

DEVELOPMENT AND VALIDATION OF RAPID UHPLC METHOD FOR DETERMINATION OF RISPERIDONE AND ITS IMPURITIES IN BULK POWDER AND TABLETS

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ABSTRACT

The aim of this study was to develop a new, rapid and highly sensitive UHPLC method with UV detection for simultaneous determination of risperidone and four other related substances possibly present in tablets. The active substance, risperidone, is the most frequently used atypical antipsychotic drug for a treatment of schizophrenia, bipolar disease and behavioral disorders in young patients, up to 17 years of age. The study is based on main impurities specified in USP35 and Ph. Eur. 7 (Imp A, B, C, and E). Tablet sample preparation was very rapid and consisted of dissolution, sonication and filtration through a 0.22 μm membrane filter. The newly developed method is based on an innovative UHPLC that provides excellent separation efficiency within a very short analysis time. Binary gradient was optimized using RP-18 chromatographic column (100 mm \times 3.5 mm, 1.7 μm). Ammonium acetate buffer pH 6.8 and acetonitrile were used as mobile phases in gradient mode with the flow rate of 0.5 mL \cdot min $^{-1}$ and temperature equal to 40 $^{\circ}\text{C}$. The wavelength of UV detector was set to 260 nm. The developed method allows four times shorter analysis time and consumes twenty two times less solvents compared to conventional HPLC method used by USP35 for a determination of related substances of risperidone in tablets. This method was validated in accordance with ICH requirements included the linearity, precision, accuracy sensitivity, and specificity.

Keywords: Risperidone; UHPLC; Method development; Validation; USP.

1. INTRODUCTION

Risperidone, with its molecular formula $\text{C}_{23}\text{H}_{27}\text{FN}_4\text{O}_2$ and IUPAC name 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]-pyrimidin-4-one [Fig. 1] belongs to the group of atypical antipsychotic drugs most commonly used in therapy of schizophrenia, bipolar disease and behavioral disorders such as autism. Risperidone was approved by the USA's FDA as the only medicinal treatment for schizophrenia for children aged 10-17 years. Pharmacological mechanism is in antagonism of dopamine (D_2), serotonin (5-HT_2) and α_1 -adrenergic receptors. The most common side effects of

risperidone are weight gain, hyperprolactinemia, insomnia, unstable blood pressure and many other side effects connected with D_2 , 5-HT_2 and α_1 antagonism.

Several analytical research publications regarding risperidone in Active pharmaceutical ingredient (API) and in pharmaceutical formulations have been published, however, none of them mentions any related substances. In human biological samples risperidone was analyzed together with its active metabolite 9-hydroxy risperidone.¹ HPLC methods were applied for determination of risperidone in bulk powder, pharmaceutical formulation² and biological samples such as

plasma¹, urine³ and postmortem fluids⁴. Methods were based on UV⁵, electrochemical⁶ or mass spectrometric detection⁷. There are just a few publications based on stability testing and degradation products identification^{8,9}. Many other analytical techniques were used for determination of risperidone such as thin layer chromatography¹⁰, dual-plate overpressure layer chromatography¹¹, gas chromatography¹² and capillary electrophoresis¹³. Many research articles focused on FT-IR and FT-Raman spectroscopy to determine polymorphs in pharmaceutical formulation¹⁴.

Unfortunately, there is not any article studying risperidone and its pharmacopoeial impurities, none of which is a degradation product, but contaminants from production, in bulk powder or tablets. In this presented paper we have focused on shortening time of single analysis for determination risperidone and its related substances in API as well as in tablets using modern UHPLC instrumentation. Method was validated with accordance to ICH guidelines¹⁵.

2. MATERIAL AND METHODS

2.1. Chemicals

Risperidone reference substance and Risperidone for system suitability chemical reference substance were purchased from the European Directorate for the Quality of Medicine and Healthcare (Strasbourg, France). Commercially available tablets of 1 mg of risperidone and their placebo were used for real samples preparation and method validation. Methanol and acetonitrile for liquid chromatography were from Sigma-Aldrich (Steinheim, Germany). Ammonium acetate was supplied by Penta chemicals (Prague, Czech Republic). HPLC grade water was prepared by Milli-Q reverse osmosis (Millipore, Bedford, USA).

2.2. Instruments

Shimadzu Nexera (Shimadzu, Japan) UFLC system with Shimadzu prominence UV-VIS detector was used for this study. The system is consisted of the autosampler SIL-30AC, two pumps LC-30AD, vacuum degasser DGU-20A5, column oven CTO-30A and UV-VIS detector SPD-20A. Data were gathered and calculated using LabSolution chromatographic software (Shimadzu, Japan).

