Available Online at

http://www.ijcpa.in

October - December 2016

DOI : http://dx.doi.org/10.21276/ijcpa

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-4 | Issue-1 | Article ID: 1150

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD OF ESTIMATION OF TERBUTALIN SULPHATE IN BULK DRUG USING NINHYDRIN

Gajanan A Vaishnav[1*], Sushama Vaishnav-Hota[2], Shital Godse[1], Angadi S.S.[3] and Patil S. J.[3]

[1]Department of Pharmaceutical Analysis, Yash Institute of Pharmacy, South City, Waluj Road, Aurangabad 431134, Maharashtra, India.

[2]Department of Pharmaceutical Chemistry, Yash Institute of Pharmacy, South City, Waluj Road, Aurangabad 431134, Maharashtra, India.

[3]Department of Pharmacology, Yash Institute of Pharmacy, South City, Waluj Road, Aurangabad 431134, Maharashtra, India.

Corresponding Author:** Email: **gajananvaishnav@gmail.com

Received: 24 October 2016 / Revised: 24 November 2016 / Accepted: 2 December 2016 / Available online: 31 December 2016

ABSTRACT

An accurate, precise and simple spectrophotometric method for the determination of Terbutalin sulphate based on the formation of a yellow color product with Ninhydrin in the presence of sodium bicarbonate with an λ_{max} at 346 nm. The Reaction heating temperature was 80°C for 30 min. The calibration curve was linear over the range of 10-60 µg/ml and the regression equation obtained was y=0.0263x+0.2 with a regression coefficient (r) of 0.98 (n=10). The limit of Detection (LOD) and limit of Quantification (LOQ) calculated as per ICH guidelines were 18.15µg/ml and 55µg/ml, respectively.

Keywords: - *Terbutalin sulphate, UV-Spectrophotometer, Ninhydrin.*

1. INTRODUCTION

Bronchial asthma is characterized by hyper responsiveness of tracheobronchial smooth muscle to a verity of stimuli, resulting a narrowing of air tubes, often accompanying by increased secretion, muscle edema and muscle plugging, symptom include dysporea, wheezing, cough and may limitation of activity. Terbutalin sulphate it is used in Bronchial asthma[1] is a (RS)-2-(tert-butylamino)-1-(3-5 dihydroxyphenyl) ethanol sulphate, is β_2 bronchodilator [2]. Ammonia, primary amine and secondary amine are detected by ninhydrin. Ammonia and primary amine produce purple color with ninhydrin and form purple color product. And secondary amine produce yellow color product, imines such as pipecolic acid and prolin, arginine, asparagin, the indol ring of tryptophan, the sulfhydril group of cystin and guanine and cynide also reacting with ninhydrin and produces yellow colored complex [3]. Reaction of secondary amine with ninhydrin used for detection of herbal constituent[4, 5], detection of basic organic drug and their metabolites in urine [6] and ninhydrin based forensic investigation [7]. It is official in I.P.[8], B.P.[9], U.S.P.[10], E.P.[11], at present, different methods to determine Terbutalin sulphate have been employed, liquid chromatography [12,] HPTLC [13], Gas chromatography, Mass Spectrometry [14] and Flourimetry [15].

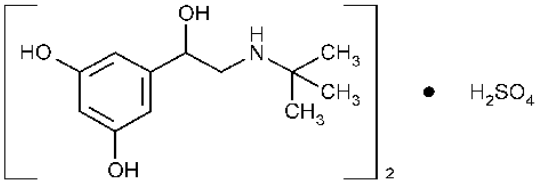

Fig.1 : Structure of Terbutalin sulphate

2. MATERIALS AND METHODS

2.1 Apparatus

The spectral measurements were carried out by using a Shimadzu UV-Visible spectrophotometer (model no.1800japan) with matched quartz cell of 1cm optical path length. Other includes weighing balance, vortex mixer etc.

