SPECTROPHOTOMETRIC OXIDATION METHOD FOR THE DETERMINATION OF RISPERIDONE IN THE PRESENCE OF TRIHEXYPHENYDYL HCl BY USING BROMATE – BROMIDE MIXTURE AS AN OXIDANT

I. Lakshmi Prasanna*[1], G.T. Naidu[1], Nuzhath Fathima[2], I.E.Chakravarthy[2], G. Abdul Huq[3]

*[1]Department of Physics, Rayalaseema University, Kurnool.518007, AP.
[2]Department of Chemistry, Rayalaseema University, Kurnool.518007, AP.
[3]Departments of Chemistry, School of Sciences, Maulana Azad National Urdu University, Hyderabad, 500032, TS.

***ABSTRACT*: A simple, sensitive, precise and accurate spectrophotometric method has been developed for the estimation of Risperidone in the presence of Trihexyphenydyl in pharmaceutical formulations and in the drug dosage form. During the study, it is observed that acidic solution of the drug formed the oxidation product with Bromate – Bromide mixture. This property of the drug is exploited for the development of spectrophotometric method for the determination and analysis of the drug. The oxidation product showed λ_{max} at 320 nm. The linearity range for Risperidone in the presence of Trihexyphenydyl is found to be 10 µg/ml to 250 µg/ml. Recovery studies gave satisfactory results indicating that none of the common additives and excipients interfere in the assay method. The molar absorptivity and the sandell sensitivity of the method are evaluated and the values are found to be to be 0.9153×10^4 lit/ mole/cm and 0.0448 µg/ ml/ cm^2 respectively.**

***KEYWORDS*: Spectrophotometry, Risperidone, Trihexyphenydyl HCl, Bromate-Bromide oxidant, Pharmaceutical formulations.**

INTRODUCTION:

Risperidone, 3-[2-[4-(6-fluoro-1,2-benzisoxazole-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-Tetrahydro-4H-pyrido [1, 2-*a*] pyrimidin-4-one, is a benzisoxazole antipsychotic, reported to be an antagonist to dopamine D2 and serotonin (5HT2), adrenergic, and histamine (H1) receptors. A few chromatographic methods have been reported in the literature for the analysis of risperidone in pharmaceutical preparations either alone, with its degradation products or with other compounds. Other techniques for the determination of Risperidone from pharmaceutical dosage form also have been reported. These techniques include extractive colorimetry, chemiluminescence, capillary zone electrophoresis and non-aqueous titration. There are

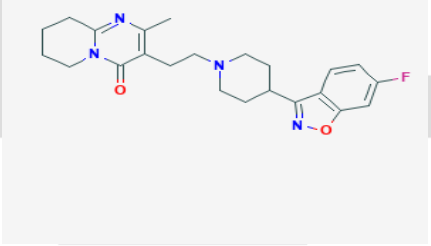

Fig: 1 Structure of Risperidone

Numerous methods to quantify Risperidone and 9-OH-Risperidone[1-6] enantiomers in biological fluids, including HPLCDAD, HPLC with electrochemical detection, MEPS–LC–UV, LC-MS/MS and affinity capillary electrophoresis and H1 NMR spectroscopy. These methods are complicated, costly and time-consuming in comparison to the simple proposed UV method.
Trihexyphenidyl HCl is used to treat symptoms of Parkinson's disease or involuntary movements due to the side effects of certain psychiatric drugs (antipsychotics such as chlorpromazine/haloperidol). Trihexyphenidyl HCl belongs to a class of medication called anticholinergics that work by blocking a certain natural substance (acetylcholine). This helps decrease muscle stiffness, sweating, and the production of saliva, and helps improve walking ability in people with Parkinson's disease. Anticholinergics can stop severe muscle spasms of the back, neck, and eyes that are sometimes caused by psychiatric drugs.

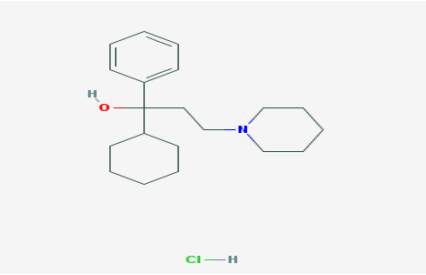

Fig: 2 Structure of Trihexyphenidyl HCl

It can also decrease other side effects such as muscle stiffness/rigidity (extrapyramidal signs-EPS). Further, it is noticed that there are a very few methods reported on the development and validation of the estimation of Risperidone[7-13] which include High-Performance Liquid Chromatography (HPLC) methods. Since not much attention has been given for developing newer analytical UV Spectrophotometric methods for the quantitative determination of such an effective and potential drug in the dosage form and in the pharmaceutical formulations form, the authors are prompted to take up this study and develop suitable new, rapid, sensitive, precise and accurate method for the determination of Risperidone. The results obtained in the present investigations are communicated in this paper.

