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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RISPERIDONE AND BENZOIC ACID IN ORAL SOLUTION

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ABSTRACT

Using less toxic chemicals and time saving method for determination of the Risperidone (RIS) and Benzoic Acid (BA) was achieved with Waters XTerra (4.6 × 150mm, 5µm) stationary phase, using a UV detector at 275nm wavelength. The optimized mobile phase was consisted of potassium dihydrogen phosphate buffer (pH-3.00) and methanol (50:50 v/v). The retention times were 3.07 and 4.18 for RIS and BA respectively. Run time was 8 min, the flow rate was 0.8 ml/min and injection volume was 10 µl. The method was validated for specificity, linearity, solution stability, accuracy and precision. The method was specific, linear, accurate and precise. The method was linear within the range of 25% to 200% of the assay concentration (200 for RIS and 400 for BA). The method was accurate within the range of 50% to 200% of assay concentration. These methods can be used for qualitative and quantitative analysis of Risperidone and Benzoic Acid in a risperidone oral solution.

KEY WORDS: **Risperidone, Benzoic acid, Analytical Method Development, HPLC.**

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INTRODUCTION

Risperidone (RIS) is an antipsychotic of the benzisoxazol derivatives. It is a selective monoaminergic antagonist. It has affinity for serotonin-5-HT2, dopamine-D2, H1-histamine alpha1–and alpha2–adrenergic receptors. Risperidone has no affinity for cholinergic receptors. It is a potent D2–antagonist. Benzoic acid (BA) is used as preservative in the Risperidone oral solution double the amount of risperidone i.e. 2mg/ml.

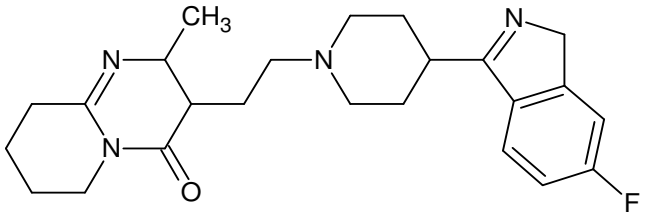

Figure 1
Chemical structure of Risperidone

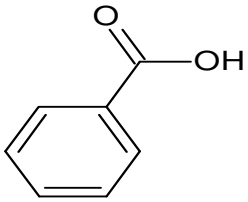

Figure 2
Chemical structure of Benzoic Acid

Blockade of dopaminergic D2 receptors in the limbic system alleviates positive symptoms of schizophrenia such as hallucinations, delusions, and erratic behavior and speech. Blockade of serotonergic 5-HT2 receptors in the mesocortical tract causes an excess of dopamine, resulting in an increase in dopamine transmission and an elimination of core negative symptoms. It has high affinity for D2dopaminergic receptors. It has actions at several 5-HT (serotonin) receptor subtypes. These are 5-HT2C, linked to weight gain, 5-HT2A , linked to its antipsychotic action and relief of some of the extrapyramidal side effects experienced with the typical neuroleptics through action at 5-HT1A . Like other 5-HT2 antagonists, risperidone also binds at alpha (1)-adrenergic receptors and, to a lesser extent, at histamineH1 and alpha (2)-adrenergic receptors. Several analytical methods have been reported in the literature for the analysis of Risperidone from pharmaceutical dosage form. The quality, safety and efficacy of a pharmaceutical product is monitored and maintained throughout the process of manufacture and stability of product by a series of tests for quality control. The quality control test involves methods which embrace chemical, instrumental, microbiological or simply biological procedures. The pharmaceutical medicines testing is based on the separation, identification and purification of pharmaceuticals. Analytical chemistry includes two important steps in analysis, are identification and estimation of the component of a compound. These techniques are also described as qualitative analysis and quantitative analysis. Qualitative analysis is a technique which estimates the particular

compound presence or absence and quantitative analysis is a technique which estimates, how much quantity present in mass or chemical mixture. The qualitative method is relatively simple, but quantitative analysis is more complicated. Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods [1]. Analytic method development and validation are key elements of any pharmaceutical development program. An HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use"[2-5].