2.2 Reagent and chemical

Terbutalin sulphate bulk drug (gift sample from Shreya life science Ltd., Aurangabad, Ninhydrin power purchased from Deepa Chemical Industries, (Aurangabad, Maharashtra) all reagent and solvent A.R. grade from S.D. fine chemical, Mumbai, distilled water.

2.3 Mehodology

2.3.1 Preparation of standard stock solution

i) Preparation of standard stock solution

stock solution (A): Accurately weighed 100 mg Terbutalin was dissolved in freshly prepared distilled water in 100 ml volumetric flask and volume was made up to the mark with the distilled water i.e.1000µg/ml.

Stock solution (B): From the stock solution (A), pipette out 10ml of solution and diluted up to 100ml with distilled water to prepare stock 2. From the stock 2 solution (100µg/ml), pipette out 1 ml ,2ml,3ml,4ml,5ml dilute each up to 10 ml respectively to prepare 10-100 µg/ml solution.

ii) Preparation of 0.4% Ninhydrin: Accurately weighed 400 mg of Ninhydrin was dissolved in 100 ml of distilled water.

iii) Preparation of Sodium bicarbonate: Accurately weighed 25 gm of sodium bicarbonate was dissolved in 100 ml of distilled water.

2.3.2 Preparation of calibration curve

Scanning of Terbutalin sulphate by UV- Spectrophotometry in Ninhydrin (0.4%).

Fresh aliquots from standard stock solution was pipette out separately and added 1 ml of Ninhydrin and 1 ml of sodium bicarbonate and final volume was made up to 10 ml with distilled water so as to obtain final concentration range in between 10-50 µg/ml. The solution of Terbutalin in bulk drug at highest dilution i.e. (40µg/ml) were scanned in the spectrum mode between 400nm to 200 nm. Wavelength maxima λ_{max} was obtained at 346nm (fig.2).

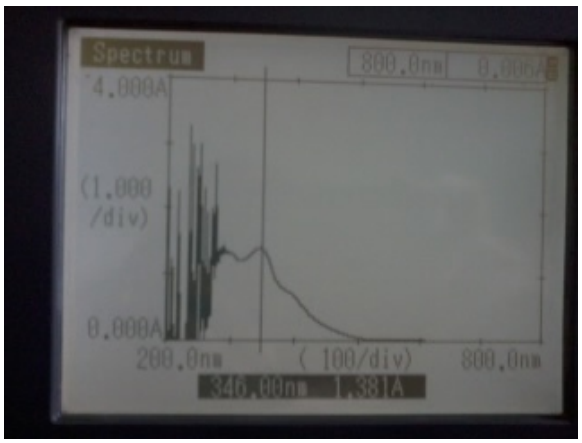

Fig. 2: Spectra of Terbutalin Sulphate

3. METHOD VALIDATION

3.1 Linearity

Calibration Curve was constructed by plotting absorbance against concentration for Terbutalin sulphate. Beer's law limits, molar absorptive, linear regression equations and sandell sensitivity were calculated for drug (Table 1). The correlation coefficient was found to be 0.982indicating excellent linearity over beer's law limit.

Table 1: Observation table for calibration curve of Terbutalin sulphate

Sr. No	Concentration range(µg/ml)	Absorbance(nm)
1	10	0.36
2	20	0.66
3	30	1.01
4	40	1.39
5	50	1.61
6	60	1.92
7	70	2.01
8	80	2.14
9	90	2.48
10	100	2.90

