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A Validated Rapid Reversed-Phase High Performance Liquid Chromatographic Method for Determination of Risperidone and Benzoic Acid

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Abstract: A simple, sensitive and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed for determination of risperidone (RSP) and benzoic acid (BZA) preservative. Analysis was achieved on an Equisil® BDS C18 column (250 x 4.6 mm, 5 µm) with an isocratic binary mobile phase composed of 0.05 M potassium dihydrogen phosphate (pH 2.5) and acetonitrile (65:35; v/v). The flow rate was 1.0 mL/ min and detection was carried out at 260 nm. Linear regression study revealed a good linear relationship over a range of 50–150 µg/mL for RSP and BZA, with a correlation coefficient (R^2) of more than 0.999. Limit of detection (LOD) and limit of quantitation (LOQ) were 4.69 and 14.20 µg/mL, and 2.72 and 8.24 µg/mL for RSP and BZA, respectively. Intra- and inter-day precisions, calculated as percentage relative standard deviation (% RSD), were lower than 2.0 %. The proposed method was applied for the quantitation of RSP and BZA in pure form and in RSP pharmaceutical dosage form.

Keywords: Risperidone, Benzoic Acid, RP-HPLC, UV Detection.

INTRODUCTION

Risperidone (RSP) is a benzisoxazole derivatives and chemically; it is 3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (Fig. 1 A). RSP is a dopamine D_2 receptor antagonist, serotonin $5HT_2$ receptor antagonist, neuroleptic (British Pharmacopoeia, 2009). RSP is mostly metabolized by alicyclic hydroxylation and oxidative N-dealkylation (Suthar et al., 2009).

RSP is used to treat schizophrenia, symptoms of bipolar disorder (manic depression) and irritability in autistic children (Haas et al., 2009; Shea et al., 2004).

Benzoic acid (BZA), is an organic compound belonging to the family of carboxylic acids, it is benzene carboxylic acid (Fig. 1 B), widely used as an antimicrobial preservative (Mutunga, 2014).

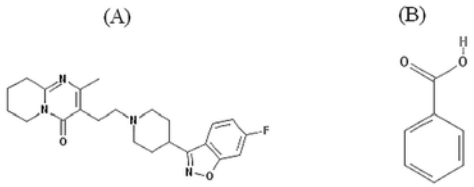

Fig. 1 : Chemical structure of RSP (A) and BZA (B)

A literature survey revealed different liquid chromatography methods for the determination of RSP in its oral solution (Bhusnure, 2015; Bhusnure et al., 2015; *USP 31*, 2008), in biological fluids (Avenoso et al., 2000; Huang et al., 2008; Moody et al., 2004; Petruczynik et al., 2016), and in tablet dosage form (Baldania

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et al., 2008; Dedania et al., 2011; Suthar et al., 2009; Svirskis et al., 2011). BZA was officially determined by an acid-base titration method (USP 31, 2008). It was also determined in foodstuffs by different chromatographic techniques (Aresta and Zambonin, 2016; Javanmardi et al., 2015; Saad et al., 2005).

The addition of antimicrobial preservatives in a medical formulation requires special justification (European Medicines Agency (EMA), 2007), so the release specifications for a finished product should include both identification and content determination tests for each antimicrobial preservative included in the formulation. Using benzoic acid as a preservative in RSP oral solution makes it an integral part of the product formulation, so it was important to develop a HPLC method capable of quantifying target analyte and preservative.

The advantages of the presented method compared with the previously reported ones (Bhusnure, 2015; Bhusnure et al., 2015) for simultaneous determination of RSP and BZA include achieving a reasonably low LOD and LOQ, better resolution and column effeciency, as well as, less run time, and more eco friendly.

The developed method is simple, precise, accurate and can be used in the quality control routine analysis for the simultaneous determination of both compounds in pure form and commercial oral solution.

EXPERIMENTAL

Reagents and Chemicals

RSP (USP R.S Lot No. G0J122, purity 99.9; w/w) and BZA (USP R.S Lot No. G0D223, purity 1.0). Risidone® oral solution (DEEF Pharmaceutical Ltd. Alqassim, KSA) was purchased from the local market and labeled to contain I mg/mL RSP and 1 mg/mL BZA preservative. Acetonitrile (HPLC grade) was obtained from Scharlau (Barcelona, Spain). Potassium dihydrogen phosphate was obtained from Techno pharmChem (India). Ultra-pure water (Milli-Q) (Millipore Corporation, Billerica, MA, USA) was used.

Instrumentation and Chromatographic Conditions

The HPLC system (Waters, USA) was equipped with an autosampler, binary HPLC pumps, dual lamb absorbance detector, and in-line degasser ISA card. Data acquisition was performed on Empower software. The detector was set at 260 nm. All determinations were performed at ambient temperature on an Equisil® BDS C18 (250 x 4.6 mm, 5 µm) column (Dr. Maisch HPLC GmbH, Germany). The isocratic mobile phase was prepared by mixing 0.05 M potassium dihydrogen phosphate (pH 2.5) and acetonitrile in a ratio of 65:35 (v/v), then filtered through 0.45 µ membrane filter (Millipore, Milford, MA, USA). The injection volume was 20 µL with a flow rate of 1.0 mL/min.

