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Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE AND CETIRIZINE HYDROCHLORIDE IN COMBINED DOSAGE FORM

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ABSTRACT
Salbutamol Sulphate (SAL) and Cetirizine HCl (CET) is used for the treatment of asthma and allergy. A simple, economical, accurate and precise method for simultaneous estimation of Salbutamol Sulphate (SAL) and Cetirizine HCl (CET) in combined dosage form has been developed. Simultaneous equation method based on measurement of absorbance at two wavelengths 276 nm and 230 nm, λ_{max} of Salbutamol Sulphate (SAL) and Cetirizine HCl (CET) in 6.8 pH phosphate buffer. Both these drugs obeyed Beer Lambert's law in the concentration range of 10-100 µg/ml for SAL and 2-20 µg/ml for CET. The high values of correlation coefficient (R^2) indicated good linearity of calibration curve for both the drugs. The accuracy and precision of method was determined and the method validated stastically. Result of percentage recovery study confirms the accuracy of proposed method. The results of validation parameters indicates the accuracy of proposed methods for estimation of SAL and CET. Simultaneous equation method can be employed for routine analysis of SAL and CET in combined dosage form.
Keywords: Salbutamol Sulphate (SAL), Cetirizine HCl (CET), Simultaneous equation method, Ultraviolet Spectroscopy, Absorptivity, λ_{max}.

INTRODUCTION

Salbutamol Sulphate (SAL), **(fig. 1)** chemically known as (RS)-1-(4-hydroxy-3-hydroxy-methylphenyl)-2-(tert-butylamino) ethanol sulphate, is beta (β) - receptor agonist used for the relief of bronchospasm in condition such as asthma and chronic obstructive pulmonary disease. It is freely soluble in water, slightly soluble in ethanol (95 %) and in ether; very slightly soluble in dichloromethane. The drug is official in Indian Pharmacopoeia[1,2]. Cetirizine Hydrochloride (CET) **(fig. 2)** is [2-[4-[(4-chlorophenyl) phenyl methyl]-1 piperazinyl] ethoxy] acetic acid is a non-sedative second generation anti-histamine drug used in the treatment of seasonal allergic rhinitis, perennial allergic rhinitis, chronic urticaria also used as adjuvant in seasonal asthma. It is freely soluble in water; practically insoluble in acetone and in methylene chloride[3, 9].

The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of SAL and CET in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of SAL and CET in combined dosage forms. The present research article describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation for estimation of both drugs in the combined dosage forms.

Ultraviolet and Visible spectrophotometry is one of the most frequently employed analytical tools in the pharmaceutical industry. Spectrophotometry is mainly concerned with the following regions of spectrum: ultraviolet, visible and infrared[4]. Ultraviolet and Visible absorption spectrophotometry involves the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of 185 nm to 380 nm, and 380 nm to 780 nm of the spectrum, respectively [5] .The amount of absorption depends on the wavelength of radiation and the structure of the compound. The absorption of radiation is due to the subtraction of energy from the radiation beam when electrons in orbital of lower energy are excited into orbital of higher energy. Since, it is an electron transition phenomenon; UV is sometimes called electronic spectroscopy [6].

The various spectrophotometric methods which are used for estimation of drug in combine dosage form include [7].

- Simultaneous equation method
- Absorption ratio method (Q-ratio method)
- Geometric correction method
- Orthogonal polynomial method
- Difference spectrophotometry
- Derivative spectrophotometry
- Chemical derivatisation
- Absorption correction method
- Multi-component method of analysis

SIMULTANEOUS EQUATION METHOD

If a sample containing two absorbing drugs (X and Y) each of which absorbs at the λ_{max} different from the other, it may be determine both the drugs by the technique of simultaneous equations. The absorptivities of drug X at λ_1 and λ_2 are ax_1 and ax_2 respectively, absorptivities of drug Y at λ_1 and λ_2 are ay_1 and ay_2 respectively and the absorbances of diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively. Let C_x and C_y be the concentrations of X and Y respectively in the diluted sample. L is the path length.