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures is equally important to those listed herein and may be addressed in subsequent documents. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated.

Typical validation characteristics which should be considered are listed below

1. Accuracy
2. Precision
3. Repeatability
4. Intermediate Precision
5. Specificity
6. Detection Limit
7. Quantitation Limit
8. Linearity
9. Range

Table 1
Requirement of Validation Characteristics in Analytical Test Procedure as Per ICH Guidelines Q2 R1

Characteristics	Type of analytical procedure			
	Identification	Testing For Impurities		Assay
		Quantitative Tests	Limit Tests	
Accuracy	√	√	–	√
Precision				
Repeatability	–	√	–	√
Intermediate Precision	–	√	–	√
Specificity	√	√	√	√
Limit of Detection	–	–	√	–
Limit of Quantitation	–	√	–	–
Linearity	–	√	–	√
Range	–	√	–	√

Experimental

A. Materials & Reagents

Risperidone USP (RIS) was provided by Janssen Pharmaceutica Ltd. Wallingstown, Little Island, Ireland. Benzoic Acid (BA) was provided from Panreca Quimica SLV, Belgium. Methanol and acetonitrile were of Analytical Grade and purchased from Finar chemicals pvt ltd company (Gujarat India). Pottasium Dihydrogen Phosphate (KH_2PO_4) purchased from SDF chemicals, Ltd. Orthophosphoric acid was of analytical grade and procured from Rankem Chemicals Ltd. Mili-Q (0.22µ) water was used for preparing mobile phase and other solutions. Pharmaceutical finished dosage forms utilized in the present work was provided by Janssen Pharmaceutical Ltd. Wallingstown, Little Island, Ireland. Product was marketed as RISPERDAL® 1mg/ml oral solution.

B. Instruments

The liquid chromatographic system, used in the present study, consisted of Agilent technologies 1200 series instrument equipped with a quaternary solvent delivery system and a model is Agilent series Infinity-1200 Diode Array Detector (DAD), with auto sampler and column thermostat. Chromatographic data were collected and processed using Agilent chromeleon software. Weighing Balance of Sartorius Minebea co. Ltd having balancing range 5-200mg. pH- Meter of Lab India Pvt Ltd with pH ranges 1-14.

C. Chromatographic Conditions

Separation was achieved on Waters Xterra C-18 column (150 mm × 4.6 mm, 5 µm pore size) maintained ambient column oven temperature and sampler temperature. Isocratic elution with methanol: water (50:50% v/v) mobile phase at the flow rate of 0.8 ml/min was carried out. The detection was monitored at 275 nm and injection volume was 10 µl. The peak purity was checked with the DAD detector.

D. Methods

i. Standard stock solutions

Stock solutions of 1 mg/ml and 2 mg/ml of RIS and BA respectively were prepared by dissolving them in acetonitrile and water. Standard calibration solutions were prepared by dilution of the stock solutions using the diluent. These solutions were considered at seven different levels which were 25%, 50%, 100%, 150% and 200% of the test concentration. For RIS and BA mixtures, standard solutions of RIS and BA containing a constant concentration of 0.2 mg/ml and 0.4 mg/ml respectively (internal standard) were prepared in diluent by maintaining the

concentrations in the range of 0.05 mg/ml to 0.4 mg/ml and 0.1 mg/ml to 0.8 mg/ml respectively. The calibration curves for RIS and BA mixtures were constructed by plotting the peak area against the drug concentration. The diluent is prepared by adding water and acetonitrile (80:20 v/v).

ii. Selection of detection wavelength

The detection wavelength was selected by scanning the 10 µg/ml concentration solution of Risperidone and Benzoic Acid in the mobile phase in UV spectrophotometer and maximum absorption was selected as 275 nm.

