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VALIDATED COLORIMETRIC METHOD FOR THE ESTIMATION OF SUCCINYL CHOLINE CHLORIDE IN FORMULATION

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ABSTRACT

A simple and new colorimetric method was developed for the estimation of succinyl choline chloride. The proposed colorimetric method is based on the reaction between succinyl choline chloride and bromothymol blue reagent, followed by colour complex formation. Parameters affecting the reaction were studied and conditions were optimized. The absorption maximum for the colour complex was observed at 450 nm. Linearity was obtained in the concentration range of 70-600 µg/ml for succinyl choline chloride colour complex. The developed method was optimised and validated. The method was successfully applied for the estimation of succinyl choline chloride in bulk and in injection.

1. INTRODUCTION

A quaternary skeletal muscle relaxant usually used in the form of its bromide, chloride, or iodide. It is a depolarizing relaxant, acting in about 30 seconds and with duration of effect averaging three to five minutes. Succinylcholine is used in surgical, anesthetic, and other procedures in which a brief period of muscle relaxation. It is very essential to know the quality of the drug in order to prevent the harmful effects of the drug on the human body. In view of that point a new colorimetric method was developed and validated to estimate succinyl choline chloride in formulation. Literature survey revealed no method was developed with bromo thymol blue reagent.[1, 2]

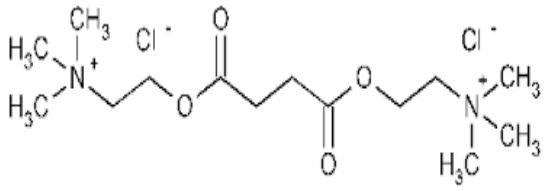

Figure 1. Structure of succinyl choline chloride

2. MATERIALS AND METHODS

Succinyl choline chloride is procured from Sequent scientific limited, Mangalore. Bromo thymol blue is from S.S Chemicals, Anantapuramu. Distilled water is obtained from Double distillation unit within the Institution. All

measurements were made with Elico CL 157 Colorimeter.

2.1 Method Development involves the Following steps

2.1.1. Solvent selection: Solubility studies were conducted to the succinyl choline chloride with various solvents[1]. It was found that succinyl choline chloride was freely soluble in distilled water.[3,4]

2.1.2. Preparation of standard stock solution

Standard succinyl choline chloride of 100 mg was weighed and transformed to a 100ml volumetric flask and dissolved in 25ml of distilled water. The flask was shaken and volume was make upto the mark with distilled water to give a solution containing 1000µg/ml (Stock solution).[5,6]

2.1.3. Selection of reagent

Reagent was selected based on the chemical structure. According to the functional groups in the structure, reagent was selected. Linearity of the drug was checked with various reagents like dragendorffs reagent, sodium nitrite, bromo thymol blue. There is good linearity of drug with bromo thymol blue. [7]

Figure 2. 2% Bromo thymol reagent

2.1.4. Determination of maximum absorbance wavelength of 10 µg/ml colour complex

Stock solution of succinyl choline chloride was further diluted with distilled water to get concentrations of **10 µg/ml** and 2 ml of reagent was added. Absorbance was checked at various wavelengths and it was found that 450 nm is the maximum absorbance wavelength.

2.1.5. Selection of concentration of reagent

Various trails were made with 0.1, 0.2, 0.4, 1, 2% reagent with drug solution. Better linearity was obtained with 2% reagent.

2.1.6. Effect of time on the linearity of the colour complex

The colour of the drug solution with reagent was stable up to 15 min after that the colour degrades. At 15 min there is good linearity of coloured complex.[8]

2.1.7. Effect of volume of reagent on the linearity of colour complex

Linearity of coloured complex was measured with different volumes of reagent. It was found that with 2 ml of reagent linearity of coloured complex is good.

2.1.8. Selection of analytical concentration range: From standard stock solution of succinyl choline chloride, working standard solutions of concentrations from 70-600µg/ml were prepared. Absorbance for these solutions was measured at 450nm. These concentrations showed linear values.

2.1.9. Construction of Calibration Curve: From standard succinyl choline chloride stock solution along with reagent concentrations of 100,200,300,400,500,600 µg/ml were prepared. Absorbance value of each solution against distilled water and reagent as a blank were measured at 450nm.