Absorbance of pure compound X at λ_1 and λ_2

At λ_1 $A_1 = ax_1C_xL$
At λ_2 $A_2 = ax_2C_xL$

Absorbance of pure compound Y at λ_1 and λ_2

At λ_1 $A_1 = ay_1C_yL$
At λ_2 $A_2 = ay_2C_yL$

Absorbance of mixture of compound X and Y at λ_1 and λ_2

At λ_1 $A_1 = ax_1C_xL + ay_1C_yL$ …….. (1)
At λ_2 $A_2 = ax_2C_xL + ay_2C_yL$…….. (2)

Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of components of the mixture. The

criteria are that the ratios should lie outside the range 0.1 – 2.0 for the precise determination of Y and X respectively.

$$\frac{A_2/A_1}{ax_2/ax_1} \text{ and } \frac{ay_2/ay_1}{A_2/A_1}$$

These criteria are satisfied only when the λmax of two compo nent are reasonably dissimilar. An additional criterion is that the two components don't interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorptions. The additivity of absorbances should always be confirmed in the development of a new application of this technique [8].

MATERIALS AND METHODS

Apparatus

A double beam Shimadzu UV-1800 series spectrophotometer was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell at fast scanned speed and fixed slit width of 1.0 nm. All weighing of ingredients were done on Ohaus digital weighing balance and bath sonicator was also used in study.

Reagents and Materials

Salbutamol Sulphate (SAL) and Cetirizine HCl (CET) were supplied as gift sample by Trojan Pharma Baddi, India. All other chemicals and reagents used were of analytical grade.

Preparation of standard stock solution

Standard stock solutions of both Salbutamol Sulphate (SAL) and Cetirizine HCl (CET) were prepared by dissolving 20mg of SAL and 20 mg of CET separately in 20ml of 6.8 pH phosphate buffer solution, sonicated for 15 minutes in bath sonicator and filtered through whatman filter paper in order to get dilution of 1mg/ 1ml i.e. 1000µg/ml.

Determination of λ maximas

By appropriate dilution of standard stock solutions of SAL and CET with 6.8 pH phosphate buffer, solutions containing 10 µg/ml of SAL and 10 µg/ml of CET were scanned separately in the range of 200-400nm. Wavelength of absorption maximas was determined for both drugs. SAL showed absorption maximas one at 224 nm and other at 276 nm respectively **(fig.3).** 276 nm was selected as λ_{max} of Salbutamol sulphate. CET showed maximum absorbance at 230 nm **(fig. 4).**

Development of Simultaneous Equation

From the overlain spectra of SAL and CET **(fig. 5);** two wavelengths namely 276 nm and 230 nm, λ_{max} of Salbutamol Sulphate and Cetirizine Hydrochloride were selected. The calibration curves were constructed in concentration range of 10-100 µg/ml for Salbutamol Sulphate **(Fig. 6)** and 2-20 µg/ml for Cetirizine HCl **(fig. 7)** at each wavelength i.e. 276 nm and 230 nm. The linearity was observed in the concentration range of 10-100 µg/ml for Salbutamol Sulphate and 2-20 µg/ml for Cetirizine HCl. The absorbances were measured at the selected wavelengths and absorptivities for both drugs **(Table 1, Table 2)** were determined at both wavelengths. The concentrations of drugs in sample solution were determined by using following formula

At 276 nm $A_1 = ax_1C_s + ay_1C_c$ …… (1)

At 230 nm $A_2 = ax_2C_s + ay_2C_c$ …… (2)

Where C_s and C_c are the concentration of Salbutamol Sulphate and Cetirizine Hydrochloride respectively, A_1 and A_2 are absorbance at 276 nm and 230 nm respectively, ax_1 and ax_2 are absorptivities of Salbutamol Sulphate at 276 nm and 230 nm respectively; ay_1 and ay_2 are absorptivities of Cetirizine Hydrochloride at 276 nm and 230 nm respectively.

Substituting the values of ax_1, ax_2, ay_1 and ay_2 from **Table 3** the equation could be rearranged as:

At 276 nm $A_1 = 0.0066C_s + 0.0050C_c$ …… (3)

At 230 nm $A_2 = 0.023C_s + 0.0338C_c$ …….... (4)

Where C_s and C_c are the concentration of Salbutamol Sulphate and Cetirizine Hydrochloride in µg/ml

By putting the values of A_1 and A_2 at their respective wavelengths, the concentrations of Cetirizine Hydrochloride and Salbutamol Sulphate in sample solutions were obtained.