Preparation of Standard Solutions

RSP and BZA stock solution containing 1 mg/mL of each analyte was prepared by dissolving 50 mg of RSP and 50 mg of BZA in 50 mL mobile phase. A standard solution containing 0.1 mg/mL of RSP and BZA was prepared by diluting 5 mL of stock standard solution into 50 mL with mobile phase.

Preparation of Sample Solutions

An aliquot of Risidone® oral solution, containing 5 mg RSP and BZA, was transferred into a 50 mL volumetric flask and diluted to volume with mobile phase, prepared solution was incubated in an ultrasonic bath for 10 min then filtered through 0.45 µm membrane filters.

Validation

The method was validated in accordance with the International Conference on Harmonization (ICH) requirements (ICH Harmonized Tripartite Guideline, 2005), which involved system suitability, repeatability, selectivity, ruggedness, linearity, LOD, LOQ, accuracy, and stability of the standard solution.

System Suitability

The system suitability parameters such as resolution (Rs), theoretical plates and asymmetry factor (As) were calculated as previously reported (ICH Harmonized Tripartite Guideline, 2005),.

Selectivity

Selectivity is the capability of the analytical method to distinguish between the target analyte and other components that may be present. To evaluate the method selectivity, the excipients used in Risidone® oral solution (tartaric acid and propylene glycol) were injected and compared with the chromatograms of RSP and BZA.

Linearity, LOD and LOQ

The linearity of a method is the ability to obtain results which are proportional to the analyte concentration, within a given range, either directly, or through a transformation. The linearity of the method was assessed at five concentration levels containing 50, 80, 200, 120, and 150 µg/mL of RSP and BZA (50–150 % of a target concentration of 100 µg/mL of both analytes. Calibration curves were constructed by plotting the peak areas against concentrations. LOD is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. LOQ is defined as the lowest concentration of an analyte in a sample that can be measured with acceptable precision and accuracy under the proposed conditions. LOD and LOQ were calculated as 3.3 (SD/S) and 10 (SD/S), respectively; where (SD) is the standard deviation of intercept of the regression line and (S) is the slope of the calibration curve.

Precision

Repeatability (intraday precision) was evaluated by analyzing six replicates of 100% of target concentration; on the same day by the same analyst and same equipment. % RSD was calculated.

Ruggedness is the extent of reproducibility of test results under regular operational conditions such as day to day and analyst to analyst. The ruggedness of the method was studied by analysis of the same sample in triplicate on different days, by different analysts.

Accuracy and Recovery

Accuracy is the closeness of test results obtained by the analytical method to the nominal value. The % recoveries of test solutions (in triplicate) at three different concentrations 50, 100 and 150 µg/mL were calculated.

Application of the Proposed Method on Pharmaceutical Oral Solution

The developed method was applied for simultaneous determination of RSP and BZA in Risidone® oral solution by analyzing sample solutions (in triplicate) at three concentration levels of 50, 100 and 150 µg/mL using standard addition technique.

Stability of Working Standard Solution

Standard solutions of 100 µg/mL of RSP and BZA were tested for storage stability prior to HPLC analysis over 11 h at room temperature. The samples were analyzed by the optimized HPLC method in fresh and stored solutions.

RESULTS AND DISCUSSION

Method Development

Different C18 columns were investigated initially, however, Equisil® BDS C18 (250 x 4.6 mm, 5 µm) column offered excellent peak shape, significant low back pressures, excellent efficiency, this column is also known to be stabile at elevated temperature and has wide pH compatibility. The highest intensity of both compounds was obtained at 260 nm. All determinations were performed at ambient temperature. Different chromatographic conditions such as mobile phase strength, pH, and the flow rate were changed to optimize peak shape, elution time and the separation of the analytes. Different mobile phase compositions were tried including acetate buffer and methanol. Using acetate buffer retarded RSP elution, while BZA was eluted in the dead time. In addition, using methanol instead of acetonitrile showed poor chromatographic resolution. Optimum resolution with a good separation of RSP and BZA was achieved within reasonable runtime (less than 8 min) using isocratic elution system of 0.05 M potassium dihydrogen phosphate (pH 2.5) and acetonitrile (65:35; v/v). The retention time was 4.000 and 5.819 min for RSP and BZA, respectively, using a flow rate of 1.0 mL/min (Fig. 2).