Table 1: Linearity of succinyl choline chloride

S.No	Conc(µg/ml)	Absorbance ±SD	%RSD
1	70	0.08 ± 0.0052	1.90
2	80	0.13 ± 0.0041	1.97
3	90	0.18± 0.0058	0.87
4	100	0.27± 0.005	1.82
5	200	0.56± 0.0065	1.34
6	300	0.81± 0.0082	0.895
7	400	1.11± 0.0075	0.98
8	500	1.38± 0.0049	0.096
9	600	1.62±0.0072	1.53

Table 2. Selection of Reagent

S.No	Reagent	R^2
1	Dragendorffs	0.920
2	Sodium nitrite	0.963
3	Bromo thymol blue	0.999

Table 3. Selection of concentration of reagent

S.No	Concentration of Reagent	R^2
1	0.1%	0.981
2	0.2%	0.962
3	0.4%	0.995
4	1%	0.997
5	2%	0.999

Table 4. Effect of time on the linearity of colour complex

S.No	Time (min)	R^2
1	0	0.981
2	5	0.985
3	10	0.992
4	15	0.999
5	20	0.995

Table 5. Effect of volume of reagent on the linearity of colour complex

S.No	Volume (ml)	R^2
1	1	0.981
2	2	0.999
3	3	0.992

Table 6. Regression and Analytical parameters

S.No	Parameter	Result
1	Maximum absorbance wavelength (nm)	450
2	Molar absorptivity(mol/l)	0.0027
3	Range (µg/ml)	70-600
4	Sandell's sensitivity(µg/cm^2)	0.37
5	Limit of detection (µg/ml)	8.25
6	Limit if quantification (µg/ml)	25
7	Regression equation	Y = 0.002x + 0.007
8	Slope	0.002
9	Intercept	0.007
10	Correlation coefficient	0.999

Table 7. Precision studies of succinyl choline chloride

S.No	Sample	Intra day (%RSD)	Inter day (%RSD)
	Succinyl choline chloride		
1	LQC (lower quality control)	1.9	1.86
2	MQC (middle quality control)	1.72	1.989
3	HQC (high quality control)	0.87	0.957

Table 8. Recovery Studies

S.NO	Name of the drug	Amount of sample(µg/ml)	Recovery level	Amount of drug added (µg/ml)	Total amount found(µg/ml) ± SD	%Recovery	%RSD
1	Succinyl choline chloride	200	80% 100% 120%	160 200 240	356.5±0.9 411.5±0.7 431.5±0.51	99.02 102.8 98.06	1.04 1.81 1.55

Table 9. Assay Studies

S.No	Drug	Lable claim	Amount found	%Recovery	%RSD
1	**succinyl choline chloride**	200mg/10 ml	206.5	103.25	1.46

Table 10. Ruggedness

S.No	Sample	Same instrument different analysts	Same analyst different Instruments
	Succinyl choline chloride		
1	LQC (lower quality control)	1.9	1.86
2	MQC (middle quality control)	1.72	1.989
3	HQC (high quality control)	0.87	0.957

From those absorbance values, calibration curve was constructed. Regression equation and correlation coefficient (R^2) are determined.

2.2. Assay: Label claim of succinyl choline chloride injection is 200 mg/10 ml. Drug equivalent to 200 **µg/ml** was taken from injection. Its absorbance was noted at 450 nm. Amount of drug in the injection was calculated from regression equation. [9]

2.3. Method Validation: The method validation was performed in terms of linearity, LOQ, LOD, Precision, accuracy, and ruggedness. [10,11]

2.3.1. Linearity: From standard stock solution of succinyl choline chloride, working standard solutions of concentrations from 70-600µg/ml were prepared. These concentrations showed linear values.

2.3.2. Precision

Precision of methods was studied as intraday and inter day. Precision was performed by analysing three different concentration of drug like LQC, MQC, and HQC.

2.3.3. Accuracy: The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e., 80%, 100%, 120%.

2.3.4. Limit of detection (LOD): The limit of Detection was found by formula method. LOD=3.3σ/slope, where σ is standard deviation.

2.3.5. Limit of Quantification (LOQ): The limit of quantification was found by formula method. LOQ= 10 σ/slope, where σ is standard deviation. [5]

2.3.6. Ruggedness: Absorbance values were taken by two analysts with the same instrument and with the two instruments by the same analyst.

4. DISCUSSION

The selected succinyl choline chloride was estimated by colorimetry. Bromo thymol blue was selected as the coloring reagent. The method was validated for all validation parameters as per ICH guidelines. The linearity range for succinyl choline chloride was 70-600µg/ml with R^2 value 0f 0.999. The %RSD for intraday and interday was <2%. The assay of dosage form was performed. The accuracy of the method was validated by recovery studies and found to be significant under specification limits with %Recovery (99-101) (within acceptable range 98-102%). The assay results were found to be (98.9%) (I.e. within 95-105).

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