Validation of proposed method The method was validated according to ICH guidelines to study linearity, accuracy and precision

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of SAL and CET. For simultaneous equation method, the Beer Lambert's law was obeyed in the concentration range 10-100 µg/ml and 2-20 µg/ml for SAL and CET respectively. The correlation coefficient was found to be 0.999 for both SAL and CET.

Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 3) for SAL and CET (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method **(Table 4).**

Recovery Studies

In order to check the accuracy, reproducibility and precision of the proposed method, recovery study was carried out by taking standard mixture solution of both SAL and CET and absorbances was determined at 276 nm and 230 nm respectively. **(Table 5)**

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for simultaneous analysis of SAL and CET. In simultaneous equation method, wavelengths selected for analysis were 276 nm for SAL and 230 nm for CET. SAL and CET showed linearity with absorbance in the range of 10-100 µg/ml and 2-20 µg/ml at their respective absorption maxima, which were validated by least square method. Coefficient of correlation was found to be 0.999 for both SAL and CET. The observations are presented in **fig. 6, fig. 7** and in **Table 1,Table 2**.The Absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer Lambert's law in indicated concentration range. The high value of correlation coefficient (R^2) also indicates good linearity of calibration curve for both the drugs. The overlain UV absorption spectra of SAL (276 nm) and CET (230 nm) in 6.8 pH phosphate buffer is shown in **Fig. 5**. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined **(Table 5).** The results of validation parameters indicates the accuracy of proposed methods for estimation of SAL and CET. Simultaneous equation method can be employed for routine analysis of SAL and CET in combined dosage form.

CONCLUSION

The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of SAL and CET. The developed method was validated as per ICH guidelines. The results demonstrated that simultaneous equation method by UV/Visible spectrophotometer could be useful technique for determination of Salbutamol Sulphate and Cetirizine Hydrochloride when they are given in same dosage form.

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REFERENCES

1. Indian Pharmacopoeia, Published by the controller of Publication: New Delhi; 2007; vol. 3: p.1063-1064.
2. The Merk Index, An encyclopedia of chemicals, drugs and biological, 14 th ed. published by Merk Laboratories; 2006 : p.216
3. Indian Pharmacopoeia. Published by the controller of Publications: New Delhi; 2007 vol. 2: p.274-275.
4. Mendham J, Denney RC, Barnes JD, Thomas MJK. Vogel's Quantitative Chemical Analysis. 6th ed. Published by Pearson Education, Delhi, India, 2004: p.630-636.
5. Indian Pharmacopoeia. Published by Controller of publications, New Delhi, 2007, Vol 1: p.112-114.
6. Kalsi PS, In: Spectroscopy of organic compounds. 5th ed. Published by New Age International, New Delhi; 2002: p.7-8.
7. Beckett AH and Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. – Part One. Published by CBS Publishers and Distributors, New Delhi, India; 2005: p.133-136.
8. Beckett AH. and Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. – Part Two. CBS Published by Publishers and Distributors, New Delhi, India; 2005: p.275-306.
9. Tripathi, KD. Essential of Medical Pharmacology. 6th ed. Jaypee brothers medical publisher, New Delhi; 2008: p.159.

Table1: Absorbance and Absorptivity of Salbutamol Sulphate at λ_{max} 276 nm and 230 nm respectively

Sr. No.	Concentration (µg/ml)	*Absorbance at 276 nm± S.D	*Absorbance at 230 nm ± S.D	Absorptivity at 276nm	Absorptivity at 230 nm
1	10	0.071 ± 0.002	0.250 ± 0.003	0.0071	0.025
2	20	0.138 ± 0.003	0.485 ± 0.002	0.0069	0.024
3	30	0.211 ± 0.002	0.713 ± 0.001	0.0070	0.024
4	40	0.264 ± 0.001	0.930 ± 0.002	0.0066	0.023
5	50	0.327 ± 0.002	1.151 ± 0.003	0.0065	0.023
6	60	0.385 ± 0.003	1.362 ± 0.002	0.0064	0.023
7	70	0.459 ± 0.001	1.599 ± 0.002	0.0066	0.023
8	80	0.512 ± 0.001	1.794 ± 0.004	0.0064	0.022
9	90	0.571 ± 0.001	1.969 ± 0.002	0.0063	0.022
10	100	0.628 ± 0.002	2.125 ± 0.003	0.0063	0.021
			Mean	**ax_1** = 0.0066	**ax_2** = 0.023