MATERIALS AND METHODS

(A) Instruments used

(i) Spectrophotometer: A Single beam UV-Spectrophotometer Model SP-UV200 with 1 cm matched quartz cuvettes is employed throughout the study for all absorbance measurements.

(ii) pH Meter: A digital ELICO-pH Meter Model LI-120 is used for pH measurements.

(B) Preparation of Reagents and Solutions

(i) Risperidone solution: 50 mg of pure Risperidone is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol.

This is used as the stock solution of the drug. The working solution with concentration 100 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

(ii) Trihexyphenidyl HCl solution: 50 mg of pure Trihexyphenidyl HCl is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol. This is used as the stock solution of the drug. The working Solution with concentration 100 µg /ml of the drug is prepared by suitably diluting the stock solution as and when required.

(iii) 5M HCL solution: 425 ml of Conc Hydrochloric acid (Merck) is diluted to 1000 ml with distilled water to get about 5 M Hydrochloric Acid solution.

(iv) Bromate - Bromide Mixture preparation: 0.835 g of Potassium Bromate and 6 g of Potassium Bromide are dissolved in distilled water and diluted to one litre. The solution is appropriately diluted to get 10 µg/ml with respect to Potassium Bromate.

(v) Methyl Orange solution: 50 mg of Methyl Orange is dissolved in 50 ml water and is diluted to get 50 µg/ml. By taking 5 ml and adding 100 ml water such that the concentration becomes equal to 50 µg/ml.

All other chemical substances and reagents employed in the present investigations are of AR Grade only.

RESULTS AND DISCUSSION: Risperidone when treated with Bromate- Bromide mixture with Methyl Orange results in an oxidation reaction. This oxidation formation reaction is spectrophotometrically monitored to develop a method for the determination of the drug. In the process of carrying out detailed investigations, first of all, optimisation of various parameters such as the wavelength of maximum absorbance (λ_{max}), the effect of the concentration of oxidizing agent Bromate -Bromide mixture and the effect of Methyl Orange on the absorbance of the oxidation reaction are established and the procedures adopted in each case are described as follows:

Absorption Spectrum of Oxidation reaction: The absorption spectrum of the oxidation reaction formed between Risperidone in the presence Trihexyphenydyl and Bromate –Bromide mixture is obtained in order to fix the wavelength of maximum absorbance in the present study. The experimental procedure adopted is as follows:

1 ml of Risperidone solution (50 µg/ml), 1 ml of Trihexyphenydyl solution (50 µg/ml) , 1 ml of 5M HCl, 1 ml of Bromate-Bromide mixture, 1 ml of Methyl Orange are taken in a 10 ml standard flask. The resulting solution is made up to the mark with distilled water. The contents of the flask are shaken well and allowed to stand for a minute for equilibration. Then the absorbance values of the oxidation reaction formed are measured in the wavelength range 250 nm to 360 nm against the reagent blank. The results obtained are used to draw a graph between the wavelength and the absorbance values. This graphical representation is called the Absorption spectrum which is shown in figure 3 below.

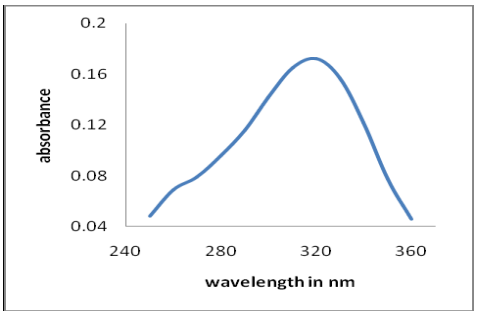

Fig.3 Absorption Spectrum of Oxidation reaction of Risperidone with Bromate – Bromide mixture

It is seen from the above Fig.3 of the absorption spectrum, that the maximum absorbance is obtained at 320 nm. Hence for all further studies, the wavelength 320 nm is fixed

Effect of Bromate-Bromide: The effect of Bromate-Bromide mixture on the absorbance of the oxidation reaction is studied by taking varying volumes (x ml) of Bromate-Bromide mixture in a series of 10 ml standard flasks. 1 ml of Risperidone solution (50 µg/ml) , 1ml of Trihexyphenydyl HCl solution(50 µg/ml), 1ml of 5M HCl, x ml (0.5 ml to 2.5 ml) of Bromate-Bromide mixture , and 1 ml of Methyl Orange are added and the resulting solution is made up to 10 ml using distilled water. The absorbance of each solution is recorded at 320 nm against a suitable blank. The results obtained are shown in Table 1 below.

