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Research Article

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Development and Validation of New Colorimetric Method for the Estimation of Salbutamol, Chlorthalidone and Metoprolol Succinate

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ABSTRACT

Colorimetric methods have been developed for the estimation of Salbutamol, Chlorthalidone & Metoprolol Succinate. Here Methods were based on reaction involving the formation of blackish colored complex with Potassium Ferricyanide & Ferric Chloride. The maximum absorbance of the solution was measured at 540 nm, 545 nm & 534 nm against blank respectively. The calibration curve calculated obeys Beer's law over the concentration range of 15-75, 14-70 & 25-125 µg/ml. The method was validated based on ICH guidelines. The high recovery and low relative standard deviation confirms the suitability of the method for determination Salbutamol, Chlorthalidone & Metoprolol Succinate. Hence it is useful for the routine analysis of Salbutamol, Chlorthalidone and Metoprolol Succinate.

Keywords: Salbutamol, Chlorthalidone, Metoprolol Succinate, Potassium Ferricyanide & Ferric Chloride UV spectrophotometric and validation parameters.

ARTICLE INFO

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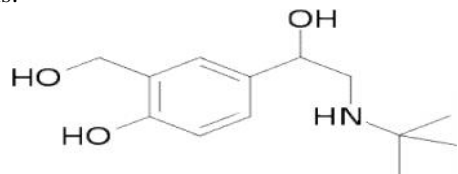
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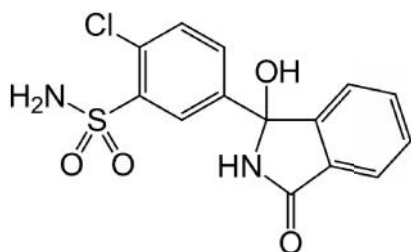
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1. Introduction

Salbutamol is a short-acting, highly selective 2-adrenergic stimulant, used in the treatment of bronchial asthma and other forms of reversible airways obstructive diseases. Salbutamol is rapidly absorbed after oral administration and undergoes presystemic metabolism in the gut, oral bioavailability is 50%. Its chemical name is 4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol. Its Molecular formula and molecular weight are C₁₃H₂₁NO₃ and 239.311 respectively. The structural formula is:



It's sparingly soluble in water, soluble in ethanol (96%), slightly soluble in ether. Salbutamol is a beta (2)-adrenergic agonist and thus it stimulates beta (2)-adrenergic receptors. Binding of albuterol to beta (2)-receptors in the lungs results in relaxation of bronchial smooth muscles. It is believed that salbutamol increases cAMP production by activating adenylate cyclase, and the actions of salbutamol are mediated by cAMP.¹ Chlorthalidone is an antihypertensive/diuretic tablets for oral use. It is a monosulfamyl diuretic that differs chemically from thiazide diuretics in that a double ring system is incorporated in its structure. It is a racemic mixture of 2-chloro-5-(1-hydroxy-3-oxo-1-isindoliny) benzene sulfonamide, with the following structural formula:



Its Molecular formula & Molecular weight are C₁₄H₁₁ClN₂O₄S & 338.766.

Chlorthalidone is practically insoluble in water, in ether and

2. Matirials and Methods

Preparation of standard stock solution of Salbutamol, Chlorthalidone, Metoprolol:

Stock solution of drugs were prepared by weighing accurately 100mg of pure drug into a 100ml volumetric flask and dissolved it and the volume was made up to mark with Dist. Water to get a concentration of 1000 µg/ml. and further dilution were did for Salbutamol and Chlorthalidone for to get 100 µg/ml.

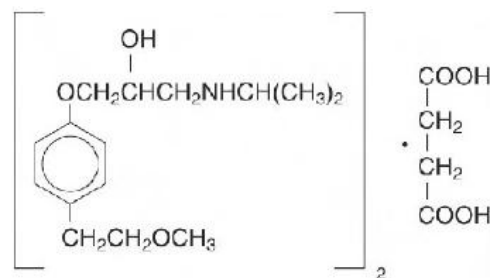
SALBUTAMOL:

Preliminary Investigation for Salbutamol:

To 1.5ml of the drug solution containing 15µg/ ml, 1 ml of 0.1% of Potassium ferricyanidewas added with 1 ml (0.1%)

in chloroform, soluble in methanol, slightly soluble in alcohol. Chlorthalidone is a long-acting oral diuretic with antihypertensive activity. Its diuretic action commences a mean of 2.6 hours after dosing and continues for up to 72 hours. The drug produces diuresis with increased excretion of sodium and chloride. The diuretic effects of Chlorthalidone and the Benzothiadiazine (thiazide) diuretics appear to arise from similar mechanisms and the maximal effect of Chlorthalidone and the thiazides appear to be similar.² Metoprolol succinate is a beta1-selective (cardio selective) adrenoceptor blocking agent, for oral administration, available as extended-release tablets. TOPROL-XL has been formulated to provide a controlled and predictable release of Metoprolol for once-daily administration. The tablets comprise a multiple unit system containing Metoprolol succinate in a multitude of controlled release pellets. Each pellet acts as a separate drug delivery unit and is designed to deliver Metoprolol continuously over the dosage interval. Its chemical name is (±) 1(isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt). Its Molecular formula & Molecular weight are (C₁₅H₂₅NO₃)₂ • C₄H₆O₄ & 652.8 Respectively.