***Each value is the average of three determinations**

Table2: Absorbance and Absorptivity of Cetirizine Hydrochloride at λ_{max} 276 nm and 230 nm respectively

Sr. No.	Concentration (µg/ml)	*Absorbance at 276 nm± S.D	*Absorbance at 230 nm ± S.D	Absorptivity At 276nm	Absorptivity at 230 nm
1	2	0.022 ± 0.004	0.077 ± 0.002	0.011	0.0385
2	4	0.038 ± 0.003	0.135 ± 0.003	0.009	0.0375
3	6	0.039 ± 0.002	0.209 ± 0.003	0.006	0.0348
4	8	0.034 ± 0.005	0.267 ± 0.001	0.004	0.0334
5	10	0.088 ± 0.003	0.332 ± 0.004	0.009	0.0332
6	12	0.035 ± 0.002	0.393 ± 0.002	0.002	0.0328
7	14	0.026 ± 0.001	0.468 ± 0.002	0.002	0.0334
8	16	0.037 ± 0.002	0.530 ± 0.003	0.002	0.0331
9	18	0.041 ± 0.003	0.585 ± 0.001	0.002	0.0325
10	20	0.050 ± 0.001	0.653 ± 0.002	0.003	0.0327
			Mean	**ay_1** = 0.0050	**ay_2** = 0.0338

***Each value is the average of three determinations.**

Table 3: Mean Absorptivity of SAL and CET at two λ_{max}

Drug	λ_{max} (nm)	Absorptivity (Mean)
SAL	276	0.0066
SAL	230	0.023
CET	276	0.0050
CET	230	0.0338

Table 4: Optical Characteristics of Salbutamol Sulphate and Cetirizine HCl

Optical Characterisitics	Salbutamol Sulphate	Cetirizine HCl
Wavelength (nm)	276	230
Beer Lambert's law limit (µg/ml)	10-100	2-20
Regression equation (y = mx + c)	y = 0.006x + 0.016	y = 0.032x + 0.011
Slope (m)	0.006	0.032
Intercept (c)	0.016	0.011
Correlation coefficient (R^2)	0.999	0.999
Precision (n = 3)	0.075	0.214

Table 5: Recovery studies for Salbutamol Sulphate and Cetirizine HCl

Salbutamol Sulphate (µg/ml)	Cetirizine HCl (µg/ml)	Salbutamol Sulphate (% recovery)	Cetirizine HCl (% recovery)
30	6	99.44±0.01	99.63±0.19
50	12	100.33±0.12	99.79±0.25
70	18	99.80±0.15	101.5±0.29

Results are shown in ±S.D (n=3)