Table 1: Effect of Bromate – Bromide mixture on oxidation

1 ml Risperidone solution (50 µg/ml) + 1 ml of Trihexyphenydyl HCl solution (50 µg/ml) +1 ml of 5M HCl +(x ml i.e;0.5 ml to 2.5 ml) of Bromate-Bromide mixture solution (10 µg/ml) +1 ml of Methyl Orange +6-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 320 nm**

S. No	Vol. of Risperidone (50 µg/ml) in ml	Vol.of Trihexy-phenydy-l(50 µg/ml) in ml	Vol. of Bromate-Bromide mixture Solution x ml	Vol. of Methyl Orange in ml	Vol. of 5M HCl in ml	Vol. of distilled water in ml (6-x)	Total Vol. in each flask in ml	Absorb-ance
1	1.0	1.0	0.5	1.0	1.0	5.5	10	0.130
2	1.0	1.0	1.0	1.0	1.0	5.0	10	0.104
3	1.0	1.0	1.5	1.0	1.0	4.5	10	0.156
4	1.0	1.0	2.0	1.0	1.0	4.0	10	0.095
5	1.0	1.0	2.5	1.0	1.0	3.5	10	0.050

It is observed that 1.5ml of Bromate-Bromide mixture is required for maximum absorbance. Hence for all further studies a volume of 1.5 ml of Bromate-Bromide solution is fixed.

Effect of volume of Methyl Orange: The effect of volume of Methyl Orange on the absorbance of oxidation reaction is studied by taking varying volumes of (x ml= 0.5 ml to 2.5 ml) of Methyl orange solution in a series of 10 ml standard flasks keeping the volume of Risperidone solution fixed at 1 ml. To each flask 1 ml of Trihexyphenydyl solution, 1 ml of 5M HCl, and 1.5 ml of Bromate-Bromide mixture are added followed by the addition of distilled water to make up each 10 ml flask to mark. The absorbance of each solution is recorded at 320 nm against the suitable blank and is as shown in Table 2 below.

Table 2: Effect of Methyl Orange on Oxidation

1 ml of Risperidone solution (50 µg/ml) + 1 ml of Trihexyphenydyl HCl solution(50 µg/ml) + x ml (0.5 ml to 2.5 ml) of Methyl Orange solution (50 µg/ml) +1 ml of 5 M HCl + 1.5 ml of Bromate- Bromide mixture (10 µg/ml) + (5.5-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 320 nm**

S. No	Vol. of Rispe-ridone (50 µg/ml) in ml	Vol.of Trihexyph enydy-l(50 µg/ml) in ml	Vol. of Methyl Orange solution x ml	Vol. of Bromate-Bromide mixture in ml	Vol of 5M Hcl in ml	Vol of distilled water in ml (5.5-x)	Total vol. in each flask in ml	Absorb-ance
1	1.0	1.0	0.5	1.5	1.0	5.0	10	0.031
2	1.0	1.0	1.0	1.5	1.0	4.5	10	0.074
3	1.0	1.0	1.5	1.5	1.0	4.0	10	0.085

4	1.0	1.0	2.0	1.5	1.0	3.5	10	0.113
5	1.0	1.0	2.5	1.5	1.0	3.0	10	0.189

It is observed that 2.5 ml of Methyl Orange solution is necessary to achieve maximum absorbance. Hence for all further studies a volume of 2.5 ml of Methyl Orange solution is required.

Effect of concentration of Drug Risperidone: This study pertains to the effect of the drug Risperidone concentration on the absorbance of the Oxidation reaction under the established optimal experimental conditions. The recommended procedure for the calibration curve and for the obedience of Beer-Lambert's Law for the quantitative spectrophotometric determination of the drug Risperidone is as follows

Calibration Curve: Obedience of Beer - Lambert's Law: Various aliquots (x ml i.e., 0.5 ml to 2.5 ml) of Risperidone solution (50 µg/ml) are taken in a series of 10 ml standard flask. To each flask,1 ml of Trihexyphanydyl HCl solution , 2.5 ml of Methyl Orange solution, 1.5 ml of Bromate-Bromide solution, 1 ml of 5M HCl are added followed by (4-x) ml of distilled water.resulting solution are made upto a total volume of 10 ml in each case. The contents of each flask are shaken well and allowed to stand for a minute for equilibration. The absorbance of each solution is measured at 320 nm against a suitable reagent blank which is prepared in a similar manner but devoid of drug solution.