iii. Selection of mobile phase

The pure drug of Risperidone and Benzoic Acid were injected into the HPLC system and run in different mobile phase system. Different mobile phases like acetonitrile, methanol, water, different pH buffer are tried. It was concluded that Potassium phosphate buffer of pH- 3.00 and methanol gives satisfactory results which passes the ICH guideline i.e. ICH Q2 (R1). Hence finalized mobile phase is Methanol and potassium phosphate buffer of pH- 3.00 (50:50).

iv. Sample preparations

The label claim of the marketed preparation is 1 mg/ml and 2 mg/ml of RIS and BA respectively. 10 ml of marketed preparation was transferred into a 50ml volumetric flask and made up volume with diluent this is the test solution concentration.

v. Preparation of Phosphate buffer

Potassium dihydrogen phosphate (2.72 g) was dissolved in 1000 mL of Mili-Q water (filtered through 0.22µm size filters) and pH 3.0 was adjusted with orthophosphoric acid. It was filtered through 0.45 µm nylon membrane filter and degassed. It was used for the preparation of mobile phase.

vi. System suitability test (SST)/ Specificity and formulation analysis

The specificity of method was established by preparing placebo solution by optimized method for assay of the samples using equivalent weight of the placebo with marketed preparation. Chromatogram of the placebo was not showing any interference at the retention time of Risperidone and Benzoic Acid (Figure). The SST ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. All critical parameters tested met the acceptance criteria on all days. Adequate resolution between the RIS and BA peaks ensured the specificity of the method. The system suitability assessment for the analytical HPLC method established instrument performance parameters such as peak area, % R.S.D., column efficiency (N) and USP tailing factor (Tf) for both the analytes.