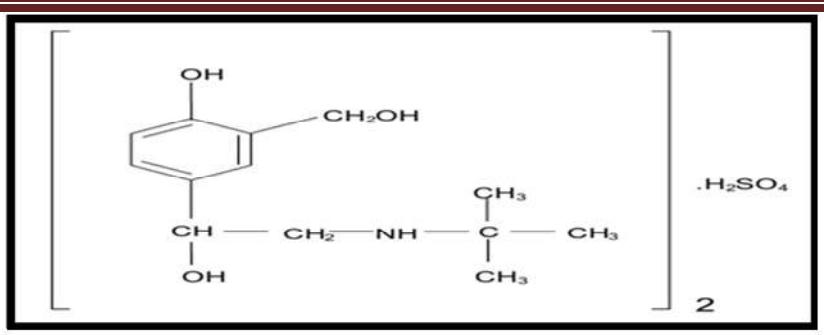

Fig. 1 : Chemical Structure of Salbutamol Sulphate

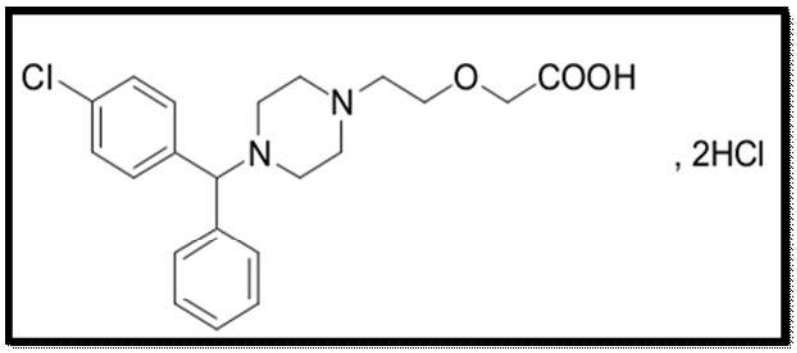

Fig. 2: Chemical Structure of Cetirizine Hydrochloride

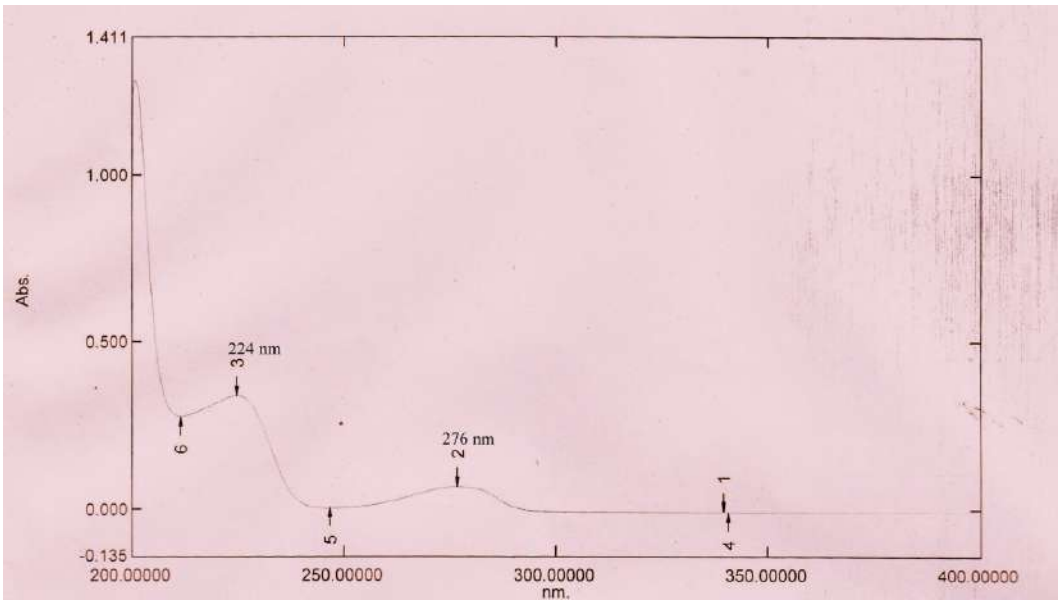

Fig. 3: UV Scan of Salbutamol Sulphate

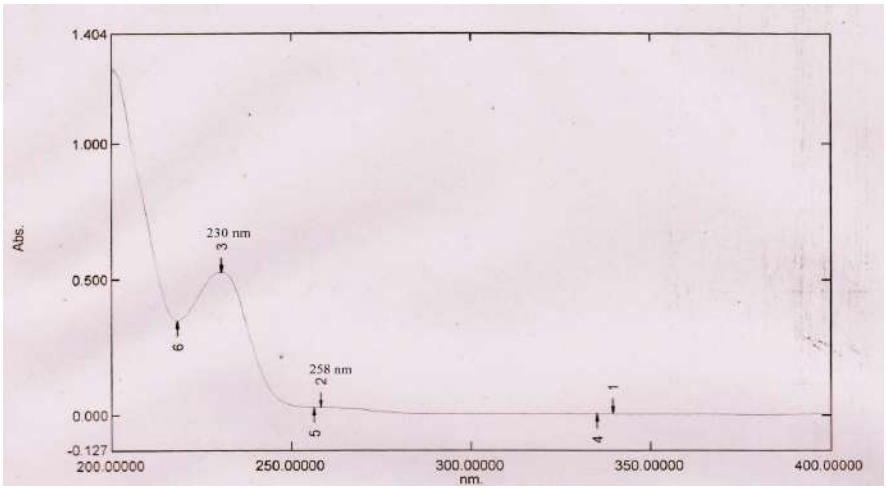

Fig. 4: UV Scan of Cetirizine Hydrochloride

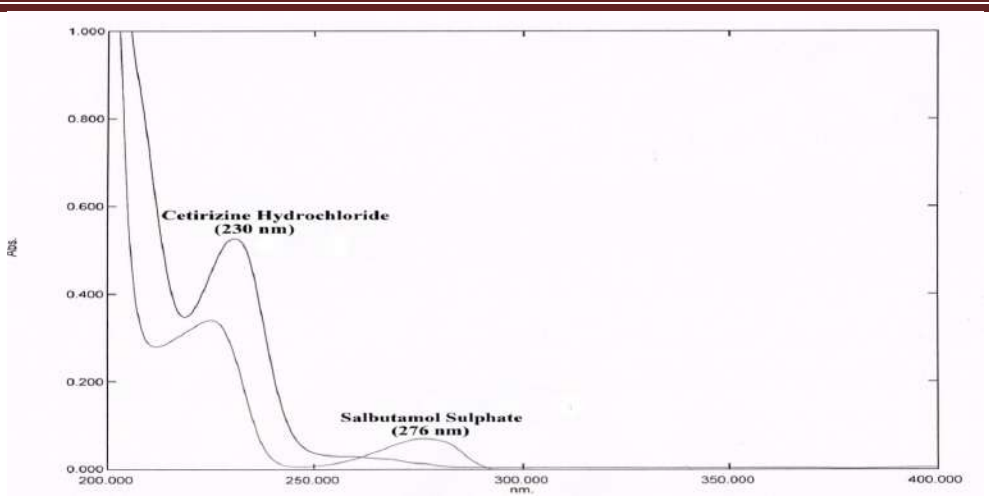