The structural formula is:



Metoprolol succinate is a white crystalline powder. It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol, slightly soluble in dichloromethane and 2-propanol, practically insoluble in ethyl-acetate, acetone, diethyl ether and heptane.³ some methods developed for same but here developed method were new by Potassium Ferricyanide.⁴⁻¹⁰ And all methods were validated by ICH guideline.¹¹

Ferric Chloride & make up volume till 10ml with Dist. Water. It will show blackish colour and measure the max. Corresponding reagent blank was prepared in the same manner omitting the drug. Shown in figure no 1.

STD Curve for Salbutamol:

From the working standard drug solution of 1.5, 3, 4.5, 6 & 7.5 ml (which gives 15-75 µg/ml) drug solution was placed in 5 different 100 ml volumetric flasks. Into this 0.6 ml of 0.06% of Potassium ferricyanide was added with 2ml of 0.5% FeCl₃ then volume was made up to 10ml with Dist. Water. It will show Blackish colour Corresponding reagent blank was prepared in the same manner omitting the drug.

The absorbance was measured against a reagent blank at 540 nm. Show in figure no. 2 and table no. 1.

Chlorthalidone:

Preliminary Investigation:

To 4ml of the drug solution containing 40 μ g/ml, 1 ml of 0.1% of Potassium ferricyanide was added with 1 ml (0.2%) Ferric Chloride & make up volume till 10ml with Dist. Water. It will show blackish colour and measure the max. Corresponding reagent blank was prepared in the same manner omitting the drug. Show in figure no. 3.

STD Curve for Chlorthalidone

From the working standard drug solution of 1.4, 2.8, 4.2, 5.6 & 7 ml (which gives 14-70 μ g/ml) drug solution was placed in 5 different 100 ml volumetric flasks. Into this 0.8 ml of 0.04% of Potassium ferricyanide was added with 1.5 ml of 0.3% FeCl₃ then volume was made up to 100ml with Dist. Water. It will show blackish colour. Corresponding reagent blank was prepared in the same manner omitting the drug. The absorbance was measured against a reagent blank at 545nm. Show in figure no. 4 and Table no. 2.

Metoprolol

Preliminary Investigation

To 2.5ml of the drug solution containing 25 μ g/ml, 1 ml of 0.1% of Potassium ferricyanide was added with 1 ml (0.1%) Ferric Chloride & make up volume till 100ml with Dist. Water. It will show blackish colour and measure the max. Corresponding reagent blank was prepared in the same manner omitting the drug. Shown in figure no. 5.

STD Curve for Chlorthalidone

From the working standard drug solution of 2.5, 5, 7.5, 10 & 12.5 ml (which gives 25-125 μ g/ml) drug solution was placed in 5 different 100 ml volumetric flasks. Into this 0.6 ml of 0.05% of Potassium ferricyanide was added with 2ml of 0.5% FeCl₃ then volume was made up to 100ml with Dist. Water. It will show blackish colour. Corresponding reagent blank was prepared in the same manner omitting the drug. The absorbance was measured against a reagent blank at 534nm. Show in figure no. 6 and Table no. 3.

Validation for All Three Colorimetry:

Linearity: A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated

directly on the drug substance (by dilution of a standard stock solution) and using the proposed procedure. This method obeys the Beer-Lambert's law in the concentration range of 15-75, 14-70 & 25-125 μ g/ml for Salbutamol, Chlorthalidone & Metoprolol respectively.

Accuracy:

Accuracy was established across the specified range of the analytical procedure. Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples.

Precision:

Repeatability

Six times Replicates of 15, 30, 45 μ g/ml concentrations of Salbutamol, Chlorthalidone & Metoprolol respectively were prepared and absorbance was measured at their respective max. SD and RSD were calculated.

Intraday Precision

Standard solutions containing 15, 30 and 45 μ g/ml of Salbutamol/ 14, 28 & 42 for Chlorthalidone / and 25, 50, 75 μ g/ml of Metoprolol were analyzed 3 times on the same day. The absorbance of solutions was measured at their respective max. SD and RSD were calculated.