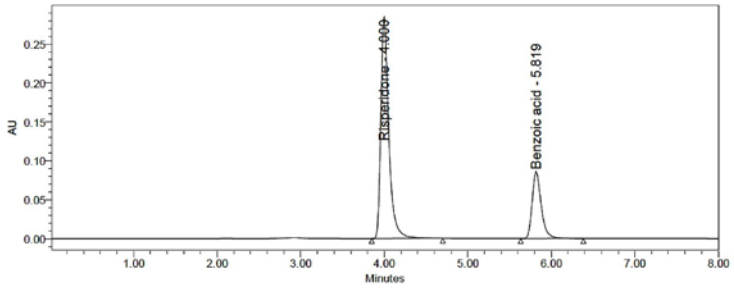

Fig. 2: HPLC chromatograms of Test solution (100 µg/mL)

Method Validation

The developed method was validated for system suitability, repeatability, selectivity, ruggedness, linearity, LOD, LOQ, accuracy, and stability of the standard solution.

System Suitability

System suitability tests were applied to verify that the system is satisfactory for the analysis to be conducted. System suitability parameters for RSP and BZA are reported in table 1.

Table 1 : System suitability parameters for RSP and BZA

Parameters	RSP	BZA	Acceptance criteria
Asymmetry	1.47	1.33	≤ 2
Resolution		9.95	> 2
Theoretical plates	9618	14539	> 2000

Selectivity

The analysis of the placebo solutions showed no interference with the target analytes at the expected retention times. These data confirmed that the presence of excipients did not interfere with the analysis, indicating selectivity of the method (Fig. 3).

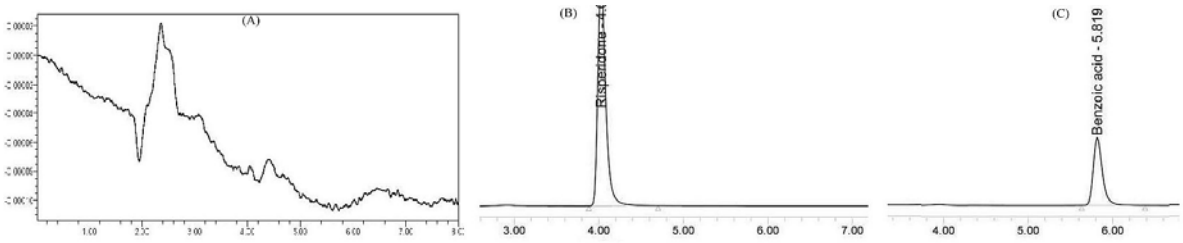

Fig. 3: HPLC chromatograms of placebo (A), RSP 0.1 mg/mL (B), BZA 0.1 mg/mL (C)

Linearity

The linearity of the HPLC method was evaluated by analyzing a series of different concentrations of each compound in the range of 50-150 µg/mL. The results of the regression statistics obtained for RSP and BZA are presented in table 2. A significant correlation between the concentration of analytes and detector response was observed, the correlation coefficient (R^2) was 0.9995 and 0.9998 for RSP and BZA, Respectively. Table 2 shows the values of LOD and LOQ for RSP and BZA. The SD of intercept was 26488 and 5331 for RSP and BZA, Respectively.

Table 2: Regression data for RSP and BZA

Analyte	Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Slope	Intercept	R^2
RSP	50-150	4.69	14.20	18653.68	39445.86	0.9995
BZA	50-150	2.72	8.24	6469.22	-1259.38	0.9998

Precision

Intra and inter-day, and analyst to analyst precision results are shown in table 3. % RSD values were within 2 % for RSP and BZA.

Table 3: Precision for RSP and BZA (n=6)

Analyte	Intra-day precision %RSD	Inter-day precision %RSD	Analyst to analyst precision %RSD
RSP	0.59	0.64	1.77
BZA	0.21	0.38	2.00

Accuracy

The proposed method showed accuracy within the proposed range. The % recovery values ranged from 99.01 to 100.11, and 99.13 to 101.11 for RSP and BZA, respectively.

Application of the Proposed Method on Pharmaceutical Oral Solution

Table 4 shows % recovery for analysis of Risidone® oral solution. The obtained results confirm the suitability of the proposed method for quality control analysis of RSP oral solution.

Table 4: Application of the proposed method for determination of RSP and BZA in pharmaceutical formulation (n=3)

Concentration	RSP % recovery ± SD	BZA % recovery ± SD
50 µg/mL	99.98 **±** 0.13	99.79 **±** 0.57
100 µg/mL	99.44 **±** 0.22	100.29 **±** 0.22
150 µg/mL	99.082 **±** 0.11	100.68 **±** 0.39

Stability of Working Standard Solution

The stability of standard solution during analysis was studied at room temperature for 11 hours. No chromatographic changes were observed. The values of % RSD of the standards were 0.75 and 0.42 for RSP and BZA, respectively.

Conclusion

A simple RP-HPLC method was developed and validated for the determination of RSP and BZA in oral pharmaceutical formulation. The proposed method showed excellent selectivity, accuracy, and precision. The method can be applied for in-process quality control studies during different manufacturing steps and to assess the quality and performance of each oral solution lot through the regular quality control testing of the pharmaceutical product.

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