Table 3: Calibration Curve: - Obedience of Beer-Lambert's Law

x ml(i.e;0.5 ml to 2.5 ml) of Risperidone solution (50 µg/ml) + 1 ml of Trihexyphenydyl HCl solution (50 µg/ml) + 1 ml of 5 M HCl + 2.5 ml of Methyl Orange solution (50 µg/ml)+ 1.5 ml of Bromate-Bromide mixture (10 µg/ml) + (4-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max}= 320 nm**

S. No	Vol. in ml Rispe -ridone (50 µg/ml) x ml	Amount of Risperi-done in µg/ml	Vol.of Trihexy-phenydyl(50 µg/ml) in ml	Vol.of Methyl Orange in ml	Vol.of Bromate-Bromide mixture in ml	Vol. of 5M HCl in ml	Vol. of distilled water in ml (4-x)	Total vol. in each flask in ml	Absor -bance
1	0.5	50	1.0	2.5	1.5	1.0	3.5	10	0.045
2	1.0	100	1.0	2.5	1.5	1.0	3.0	10	0.083
3	1.5	150	1.0	2.5	1.5	1.0	2.5	10	0.126
4	2.0	200	1.0	2.5	1.5	1.0	2.0	10	0.175
5	2.5	250	1.0	2.5	1.5	1.0	1.5	10	0.223

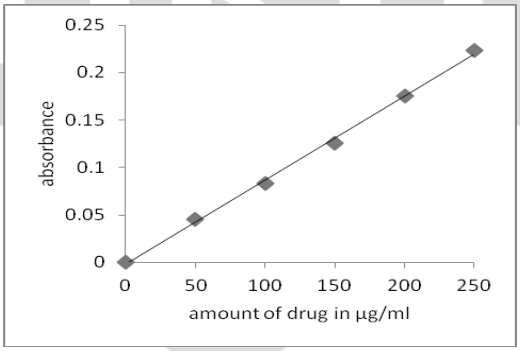

Fig.4 Calibration curve –Verification of Beer-Lambert's Law

It is obviously clear from this calibration straight line as shown in above Fig.4 that the absorbance values increased linearly with the increase in the amount of the drug. This verifies the Beer-Lambert's Law and suggests that the method can be successfully employed for the spectrophotometric quantitative determination of the drug Risperidone in the range 10 µg/ml to 250 µg/ml. The molar absorptivitiy and the Sandell Sensitivity of the method are found to be 0.9153×10^4 lit/ mole/cm and 0.0448 µg/ ml/ cm^2 respectively.

Assay of Risperidone drug in pharmaceutical formulations: -

The recommended procedure for the quantitative micro determination of Risperidone drug is applied for the assay of the drug in the dosage form of the commercial tablets and also in pharmaceutical formulations. The assay is carried out as follows:

20 tablets of Risperidone are weighed and finely powdered. An accurately weighed portion of the powdered sample equivalent to 50 mg of Risperidone is taken in a 50 ml volumetric flask containing 25 ml of methanol and is sonicated for about 20 minutes. The resultant solution is filtered through Whatman filter paper No.41 into another 50 ml volumetric flask. The filter paper is washed several times with methanol and the washings are added to filtrate. The final volume is made upto the mark with methanol. Now, 5 ml of filtrate of the sample solution is diluted to 10 ml with methanol and treated as per the recommended procedure of calibration. From this, the amount of the drug present in the sample is computed from the calibration curve. The results obtained are as shown in Table 4 below.

Table 4: Assay of Risperidone in Tablets

Sample	Labelled amount in mg	Amount found by present method ±SD*	Percentage of Label claim	*t_{cal}	% RSD
Tablet I	20	20.074±0.21	100.074	0.7879	1.05
Tablet II	20	20.104±0.12	100.104	1.9378	0.60

* Average of 5 determinations based on label claim.

CONCLUSION:

The calibration curve is linear up to 250 µg/ml indicating the suitability of the proposed method for the spectrophotometric determination of Risperidone in the range of 10 µg/ml to 250 µg/ml. The standard deviation values are found to be low showing high accuracy and reproducibility of the method. The calculated `t' values are less than the `t' theoretical values with 4 degrees of freedom at 95% level of significance. This indicates that there is no significant difference between the proposed method and the standard method. Further, there is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentration of those present in general pharmaceutical preparations. Thus the proposed method can be conveniently adopted for the routine analysis and estimation of Risperidone in pharmaceutical formulations.

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