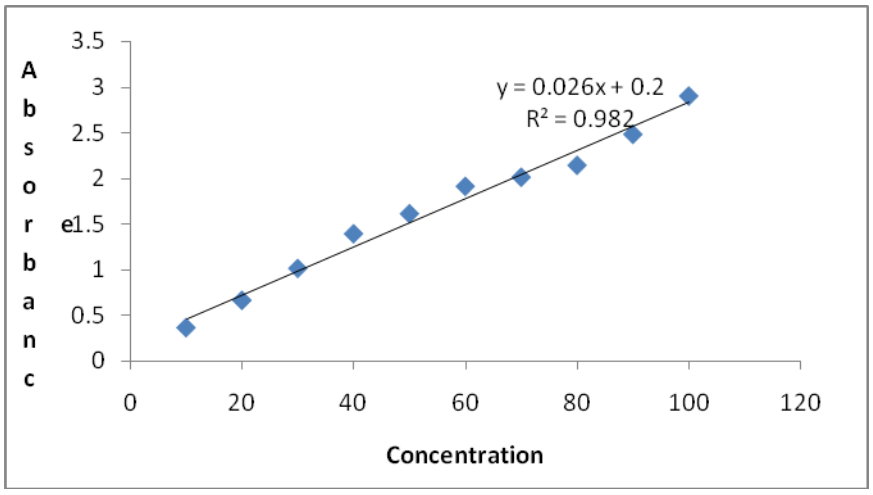

Fig.3: Calibration curve of Terbutalin sulphate at 346nm.

3.2 Sensitivity

The detection limit (LOD) and limit of quantification (LOQ) were calculated using the following equation according to ICH guideline [16]. The results obtained are compiled in Table 2.

LOD=3.3σ/s

LOQ=10σ/s

Where, σ=the std. deviation of replicate blank response.

S=the slop of the calibrated curve.

3.3 Precision

Precision of the method was calculated in the term of intermediate precision (intra-day and inter-day)[4]. Two concentration of Terbutalin sulphate were analyzed in three replicates during the same day (intra-day precision).and two days (inter-day precision). The % RSD value of intra-day and inter-day studies showed good precision. Six dilutions of 40 µg/ml concentration of Terbutalin sulphate

were prepared and assay by proposed method. An acceptance criteria of % RSD less than 2.5% was used [17]. The result obtained are compiled in Table 3.

3.4 Accuracy

Accuracy of an analytical method is the closeness between the reference value and the found value [18]. Accuracy was evaluated as % RE between the measured concentration and actual concentration for Terbutalin sulphate. The results obtained are compiled in Table 3.

3.5 Recovery Study

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. 50µg/ml solution was spiked with 100µg/ml at three concentration level (80,100 and 120%). Six dilutions of 45, 50 and 55 µg/ml concentration of Terbutalin sulphate were prepared and assayed by proposed method. An acceptance criteria of % Recovery between 95-105% was used [17]. The result obtained are compiled in Table 3.

4. RESULTS AND DISCUSSION

Now a day's Ninhydrin is used to detect ammonia, primary and secondary amine. All primary amine and ammonia produce purple color with ninhydrin and secondary amine produce yellow color complex with Ninhydrin [19]. During study, it was found that ninhydrin form yellow colored product with Terbutalin sulphate in the presence of saturated solution of $NaHCO_3$ and without employing any salt. All absorbance measured were at λ_{max} 346 nm. Reaction between ninhydrin and Terbutalin sulphate did not give any color product in the absence of $NaHCO_3$.four parameter Volume of $NaHCO_3$, concentration of Ninhydrin and heating time were optimized. Different concentration of NaOH and Acetate buffer (pH 5.5) were used to study the effect of pH on the reaction between ninhydrin and Terbutalin sulphate. Since no color was formed, the reaction was specific in bicarbonate medium. When more than 1 ml volume of $NaHCO_3$ and heating time increased violet color produce but it was, unstable and turned to stable yellow color. All measurements were made at 346 nm against reagent blank.

4.1 Optimization of the reaction conditions [20, 21].

4.1.1 Effect of heating time

The effect of heating time on the absorption intensity was studied. Different heating times in a boiling water bath (at 100°C) from 5 min until 1 hr. were tried. It was found that heating for about 30 min (at 100℃) gave maximum absorption intensity (fig 4).

