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Method Development and Validation of Risperidone by using RP - HPLC and its Stress Studies

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ABSTRACT

The objectives of the research work were focused on the development and validation of RP-HPLC for the stability assessment of Risperidone as it applies to stress studies. A new stability indicating RP-HPLC method was developed for the quantification of Risperidone in tablets. Separations module with Waters 2489 UV detector with Hypersil BDS C18 column (250 mm x 4.6 mm, 5µ) was operated with column temperature 50°C. The detector was monitored at 280 nm and the chromatographic study was run for 12 min. Linearity was observed over 40-80µg/mL with linear regression equation Y=16174x-18715 with correlation coefficient R^2 = 0.9999. The LOQ and LOD were found to be 0.0039µg/ml and 0.0119µg/ml respectively. Forced degradation studies were performed for Risperidone and the method was validated as per ICH guidelines. The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Risperidone in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Risperidone formulations. It was observed that Risperidone is stable towards the forced degradation studies as the drug decomposed is less than 5%.

Keyword- Development, validation, Linearity, Degradation.

INTRODUCTION

Analytes in the pharmaceutical analysis sector range from basic chemicals to complex biomolecules in structure. A wide range of techniques to used to develop reliable analytical methods for these substances.

A. Qualitative analysis: It deals with the identification of substances. It deals withthe determination of elements or compounds present in the sample.[1]

B. Quantitative analysis provides numerical information concerning the numberof sample species (the analyte) in a measured amount of matter in the sample.[2]

Chromatography:

Writing in color is known as Chromatography [3] (Greek khromatos: color, and Graphos:written). Mikhil Tswett discovered the word chromatography and its concepts in 1903. By exploiting the differences in partitioning behavior analytes between moving and stationary phases, chromatography is used to identify and separate components in a mixture.

High-performance liquid chromatography (HPLC):

The HPLC is the method of choice in analytical chemistry [4] since it is specific, robust, linear, precise, and accurate. The integrator itself performs the limit of detection analysescalculation.

Principles of separation[5]:

High-surface-area particles are used in adsorption chromatography to adsorb molecules of interest. Using non-polar mobile phases such as chloroform or heptane, you will need to use adsorption solid like silica gel, alumina, or even porous glass beads. The competition model and the solvent interaction model are used to describe the adsorption process in adsorption chromatography.

Instrumentation:

The essential parts of the High-Performance Liquid Chromatography are:

- Solvent reservoir and treatment system
- Mobile phase
- Pump system
- Sample injection system
- Column

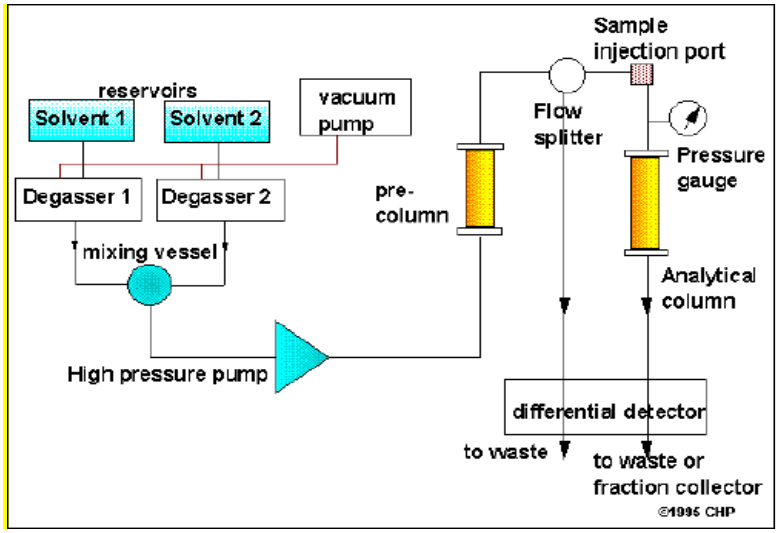


Fig. 1. An example of an HPLC flow chart

Mobile phase:

When it comes to HPLC, most mobile phases are composed of organic solvents mixed with water or aqueous buffers. It is better to use isocratic methods rather than gradient approaches.[6] Some examples of organic solvents which we are using as a mobile phase are - Acetone, Methanol, Chloroform , etc…

Aim & Objectives

- ✓ To develop and validate an analytical method for Risperidone by using RP-HPLC and performits Stability studies.
- ✓ The objectives of the research work were focused on the development and validation of RP-HPLC for the stability assessment of Risperidone as it applies to stress studies.