2.3. Chromatographic conditions

RP-18 chromatographic column Synchronis C18 (100 mm × 3.0 mm i.d. with the particle size of 1.7 µm, ThermoScientific) was used for separation of analytes. The mobile phases consisted of 60mM ammonium acetate buffer

pH 6.8, prepared by dissolution of ammonium acetate in Milli-Q water, and acetonitrile (Sigma-Aldrich, United Kingdom). Mobile phase was filtered under vacuum (0.22 µm nylon filter, Whatman) and sonicated for 10 minutes.

2.4. Preparation of the standard solutions

Standard solutions were prepared by diluting the substance with methanol to make concentration in the range from 0.5 µg·mL⁻¹ to 2 mg·mL⁻¹. Dilution of risperidone for system suitability chemical reference substance was made in accordance with the pharmacopoeia description (10 mg in 1 mL of methanol).

2.5. Sample preparation

Twenty tablets with 1 mg of risperidone were accurately weighed and powdered. Mean tablet weight was counted and an appropriate amount of powder equivalent to 10 tablets (10 mg of risperidone) was weighed and transferred into 10 mL volumetric flask. About 7 mL of methanol was added, then shaken and sonicated for 15 minutes. Afterwards, all samples were brought to volume with methanol and filtered using two syringe nylon filters (Whatman, UK), 0.4 µm and 0.22 µm.

3. RESULTS AND DISCUSSION

3.1. Method development

Since there are no scientific publication on simultaneous determination of risperidone and its related substances, the source data for this work had to be acquired from the United States Pharmacopoeia 35 where the method for determination of risperidone related substances in tablets is based on binary gradient conventional HPLC system with the flow rate of 2.5 mL·min⁻¹. The aim of this work was to shorten the time of analysis (the original analysis time being 30 minutes) using modern UHPLC system as well as lowering the high solvent consumption and simplifying of pre-analytical procedures.

C18 reverse phase silica gel column was used for a separation of analytes. We focused on the optimization of the mobile phase, such as pH, components and their ratio, flow rate, temperature, detection wavelength, and gradient scheme.

Risperidone exhibits a maximum absorption at 278 nm, nevertheless the 260 nm wave length was chosen due to its better sensitivity when there is a need to determine the impurities, because the detector responses were enhanced for most of them.

First objective of this study was to optimize the most suitable buffers pH in order to get the best separation of all peaks. Risperidone and

all related substances have basic character (Risperidone-pKa 8.8) and their chromatographic behavior on C18 sorbent is very similar. Therefore, with the help of lower pH buffer, all substances are protonated and eluted with very small differences in retention. Because of the column stability up to pH 8.0, ammonium acetate buffers with pH value from 4.5 to 6.8 and phosphate buffer pH 7.2 were tested. The lower pH buffers cause bad resolution of some pairs of peaks and pH 7.2 phosphate buffer inflicts increase of tailing factors with no benefits in analysis time and resolution. Next step was to assign the most suitable organic component of the mobile phase. After initial attempts using methanol, which provides peaks with higher symmetry factor and retention times, the components of mobile phase became acetonitrile and ammonium acetate buffer pH 6.8. For mobile phase A the ratio was ACN-buffer (3:97, v/v); for B (90:10, v/v). Having attempted many trials to get sufficient peak resolution, the flow rate was set to 0.5 mL·min⁻¹ in gradient mode (T_{time-A:B}) T_{0-0.6 min}-98:2, T_{0.6->5.4}-80:20, T_{5.4-5.8}-80:20, T_{5.8->6.6}-2:80, T_{6.6-8}-2:80. The injection volume was 3 µL.

The temperatures in the range of 25 °C to 50 °C were tested to improve the method and shorten the time of the single analysis. The best results were exhibited at the temperature of 40 °C. The higher temperature led to worse resolution of peaks and peak symmetry. The aforementioned temperature has no negative effect on solution stability.

The developed chromatographic conditions achieved resolution of all peaks more than 1.5 with symmetric peaks. The retention time of risperidone was 5.2 min and all impurities were eluted between 4 min and 5.5 min. Run time of the single analysis was 8 minutes including column reconditioning.

3.2. System suitability test

Observed data for the system suitability test were the peak resolution, peak symmetry and injection repeatability. The resolution between peaks of impurity C and risperidone has to be more than 2.0 and between all other peaks more than 1.5. Peak symmetry of risperidone peak for content determination has to be 0.8-1.5. Injection repeatability is made by five injections with maximum 2.0% relative standards deviation.