Interday Precision

Standard solutions containing 15, 30 and 45 μ g/ml of Salbutamol/ 14, 28 & 42 for Chlorthalidone / and 25, 50, 75 μ g/ml of Metoprolol were analyzed 3 times on the different days. The absorbance of solutions was measured at their respective max. SD and RSD were calculated.

Limit of Detection (LOD) & Limit of Quantitation (LOQ):

The LOD & LOQ are estimated from the set of 6 calibration curves used to determine method linearity.

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope}) \quad \text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

The entire validation summary shown in Table no. 4

3. Result and Discussion

Salbutamol, Chlorthalidone & Metoprolol respectively was estimated based on the reaction of Potassium ferricyanide & FeCl₃. The probable reaction takes place, resulting in the formation of blackish colour which showed max at 540 nm,

545 nm & 534 nm against blank respectively. The method obeyed Beer-Lambert's law in the concentration range of 15-75, 14-70 & 25-125 μ g/ml for Salbutamol, Chlorthalidone & Metoprolol respectively.

Table 1: STD Curve for Salbutamol

SL. No.	Concentration of drug taken in μ g/ml	Absorbance At 540 nm
1.	15	0.134
2.	30	0.345
3.	45	0.52
4.	60	0.752
5.	75	0.912

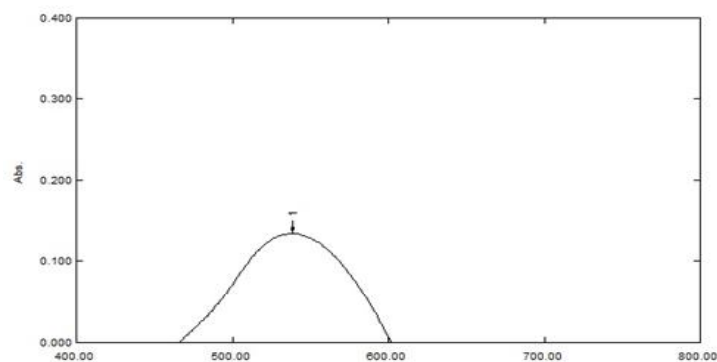


Figure 1: Potassium ferri cyanide & FeCl₃ with Salbutamol

Conclusion: The max of the colour Salbutamol was found to be 540 nm

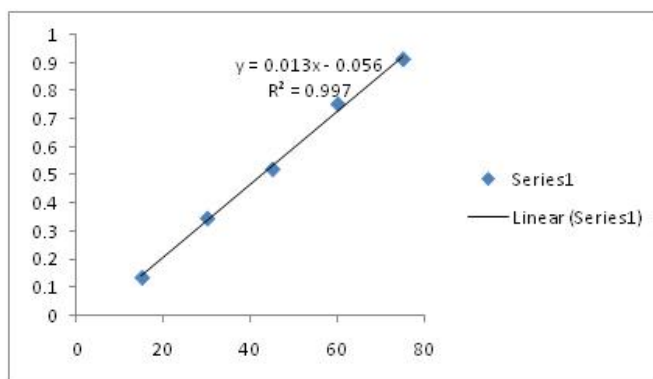


Figure 2: STD Curve for Salbutamol

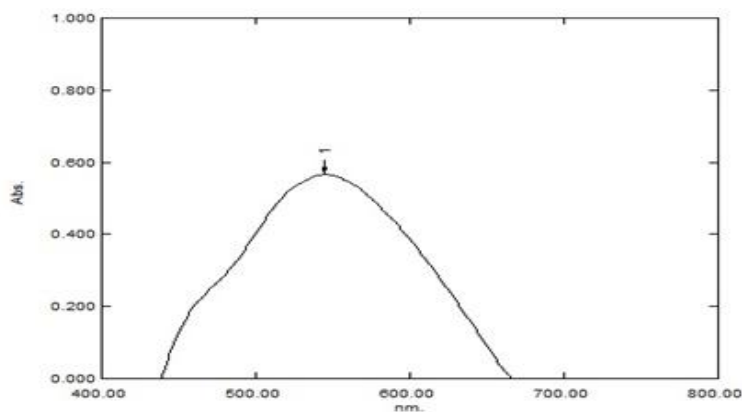


Figure 3: Potassium ferricyanide & FeCl₃ with Chlorthalidone

Conclusion: The max of the colour Chlorthalidone was found to be 545nm.