Plan Of Work:

Keeping in view the aim and objectives, the plan of work was set as follows

- Review of the Literature & Selection of the newer drugs.
- Procurement of the standard drugs samples.
- HPLC method development.
- Selection of initial chromatographic conditions.
- Identify the weakness of the developed methods.
- Optimization of chromatographic conditions.
- Assay of the marketed formulations.
- Validation of the developed method as per ICH guidelines.
- Stability studies under various stress conditions.

MATERIALS AND INSTRUMENTS

Materials used: (Table -1)

S.no	Chemicals/Solvents	Manufacturer
1	HPLC Grade Acetonitrile	Lab Fine Chem Industries Ltd., Mumbai, India
2	HPLC Grade Methanol	Lab Fine Chem Industries Ltd., Mumbai, India
3	Ammonium Acetate	Gaurav ScientificandChemicals, Mumbai, India
4	Hydrochloric acid	Lab Fine Chem Industries Ltd., Mumbai, India
5	Sodium Hydroxide	Lab Fine Chem Industries Ltd., Mumbai, India

Instruments Used (Table -2)

1	UV Spectrophotometer	Shimadzu UV1800S
2	Ultra performance Liquid Chromatography	Agilent UHPLC-MS, E6125B
3	Weighing Balance	Shimadzu

RESULT AND DISCUSSIONS

Instrument Specifications

Instrument - High Liquid Performance chromatography
Injector - Rheodyne (20µl loop)
Software - LC solutions
Detector - PDA

Selection Of Mobile Phase:

Initially the stressed samples were analyzed using a mixture of Water : Acetonitrile (75:25) with a flow rate of 0.8 ml/min in which the peak was obtained at Rt 11.85 mins and also the resolution and peak symmetry were not satisfactory. The mobile phase ratio was changed to 70:30 % v/v and the drug sample was injected in to the loop where a sharp peak was elutedat 9.16 mins with minimal tailing.

The HPLC method's PDA detector selectivity is based on the wavelength of the PDA detector. For the drug to have a specific and definite response, a precise wavelength must be chosen. A wavelength of 280 nm was selected from the UV spectra of the risperidone drug because it was the most relevant. Different mobile phases tried and their observations are given in the Table-3

Optimised chromatographic conditions Table -4

Mobile Phase condition	**Observation**
Water: Acetonitrile (75:25)	Tailing, Broad peaks
5mM K_2HPo_4: Acetonitrile pH5 (70:30)	Fronting, Tailing, Splited Peaks
5mM K_2HPo_4: Acetonitrile pH4.5 (70:30)	Tailing, Splited Peaks
5mM K_2HPo_4 : Acetonitrile pH4 1ml/min (70:30)	Tailing, Broad Peaks
Water: methanol (70:30)	Good Symmetric Peaks

Detector Wavelength	280nm
Injection volume	20µl
Column Temperature	60^0C
Auto sample Temperature	2^0C
Elution mode	Isocratic
Run time	**12** minutes
Column	C_{18} column (100 x 2 mm, 1.9)
Flow rate	1ml/min