3.3. Method validation

3.2.1 Selectivity

The tablet excipients, solvent sample and system suitability test solution were analyzed under the final chromatographic conditions and

compared with risperidone standard solution to prove the selectivity of the developed method. No peak from placebo and blank chromatogram interfered with risperidone peak as well as peaks of impurities. [Fig. 2] The system suitability test chromatogram shows one peak of risperidone and four resolved peaks of impurities with resolution more than 1.5. Method is selective for determination of risperidone and its related substances in API and tablets.

3.2.2 Linearity

The linearity of the method for determination of risperidone was measured at five concentration levels in range between 0.8-1.2 mg·mL⁻¹ for risperidone in triplicate injections. The equation of calibration curve was $A = 43899c + 228087$ where A is the peak area and c is the risperidone concentration within the sample. Linearity was proven ($r^2 = 0.9991$; $r^2 \geq 0.999$).

As we do not have any impurity standard in satisfactory purity we used diluted solutions of risperidone in appropriate concentrations for an evaluation of linearity as well as sensitivity, precision and accuracy.

Linearity for impurities determination was demonstrated in the range of risperidone standard sample concentrations: 0.5 µg·mL⁻¹ (disregard limit 0.05 %), 1 µg·mL⁻¹, 1.5 µg·mL⁻¹, 2 µg·mL⁻¹ (limit for impurities A,B,C,E) and 3 µg·mL⁻¹. The curve equation was $A = 6388.2c - 911.7$ with (A) being the peak area and (c) is risperidone concentration within the sample. Linearity was proven by correlation coefficient $r^2 = 0.9975$ ($r^2 \geq 0.99$).

3.2.3 Sensitivity

The sample with concentration of disregard limit (0.5 µg·mL⁻¹) was injected six times. Average signal to noise parameter was found to be 26. The minimum requirement for this parameter is 10 for the quantification, therefore, the sensitivity of this UHPLC method is satisfactory.

3.2.4 Precision

The method precision was determined by preparing six test solutions of approximate concentration 1mg·mL⁻¹ of risperidone substance. All samples were injected in triplicate and results were evaluated by the Relative Standard Deviation (RSD) of the risperidone peak areas. The obtained RSD value for risperidone API was 0.79 % that matches required limit of 1.0 %.

The method precision was also tested for risperidone determination in tablets. Six sample solutions with the concentration of

about 1 mg·mL⁻¹ of risperidone were prepared and every sample was injected in triplicate. RSD value from six samples was counted as 1.74 % fulfilling the requirement (up to 2.0 %). The precision for impurities was proven by analyzing six model samples prepared by risperidone substance addition to placebo in 1 µg·mL⁻¹ concentration that corresponds to the pharmacopoeial limit for unspecified impurities. The precision was proven. RSD value of three injections of each sample was found to be 0.52 % (the limit being 7 % for the determination of impurity in pharmaceutical formulation within the concentration range of 0.1 - 1.0 %).

3.2.5. Accuracy

The accuracy was evaluated by applying the developed method to the samples made from risperidone drug substance and tablet placebo powder in the amount corresponding to the proportion in real tablets. They were prepared in the following concentrations: 0.8 mg·mL⁻¹, 1 mg·mL⁻¹ and 1.2 mg·mL⁻¹, all in triplicate. The accuracy was calculated as the percentage of the drug recovered in the presence of tablet excipients. Mean recovery for risperidone tablets is 100.7 % ± 0.7 % which fulfilled the limit for accuracy in pharmaceutical formulations (98.0 %-102.0 %). The method is accurate and may be used for a determination of risperidone in 1 mg tablets.

The accuracy for impurities was proven by analyzing six model samples prepared by addition of known amount of risperidone substance to excipient sample in 1 µg·mL⁻¹ concentration that corresponds to the limit for unspecified impurities determined by the pharmacopoeia. The accuracy was proven using three injections of each sample and mean recovery was 91.5 % ± 0.5 % (limit is 85.0 % - 115.0%).

3.2.6. Robustness

A robustness test was expected to confirm the reliability of an analysis to small deviations of the method parameters. The robustness of the method was proven by analyzing the mixture of all monitored substances represented by system suitability test solution. Deliberate changes to the standard experimental conditions were made and system suitability test was performed. Monitored parameters impact was studied for column temperature (40 ± 5 °C), ammonium acetate pH value (6.8 ± 0.4) and ratio of acetonitrile in mobile phase B (90 ± 2 %). All obtained analyses results illustrated that the resolution of all peaks and peak symmetry is in accordance with method's system suitability test.