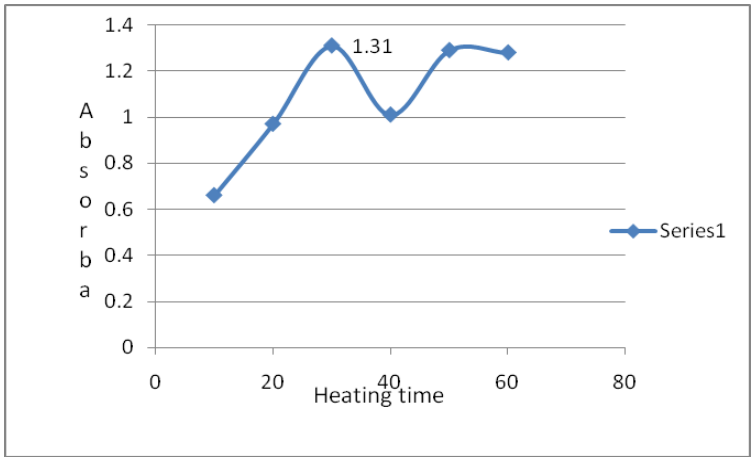

Fig.4: Effect of heating time on the reaction of Ninhydrin with 40µg/ml Terbutalin

4.1.2 Effect of pH

The effect of pH on the reaction was studied over the pH 1-9. It was found that Sodium bicarbonate of pH 9 was the optimum for Terbutalin. Different volumes of buffer ranging from 0.5ml to 3 ml were tried.

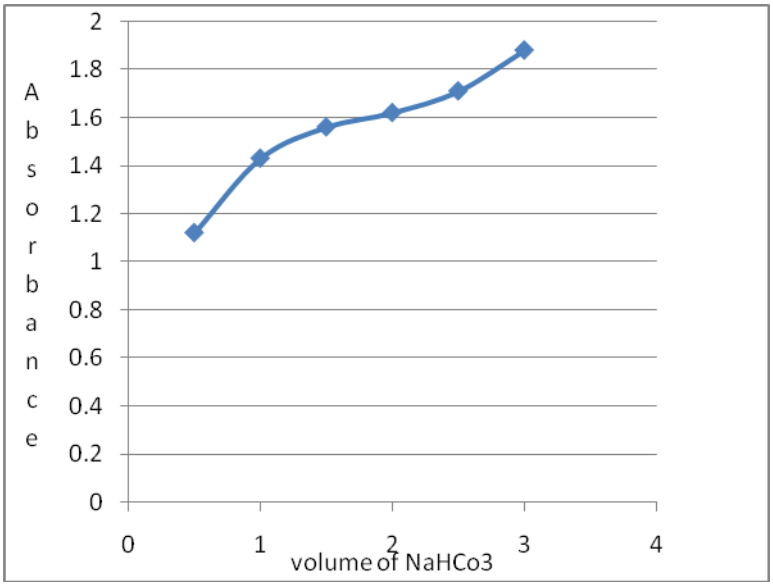

Fig.5: Effect of pH on the reaction of Ninhydrin with 40µg/ml Terbutalin.

4.1.3 Effect of Ninhydrin concentration

Different volumes of Ninhydrin ranging from 1 ml to 5 ml were tried. Maximum color intensity was increasing with the volume of Ninhydrin.