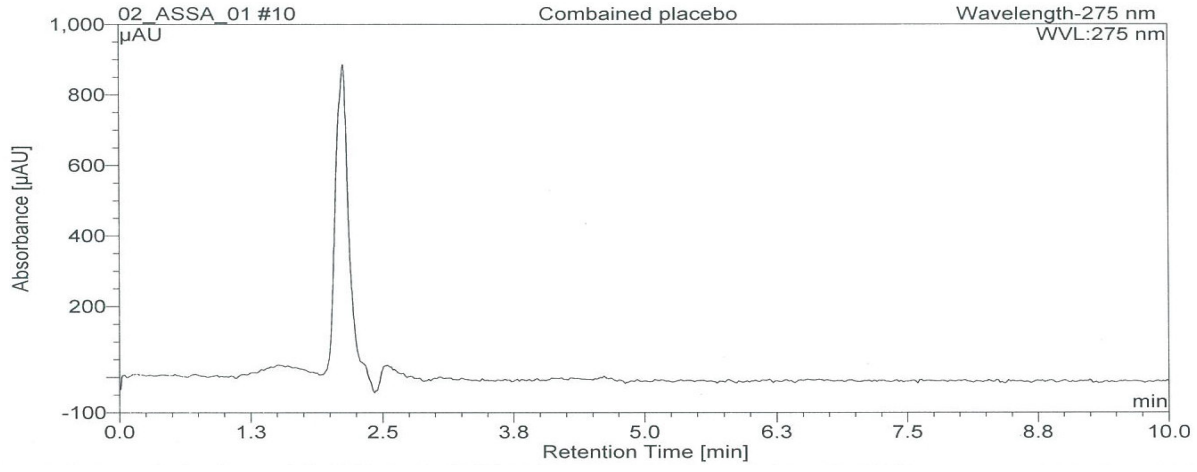

Figure 3
Chromatogram of blank/Placebo

RESULTS AND DISCUSSION

I. Method optimization

Waters Xterra C-18 column (150 mm × 4.6 mm, 5 µm pore size), column was the most suitable one since it produced symmetrical peaks with better resolution. The UV detector response of RIS and BA was studied and the best wavelength was found to be 275 nm showing highest sensitivity of both compounds. Several modifications in the mobile phase composition were made in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, flow rate, temperature and stability of RIS and BA was also studied. Initially no peaks were observed when acetonitrile and phosphate buffer in different ratios were utilized, at temperature of 30°C and 1.0 ml/min flow rate on a C8 column. So acetonitrile was replaced by methanol, at that time both drugs again didn't show peaks. Hence the C8 column was replaced by C18 column methanol and phosphate buffer (pH- 3.0) 50:50, peaks of both drugs were observed, but with less resolution and with peak broadening effect for RIS and BA at temperature of 30°C. Then ratio of buffer and methanol was changed to 40:60, peaks of both drugs were observed with good resolution without peak broadening, tailing, fronting and with good sensitivity as well, at 35°C temperature and flow rate of 1.0 ml/min. The effect of flow rate on the separation of peaks was studied by varying the flow rate from 0.5 to 1.3 ml/ min; a flow rate of 0.8 ml/min was optimal for good separation and resolution of peaks in a reasonable time as shown in Fig. 2 shows the chromatogram for a working standard mixture of RIS and BA, respectively. System suitability parameters with peak purity data are given in Table 2.

Table 2
System suitability parameters with Peak Purity Data

Sr. no.	Parameters	Risperidone	Benzoic Acid
1	Retention Time	3.045	4.162
2	Resolution USP	-	4.28
3	Theoretical Plate	2025	4381
4	Tailing Factor	1.16	1.09

II. Method validation

The method was validated according to ICH guidelines. The following validation characteristics were addressed: linearity, range, accuracy, precision, specificity, and robustness. Specificity of the method was determined by analyzing samples containing a mixture of the drug product and excipients. All chromatograms were examined to determine RIS & BA.

III. Formulation analysis and system suitability/ Specificity

The assay for the marketed oral solution was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.9 % for RIS, 100 % for BA. With % RSD for RIS and BA was 0.1 and 0.1 %.

Chromatogram of Standard Test Solution

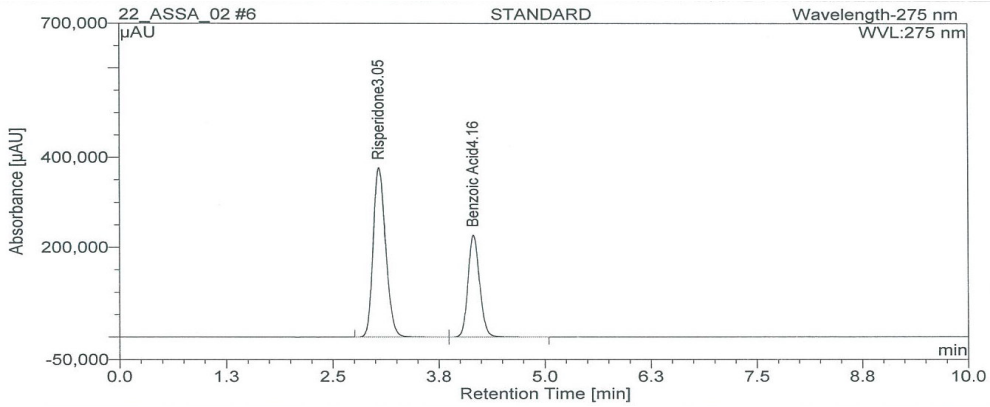

Figure 3
Chromatogram of Standard Test Solution of RIS and BA

IV. Linearity and range

Linearity was determined for RIS and BA in the range of 25% - 200% of test solution concentration. The correlation coefficient ('r^2') values were >0.998 (n = 6) indicating an excellent correlation between peak areas and analyte concentrations.