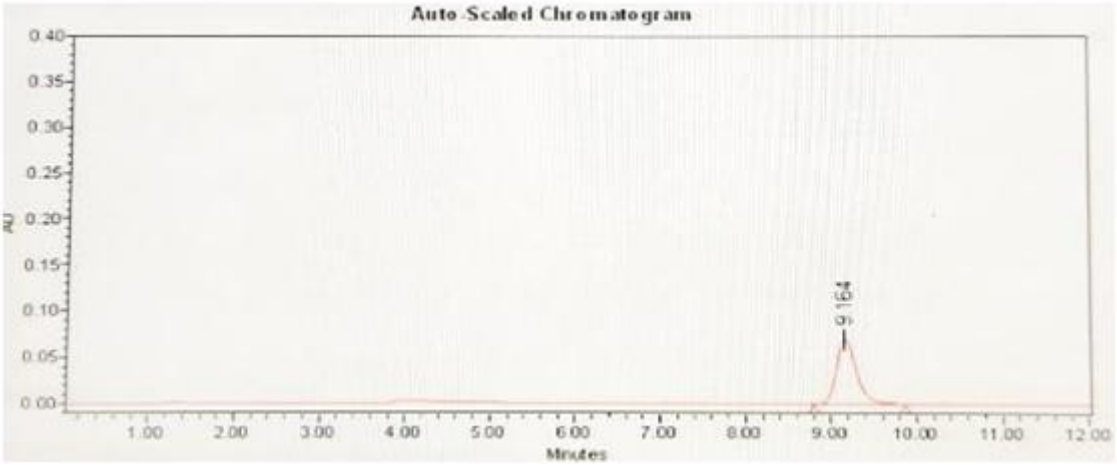
Validation of RP-HPLC Method

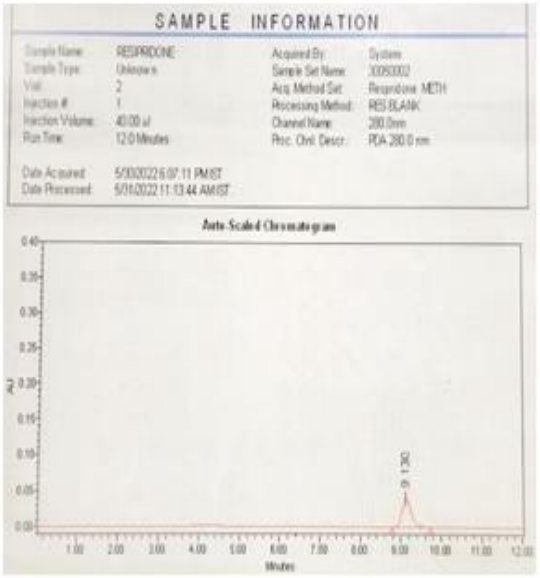
System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution (Rs) etc., were studied, and results are given in table.no.5

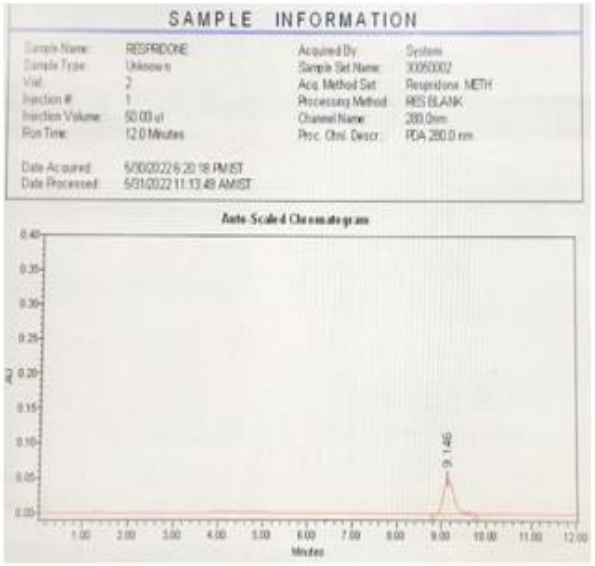
System suitability studies of Risperidone Table-5:

Drug	**Theoretical plates (N)**	**Retention time (Rt)**	**Tailing factor**
Risperidone	3718	9.164	1.5