4. CONCLUSION

A validated fast chromatographic method was developed for the determination of risperidone and its four related substances in drug substance and tablets using a modern UHPLC instrumentation and particle column Synchronis C18 (100 mm × 3.0 mm i.d. with a particle size of 1.7 µm) using gradient program and the temperature equal to 40°C. In comparison to the original pharmacopoeial HPLC method the total time of analysis was shortened more than 4 times and solvent consumption decreased 22 times. For its practical use the method was validated in accordance with ICH guidelines and requirements.

This is the first published chromatographic method for simultaneous determination of the assay of risperidone and its related substances. The newly developed method might prove useful for the pharmaceutical dosage form release quality control.

5. ACKNOWLEDGMENTS

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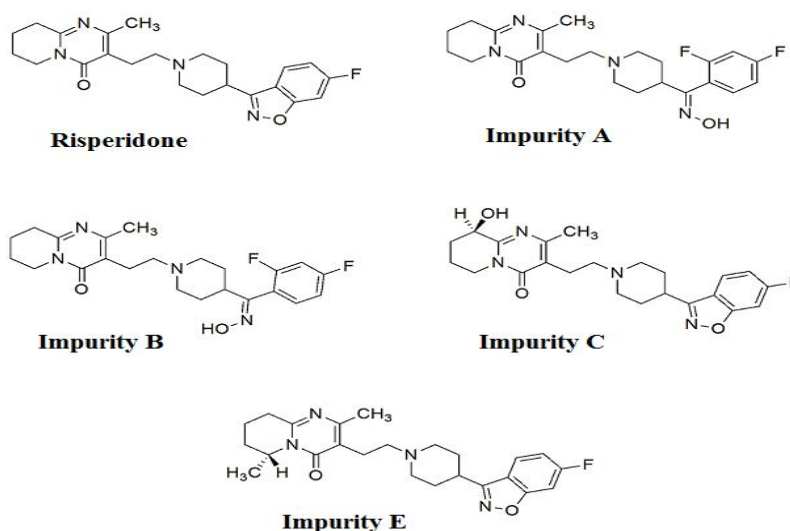


Fig. 1: Chemical structure of Risperidone and its impurities A, B, C, E

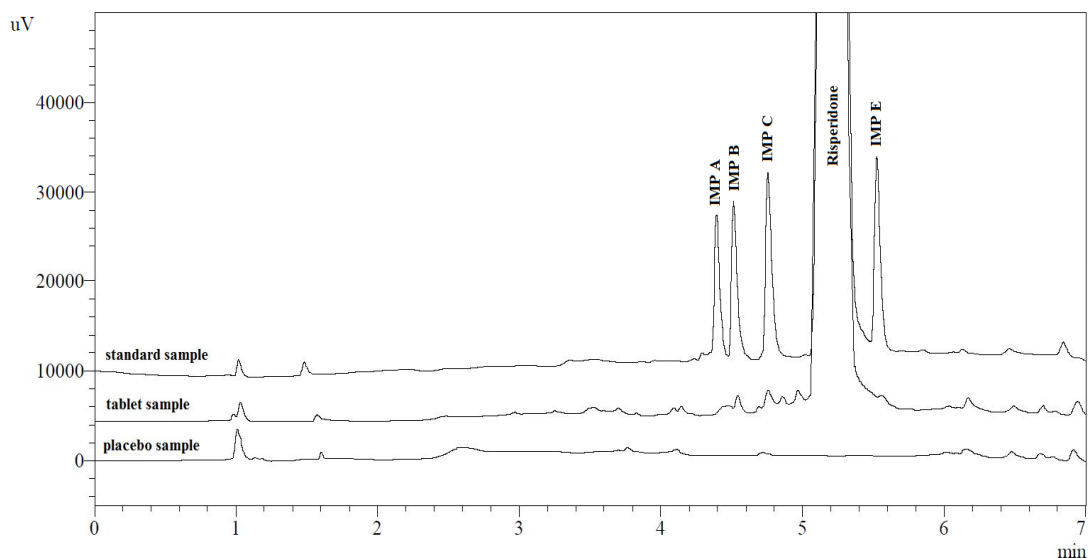


Fig. 2: Chromatograms of standard sample solution, placebo sample and real tablet sample in described chromatographic conditions

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