Table 2: STD CURVE for Chlorthalidone

SL. No.	Concentration of drug taken in µg/ml	Absorbance At 545 nm
1.	14	0.219
2.	28	0.360
3.	42	0.550
4.	56	0.724
5.	70	0.912

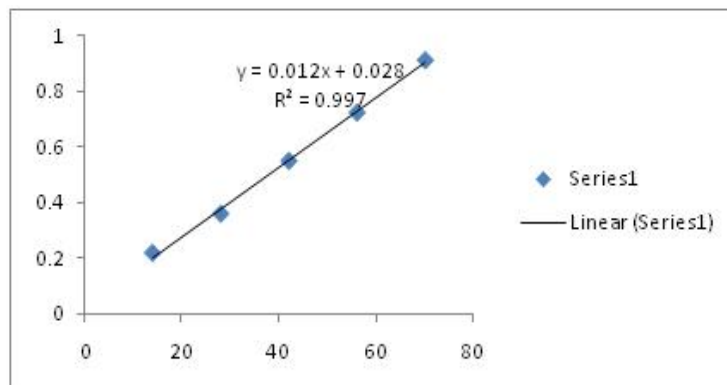


Figure 4: STD Curve for Chlorthalidone

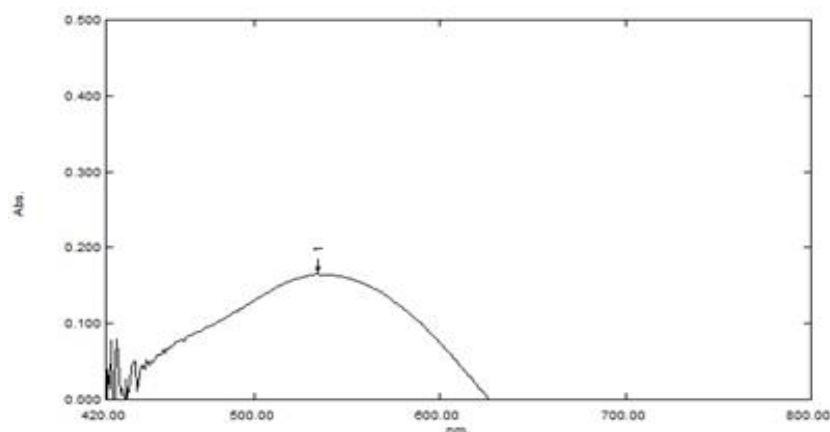


Figure 5: Potassium ferricyanide & FeCl₃ with Metoprolol

Conclusion: The max of the colour Metoprolol was found to be 534nm.

Table 3: STD Curve for Metoprolol

SL. No.	Concentration of drug taken in $\mu\text{g/ml}$	Absorbance At 534 nm
1.	25	0.165
2.	50	0.323
3.	75	0.516
4.	100	0.705
5.	125	0.89

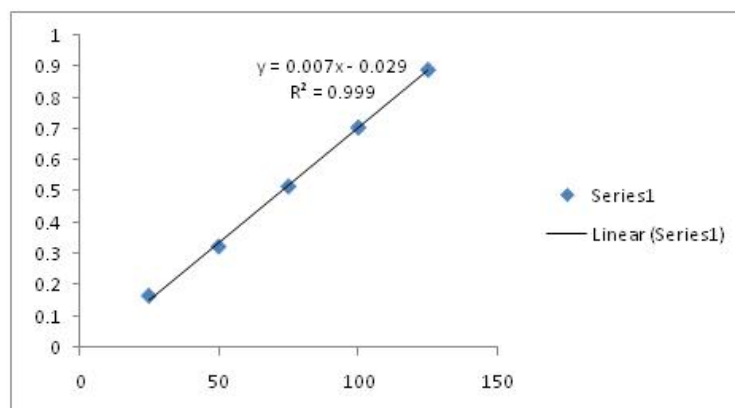


Figure 6: STD CURVE for Metoprolol

Table 4: The entire validation summary shown in Table no. 4.

Parameter	SALBUTAMOL	CHLORTHALIDONE	METOPROLOL
max (nm)	540	545	534
Beer's law limits ($\mu\text{g/ml}$)	15-75	14-70	25-125
Regression equation ($y=a+bc$)			
Slope (b)	0.0131	0.0125	0.007317
Intercept (a)	0.004802	0.028	0.002561
Correlation coefficient (r^2)	0.9975	0.9978	0.999
PRECISION			
Repetability	0.135 \pm 0.003656	0.222 \pm 0.004506	0.167 \pm 0.004517
Intraday	0.136 \pm 0.002082 0.346 \pm 0.006506 0.523 \pm 0.005508	0.220 \pm 0.005132 0.363 \pm 0.004726 0.556 \pm 0.004163	0.171 \pm 0.004583 0.328 \pm 0.008021 0.506 \pm 0.012055
Interday	0.126 \pm 0.01106 0.334 \pm 0.011136 0.510 \pm 0.011533	0.210 \pm 0.010066 0.352 \pm 0.010693 0.546 \pm 0.010536	0.152 \pm 0.012662 0.306 \pm 0.013528 0.506 \pm 0.012055
Limit of Detection	1.2	1.59	1.15
Limit of Quantitation	3.66	4.83	3.50

4. Acknowledgment

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