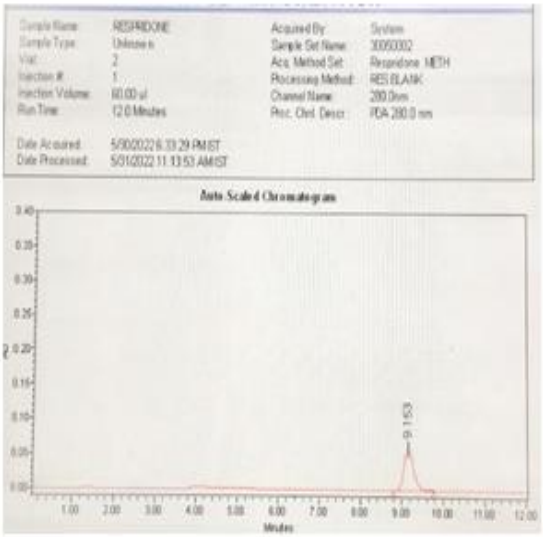




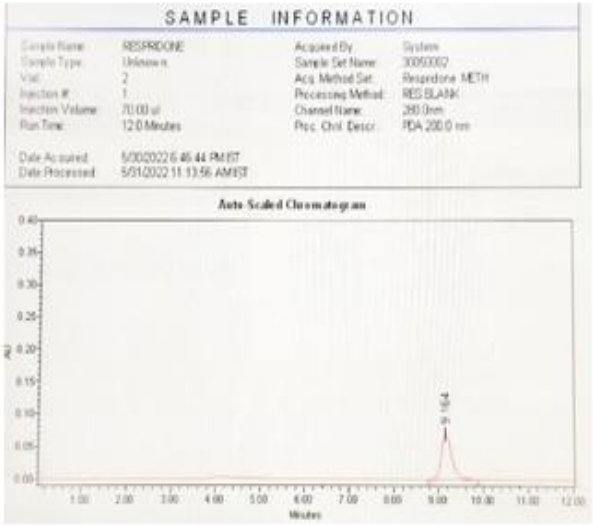
Linearity Chromatogram of Risperidone at 40 ppm**.**



Linearity Chromatogram of Risperidone 50ppm



Linearity Chromatogram of Risperidone at 60 ppm**.**



Linearity Chromatogram of Risperidone 70ppm

Linearity of Risperidone: Table-6

Concentration (µg/ml)	**Mean peak area (A.U.)**
40	628377
50	792497
60	950780
70	1117162
80	1279743
Linear regression equation (y=mx+c)	Y=16174x-18715
Slope(m)	16174
Intercept(c)	18715
Correlation coefficient (R^2)	0.9997

Table -7 Intraday precision of Risperidone

Drug	Conc (µg/ ml)	Peak area	Time intervals of samples in precision					Aver age	%RSD
Risperi done	40	628418	628310	628416	628411	628616	628419	6284 34.4	0.02
	50	792548	792537	792599	792413	792420	792620	7925 17.8	0.01
	60	950820	951821	950009	950018	953914	954001	9519 52.6	0.21
	70	1107182	1107182	1100182	1100054	1100213	1101182	1101 762.6	0.28
	80	1279780	1270780	1271780	1279780	1270001	1270180	1272 504.2	0.32

Table-8 Interday precision of Risperidone

Drug	**Conc (µg/ml)**	**Peak area**	**Time intervals of samples in precision**					**Average**	**%RSD**
Risperido ne	40	628418	62845	62840	628921	628001	628400	628437.4	0.05
	50	792548	79250	79214	792540	792064	792001	792251.8	0.03
	60	950901	95007	95045	950801	950904	950154	950458.2	0.04
	70	1107173	110752	110072	1100014	110023	110102	11017026	0.18
	80	1279702	127081	127180	1279880	127041	127042	1272506.2	0.22

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated mathematically. The LOD and LOQ of Risperidone were found tobe 0.0039µg/ml and 0.0119µg/ml respectively. It can be shown in table.no-9

Table.no.9 LOD and LOQ of Risperidone

Drug	**Limit of Detection**	**Limit of Quantification**
Risperidone	0.0039	0.0119

Robustness

It is possible to assess an analytical procedure's robustness by observing how well it holds up undertypical conditions, even when tiny but deliberate changes are made to the technique parameters. Changes in the composition of the buffer in the mobile phase and flow rate were used to test the method's robustness. Each condition's RSD percentage was computed. Although several procedure parameters were deliberately varied, the findings derived from these changes were remarkably consistent and results are shown in table.no.10

Parameters	**Modifications**	**Robustness**	
		Plate count	**tailing factor**
		Risperidone	**Risperidone**
Flow Rate	0.8	3512	1.41
	Optimized	3711	1.50
	1.2	3704	1.41

Mobile PhaseComposition	10% less	3711	1.41
	Optimized	3711	1.50
	10% more	3012	1.46
Wavelength	+5nm	3412	1.45
	Optimized	3711	1.50
	-5nm	3312	1.45
Column Temperature	$+5^{o}C$	3212	1.42
	Optimized	3711	1.50
	$-5^{0}C$	3412	1.46

Accuracy

The accuracy of the drug's recovery was determined by conducting recovery experiments. The sample formulation was reanalyzed using the suggested approach after it was mixed with a knownquantity of standard pharmaceuticals. Both a 50 and a 100 percent sample was used in this study table.no. 11 displays the recovery results.