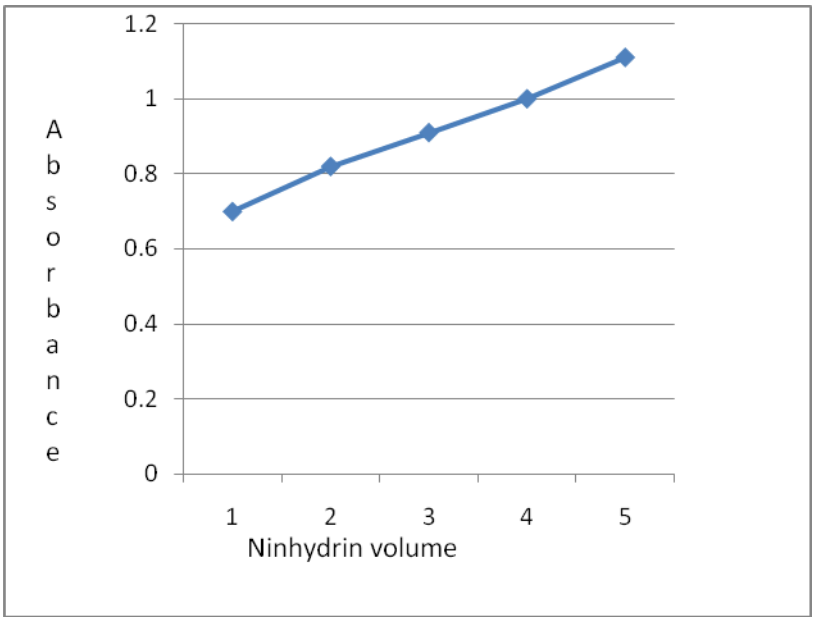

Fig.6: Effect of volume of ninhydrin on the reaction with 40µg/ml Terbutalin.

4.1.4 Effect of solvent

Water, acetone and acetonitril were tried. Water gave the best results for Terbutalin sulphate.

4.1.5 Stoichiometeric Relationship

The stoichiometery of the reaction product formed between the cited drug and ninhydrin was investigated by applying the continuous variation method. The molar ratio of ninhydrin to drug (2:1) (reagent: drug).

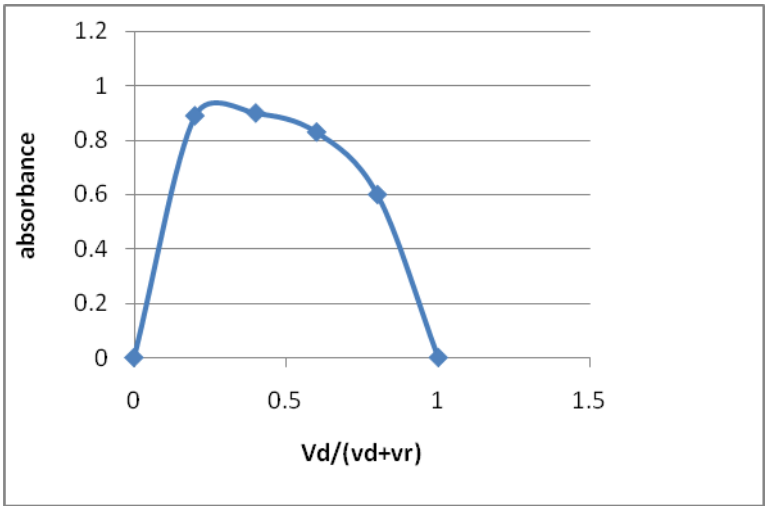

Fig.7: Continuous variation plot for Terbutalin sulphate

Table 2: Regression parameter and sensitivity value

Sr. No	Parameter	Value
1	Linearity range(µg/ml)	10-100
2	λmax(nm)	346
3	Ɛ(L/mol/cm)	-
4	Sandell sensitivity(µg/ml)	-
5	Slop(b)	0.0263
6	Intercept(a)	0.2
7	LOD µg/ml	18.15µg/ml
8	LOQ µg/ml	55µg/ml
9	Regression equation	-
10	Correlation coefficient (r2)	0.98

Table 3: % Recovery data of Terbutalin sulphate using ninhydrin

Sr.no	Level	Concentration taken	Concentration found	% RSD	% Recovery
1	80%	45µg/ml	44.01µg/ml	0.56	99.78%
2	100%	50µg/ml	49.84µg/ml	1.16	99.68%
3	120%	55µg/ml	54.18µg/ml	2.04	98.49%

5. CONCLUSION

This paper presents a spectrophotometric evaluation of Terbutalin sulphate with ninhydrin in the presence of $NaHCO_3$ which produces a yellow colored product with absorbance maximally at 346nm.The proposed spectrophotometric method developed for the determination of Terbutalin sulphate is inexpensive compared to many reported methods. The method is selective, precise and accurate.

6. ACKNOWLEDGEMENT

Author thanks to Wockhardt Pharmaceutical Ltd. (Aurangabad, India) for providing pure Terbutalin sulphate.

