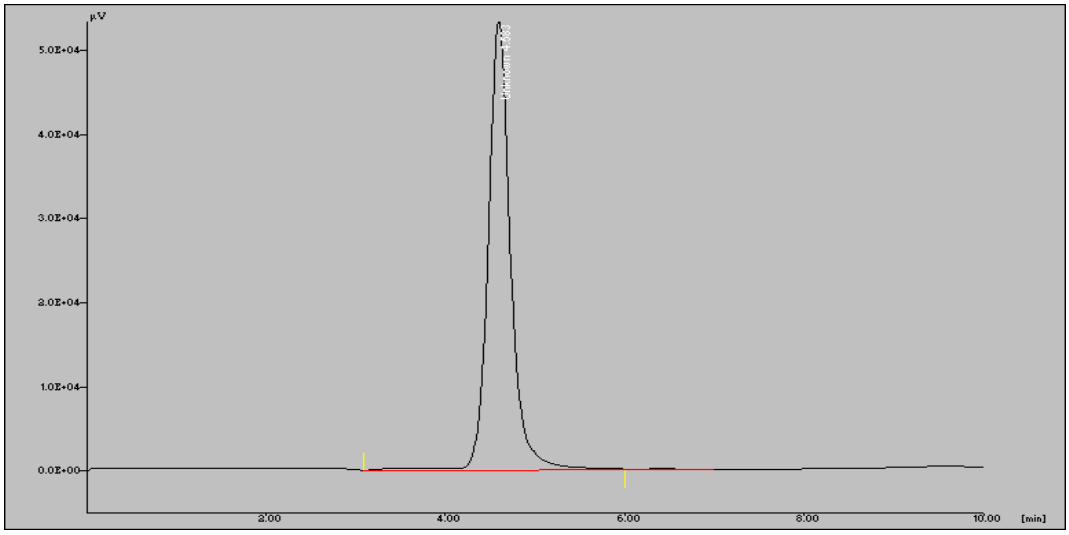


Figure 3. Representative chromatogram for furosemide (retention time = 4.583)

Table 3: Chromatographic data for Furosemide

Name	RT	Area[µV.Sec]	Resolution	Plates	Capacity	Asymmetry
Furosemide	4.583	879670.881	0.00	2197.47	549.00	1.15

Total Area of Peak = 879670.881[µV.Sec]

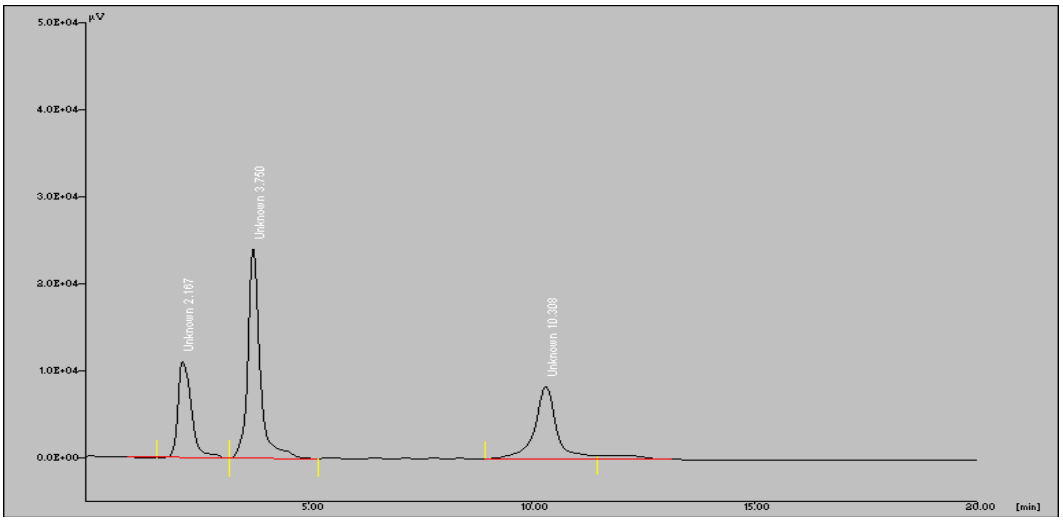


Figure 4. Representative chromatogram for SAL, THE and FUR in combination (retention Time of SAL, THE and FUR= 10.308, 2.16, and 3.7 min)

Table 4: Chromatographic data for SAL, THE and FUR

Name	RT	Area[µV.Sec]	Resolution	Plates	Capacity	Asymmetry
SAL	10.308	269748.854	12.64	3811.03	1236.00	0.87
THE	2.167	213764.458	0.00	328.49	259.00	1.61
FUR	3.750	417720.250	3.73	1626.40	449.00	1.30

Total Area of Peak = 901233.562[µV.Sec]

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