Drug	**Levels (%)**	**Amount taken(µg/mL)**	**Amount added(µg/mL)**	**Amount recovered**	**% recovered**
				(µg/mL)	
Risperidone	**80**	**50**	**40**	**89.85**	**99.83%**
	100	**50**	**50**	**100.35**	**100.35%**
	120	**50**	**60**	**120.16**	**100.13%**

Acid degradation

Over a wide range of pH, hydrolysis is a frequent degradation chemical process. Hydrolysisis a chemical process in which a chemical component is broken down by the addition of water to the reaction. Ionizable groups in molecules can be used to catalyze hydrolysis in acidic conditions. Drug substances are exposed to an acidic environment in order to produce primary degradants in a desired range of concentrations. The stability of the drug material specifies the type and concentration of acid used. For acid degradation to break down a, use hydrochloric acid (0.1 to 1 M). Co-solvents can be used to dissolve substances for stress testing that are insoluble in water. In order to choose a co-solvent, it is necessary to know the drug's structure. Normal stress testing trials begin at ambient temperature and progress to increased temperatures ranging from $50–70^{o}C$ if no degradation is seen. Stress testing should not last for longer than seven days at the most. To prevent further breakdown, an acid or buffer is used to neutralize the degraded sample. As shown in fig. and results in table.no.12.

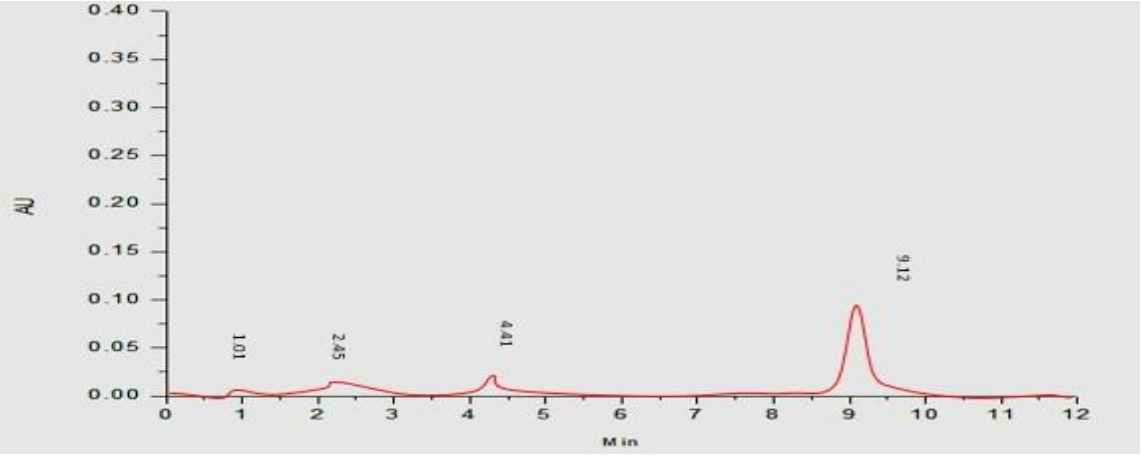


Table no -12 Acid degradation

S.no	Impurities	Peak Area	%Degradation	% Assay of Active Substance
1	Impurity-A	74552	0.2882	28.82
2	Impurity-B	14303		
3	Impurity-C	10416		

Table no -13: %Acid Degradation of Risperidone

S.no	Optimized and Degradation types	Retention time
1	Risperidone Peak	9.12
2	Impurity-A	1.01
3	Impurity-B	2.45
4	Impurity-C	4.41

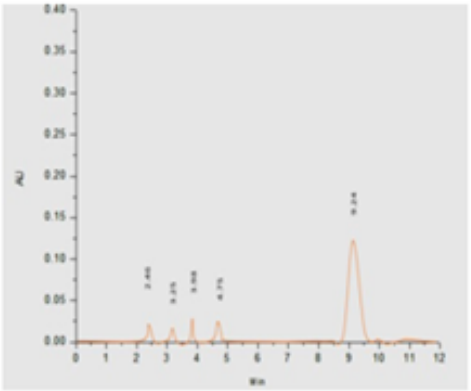
Table no -14: Acid degradation of Risperidone

S.no	Optimized and Degradation types	Retention time
1	Risperidone Peak	9.12
2	Impurity-A	1.01
3	Impurity-B	2.45
4	Impurity-C	4.41