Fig. 5: Overlay Spectra of SAL and CET for Simultaneous Equation Method

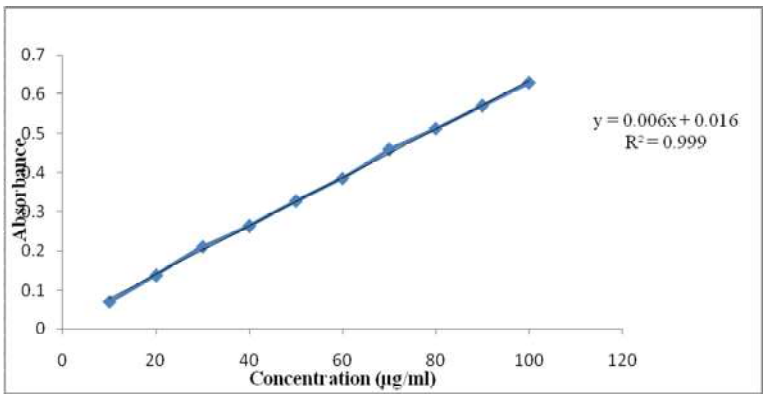

Fig. 6: Standard calibration plot of Salbutamol Sulphate at 276nm in 6.8 pH Phosphate buffer solution

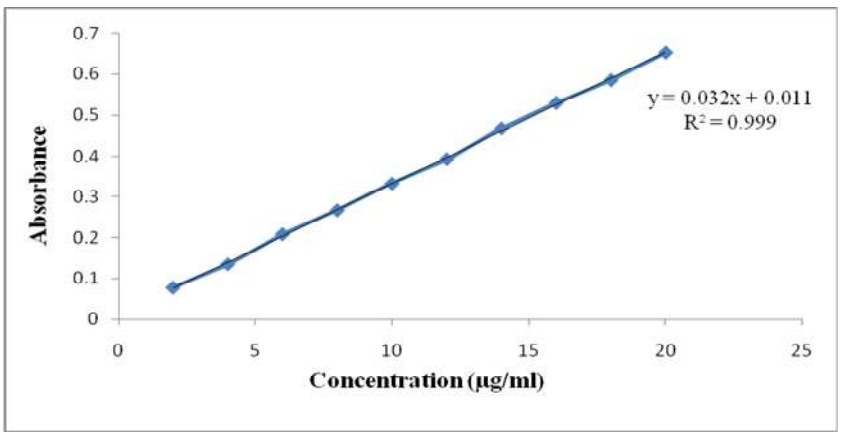

Fig. 7: Standard calibration plot of Cetirizine Hydrochloride at 230 nm in 6.8 pH Phosphate buffer solution

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