REFERENCES

1. K.D. Tripathi, Essentials of Medical Pharmacology, Medical publisher (p) Ltd, 5th edition, (2007), 197 -199.
2. Indian pharmacopoeia, 4th edition, volume II, The controller of publication, Government of India, New Delhi, (1996).
3. General Chemistry lab**,** Wednesday, January 11**.** (2012).

4. Mendel Friedman, Application of ninhydrin reaction for analysis for amino acid to agriculture and biochemical science, journal of food chemical and agriculture, ACS Publication,52(3), (2004)**,** 385-406.
5. E.A Bell**,** The isolation of L-Homarginine from seed of Lathyrus gcera, Biochemical journal, (1962), 85-91.
6. Milton l. Bastos, Gerald E. Kanaen, Randolph M. young, Josph R. Detection of basic organic drug and there metabolite in urine.
7. Gabi Drochiolli, Ion sandu Gabriel loan olteanu, lonel mangala glu, International journal of criminal investigation, volume-1, Issue - 1, 37-58.
8. Indian Pharmacopoeia, 4th edition, Vol-II, The controller of Publication, Government of India, New Delhi, 1996.
9. British Pharmacopoeia, Volume l, department of health Scottish home and health department welsh office, Department of health and serial service for Northern Irland, 1998.
10. United State Pharmacopoeia, 23rd Revision, US Pharmacopoeia conventional Inc, Rockville, MD 20852, 1995.
11. European Pharmacopoeia, 3rd edition, council of Europe, 07, strabourg codex, 1997.
12. N Daraghmeh, M.M Al-Omari, Z Sara, A.A Badwan[a], A.M.Y Jaber[b], Determination of terbutaline sulfate and its degradation products in pharmaceutical formulations using LC, J. of Pharma Biomedical Analysis Volume 29, Issue 5, 31 July 2002, Pages 927–937.
13. Md. Faiyazuddin, Abdul Rauf, Niyaz Ahmad, Sayeed Ahmad, Zeenat Iqbal, Sushma Talegaonkar, Aseem Bhatnagar, Roop K. Khar, Farhan J. Ahmad, validated HPTLC method for Determination of Terbutalin sulfate in biological samples: Application to pharmacokinetic study ,Saudi Pharmaceutical Journal, Volume 19, Issue 3, July 2011, Pages 185–191.
14. Sven-Erik Jacobsson, Sven Jönsson, Claes Lindberg* and Leif-Åke Svensson Determination of terbutaline in plasma by gas chromatography chemical ionization mass spectrometry, whelly publication ,Volume 7, Issue 6, pages 265–268, June 1980.
15. Rau HL, Arooor AR, Rao PG, Fluorimetric, Indian Journal Pharmaceutical science,1990, 52 (5),255-6.
16. International Conference on Harmonization of technical Requirement for registration of pharmaceutical for human use. ICH harmonized tripartite guideline, validation of analytical procedure text and methodology Q29 (R1) Complimentary guideline on methodology data 06 November 1996.
17. Yuri kazakevich, Rosario Lobrutto**,** HPLC for Pharmaceutical sciences, Assignment of validation parameter, Walley India**,** (2011) 463.
18. Ermer, J. and miller, J.H.(2005).Method validation in pharmaceutical analysis. Wiley -VCH, 34-114.
19. Value @ amrita virtual amrita laboratories universalizing education, Amrita vishva vidhyapeetham**,** (1956).
20. Raina Adel sayed, wafaa sayed Hassan, Magda yossif Ei-Mammli and Abdalla Shalaby, Spectrophotometric method for the Determination of cefotaxime sodium and cefoperazone sodium in pure and pharmaceutical dosage forms**.** *American Chemical Science Journal 3(4)*, (2013), 514-52.
21. Kanakapura basavaiah, kalsang tharpa, salmara ganeshbhat hiriyanna and Kanakapura basavaiah vinay, Spectrophotometric determination of lisinopril in Pharmaceuticals using Ninhydrin a modified approaches. *Journal of food and drug Analysis*, vol.17, No 2, (2009), pages 93-99.