Oxidative degradation

Other oxidizing agents, such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyroni-trile, AIBN), can also be utilized in forced degradation investigations. It is important to know the drug material before selecting an oxidising agent, its concentration, and theconditions for use. Hydrogen peroxide exposure at neutral pH and room temperature for seven days or up to a maximum of 20% degradation is stated to be able to produce meaningful degradation products. Electrons are transferred between anions and cations as a result of the oxidative decomposition of drugs. Electron transfer oxidation of amines, sulphides, and phenols produces N-oxides, hydroxylamine, sulfones, and sulfoxides. Oxidation of labile hydrogen-containing functional groups such as those in the benzylic carbon, the allylic, and the tertiary carbon positions with regard to the Hetero atom can result in hydro peroxides, hydrogen or ketoneformation. As shown in figure. and results in table.no.15.

S.no	Optimized and Degradation types	Retention time
1	Risperidone Peak	9.24
2	Impurity-A	2.46
3	Impurity-B	3.25
4	Impurity-C	3.98
5	Impurity-D	4.75



Thermal degradation:

Thermal degradation (e.g., dry heat and wet heat) should be performed under more challenging settings than those prescribed by ICH Q1A accelerated testing. Solid-state drug substances and drug products should be treated with dry and wet heat, whereas liquid drug products should be treated with dry heat. In research, higher temperatures for a shorter period of time may beused. As illustrated in tables.no.16,17

Table -16 Thermal degradation of Risperidone

S.no	Optimized and Degradation types	Retention time
1	Risperidone Peak	9.24
2	Impurity-A	2.13
3	Impurity-B	3.35
4	Impurity-C	4.63

Table – 17: %Thermal degradation of Risperidone

S.no	Impurities	Peak Area	%Degradation	% Asssay of Active Substance
1	Impurity-A	26794	0.2928	29.28
2	Impurity-B	14592		
3	Impurity-C	10600		

Table.no.18. overall Degradation of Risperidone

Stress condition	%Impurity						(%) Degradation	(%) Asssay of Active Substance
	Imp -A	Imp-B	Imp-C	Imp- D	Imp-E	Imp-F		
Acid hydrolysis	74.552	14.303	10.416	103.701	93.170	8.227	0.2882	28.82
Alkaline Hydrolysis	40.236	1.243	15.255	ND	ND	ND	0.3248	32.48
Oxidative Hydrolysis	1.708	74.775	ND	ND	ND	ND	0.4351	43.51
Thermal Hydrolysis	76.794	14.592	10.600	104.179	93.280	8.806	0.2928	29.28

CONCLUSIONS

Risperidone is an atypical antipsychotic used to treat schizophrenia and bipolar disorder. Risperidone is an anticonvulsant drug used for the treatment of epilepsy. It acts by decreasing the abnormal electrical activity in the brain. A new stability indicating RP-HPLC method was developed for the quantification of Risperidone in tablets. Separations module with Waters 2489 UV detector with Hypersil BDS C18 column (250 mm x 4.6 mm, 5µ) was operated with column temperature 50°C. The detector was monitored at 280 nm and the chromatographic study was run for 12 min. Linearity was observed over 40-80 µg/mL with linear regression equation Y=16174x-18715 with correlation coefficient R^2 = 0.9999. The LOQ and LOD were found to be 0.0039µg/ml and 0.0119µg/ml respectively. Forceddegradation studies were performed for Risperidone and the method was validated as per ICH guidelines. The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Risperidone in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Risperidone formulations. It was observed that Risperidone is stable towards the forced degradation studies as the drug decomposed is less than 5%.

BIBLIOGRAPHY

[1]. Stability Indicating Liquid Chromatographic Method for Estimation of Trihexyphenidyl Hydrochloride and Risperidone in Tablet Formulation: Development and Validation Consideration Chromatography Research International Volume 2014, Article ID 523184,

[2]. Determination of Risperidone and forced degradation behavior by HPLC in tablet dosage form, International Journal of Pharm Tech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol.1, No.3, pp 568-574 , July-Sept 2009.

[3]. Rupali Tambe RP-HPLC Method Development And Validation Of Paliperidone In Bulk And Pharmaceutical Dosage Form The International journal of analytical and experimental modal analysis Volume XII, Issue VI,June/2020 ISSN NO:0886-9367