Linearity Graph of Risperidone

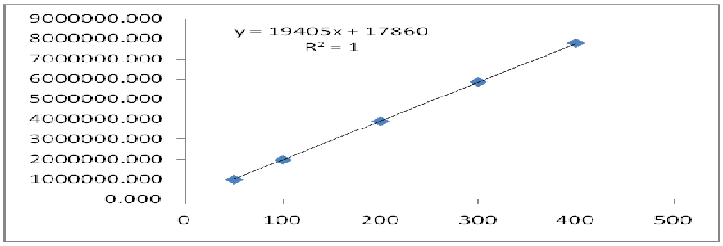

Figure 1
Linearity Graph of Risperidone

Linearity Graph of Benzoic Acid

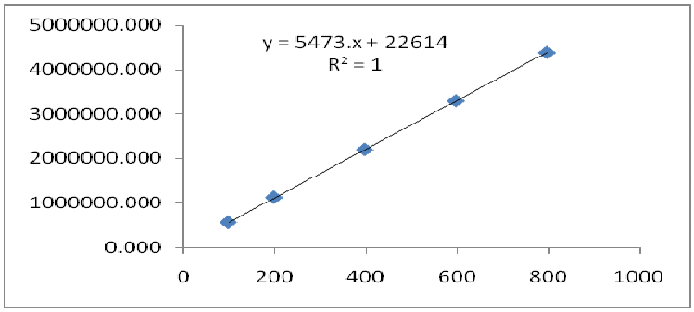

Figure 2
Linearity Graph of Benzoic Acid

V. Accuracy (% Recovery)

The mean percentage recoveries obtained were 101.3%, 100% for RIS and BA respectively. The developed method was found to be accurate as the mean percentage recoveries obtained for RIS and BA were found to be within limit as recommended by ICH guidelines. The developed method was found to be accurate as the % RSD values for accuracy studies were <2% (Table 4), as recommended by ICH guidelines.

VI. Method Precision

The system precision was demonstrated by preparing the standard solution at test concentration and injected repeatedly for six times. The % RSD for repeatability of sample preparation is 0.12 % and 0.07 % for RIS and BA respectively. The precision is satisfactory and the % RSD is not more than 2.0% as per ICH guidelines. The results are shown in Table 4.

Table 3

Precision Data of Risperidone and Benzoic Acid

Sr. No.	Risperidone		Benzoic Acid	
	Rt	AUC	Rt	AUC
1	3.043	3925181.354	4.15	2212236.043
2	3.042	3933004.578	4.148	2213473.507
3	3.040	3927837.275	4.146	2213022.934
4	3.040	3923224.770	4.149	2210530.218
5	3.040	3935301.680	4.147	2214591.977
6	3.039	3930297.746	4.145	2214911.972
Mean	3.04066667	3929141.234	4.1475	2213127.775
Std Dev	0.00150555	4617.348736	0.001871	1612.467489
% RSD	**0.04951366**	**0.117515469**	**0.045107**	**0.072859213**

Table 4

Summary of result of validation parameter

Sr. No.	*Parameter*	*Result*	
		RIS	*BA*
1	*Accuracy (% Recovery)*	*101.3*	*100*
2	*Precision(% RSD)*	*100.9(0.1%)*	*100(0.1%)*
3	*% Assay*	*100.6*	*100*
4	*Linearity(r^2 value)*	*1.000*	*1.000*

VII. Robustness and solution stability studies

The % Assay and % RSD was found to be in range 100 ± 1.5% and <2, respectively. It indicates that method follow specification of ICH guideline. Results of the stability studies were in the range of 99.5 - 101.5%. Stability as described in method development under experimental section was studied. Results of the stability studies were within the acceptable limit (98 - 102%).

VIII. Forced Degradation

As per ICH guidelines, the target degradation below 0.5% should be there for the stability indicating ability of the assay method and the same was tried in the present study. No interfering peaks were found due to degradation products at the drugs Rt's.

Abbreviations

BA- Benzoic Acid
RIS- Risperidone
HPLC- High Performance Liquid Chromatography
ICH- International Conference of Harmonization
DAD-Diode Array Detector
KH_2PO_4 -Pottasium Dihydrogen Phosphate
SST-System Suitability Test
Tf- Tailing Factor
R.S.D.- Relative Standard Deviation

CONCLUSION

The proposed method has advantage of simplicity and convenience for the separation and quantitation of RIS and BA in the combination and can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to ecofriendly chromatographic procedure. The method is accurate, precise, rapid and selective for simultaneous estimation of Risperidone and Benzoic Acid in oral suspension dosage form. Hence it can be conveniently adopted for routine analysis.

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