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Development and Validation of RP-UPLC Method for Determination of Related Substances in Risperdal® Consta®

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Abstract

In this work, a validated stability-indicating ultra-high performance liquid chromatographic (UPLC) method has been developed for quantitative determination of related substances in Risperdal® Consta®. The chromatographic separation was achieved on a Waters BEH C18 column (2.1 × 100 mm, 1.7 µm) in an isocratic elution mode. The limit of quantification is 0.1 µg/mL, and the method is linear over the concentration range of 0.1-1.5 µg/mL for risperidone. The PLGA polymers have been dissolved in organic phase (acetonitrile) and precipitated in aqueous phase (0.01 N HCl). This simple step avoids the clogging of column and makes the method more robust. The mean recovery of extracted risperidone from PLGA microspheres is 99.26 ± 1.22%. Herein, this method was proved to be highly reliable and applicable for measuring impurities in PLGA microspheres.

Keywords: RP-UPLC; Validation; Risperdal® Consta®; PLGA; Relative substances

Introduction

Risperidone, which is a novel antipsychotic medicine mainly worked for treating schizophrenia and also the psychotic, affective, or behavioral symptoms associated with other disorders [1]. The main pharmacological effects of risperidone include dopamine D2 antagonism and serotonin 5-HT2 receptor blockade [2]. The molecular formula and IUPAC name of risperidone are $C_{23}H_{27}FN_4O_2$ and 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 respectively. The chemical structure of risperidone was depicted in Figure 1.

In recent years, a sustained-release API (active pharmaceutical ingredient) delivery system has benefit patients, and embedding these drugs inside. US Food and Drug Administration (FDA)-approved carriers such as liposomes [3], polyethylene glycol (PEG) [4], or poly (lactide-co-glycolide) PLGA [5,6] were wildly developed by researchers. PLGA microspheres have been presented to be a controlled release system for various drugs in demand. It degrades and to form water-soluble and non-toxic monomers under physiologic conditions. In April 2007, the US FDA approved Risperdal® Consta® (risperidone long-acting injection; Johnson & Johnson) for the treatment of schizophrenia. It overcomes the major issue with the current oral dosage is the difficulty of making patients take the oral medication on a daily basis.

The determination of the relative substances could be a critical item to monitor the goods quality. The assay techniques for determining the impurities content for several dosage forms including oral solution, tablets and orally disintegrating tablets have been published in pharmacopoeias (e.g., USP, JP). In general, high-performance liquid chromatography (HPLC) equipped with UV detector has been utilized for the impurities assay. However, the unsuitable column selection (e.g., the production process/control by different manufacturers) and the total analysis time consuming are the major concerns. Especially, isolating and identifying the impurities/degradants is not negligible in the least amount of time for the process control monitoring of pharmaceutical industry. Hence, more rapid and simple method should be developed at the early drug discovery stage.

Ultra performance liquid chromatography (UPLC) has been a proven technique in the past decade. Due to packing with sub-2 µm particles, analytes retain less time on the stationary phase which not only increase the resolution power but also improve the sensitivity at the same time. Besides, reduced solvent consumption is another benefit compared to conventional HPLC. Bindu et al. have reported a UPLC method for quantitating six impurities of paliperidone (9-hydroxy resperidone) in 8 min [7]. Nejedly et al. developed a UHPLC method for discrimination the main degradation impurities from risperidone tablet [8]. The new UHPLC method has reduced 4 times of total analysis time comparing with original pharmacopoeial HPLC method. A comparison for the determination of related substances in risperidone tablet using HPLC and UPLC system has been evaluated by Yan et al. [9]. UPLC revealed higher efficiency for determining the generated impurities from the force degraded samples.

However, there were no reported chromatographic methods for determining the related substances in Risperdal® Consta®. Completely extracting drugs or impurity substances from PLGA microspheres and removing the high molecular weight PLGA polymer were the major challenges. Herein, the aim of this study was to develop and validate a simple and rapid UPLC method to resolve the mentioned issue.

Materials and Methods

Chemical

USP reference standard Risperidone and Risperidone Related Compounds Mixture were purchased from USP convention, Inc. (Rockville, MD). Poly (D,L-lactide-co-glycolide) 75:25 was obtained from Lakeshore Biomaterials (Birmingham, Al). Distilled water was produced by Millipore water purification system. Risperdal® Consta® 25 mg long-acting injection was purchased from Janssen

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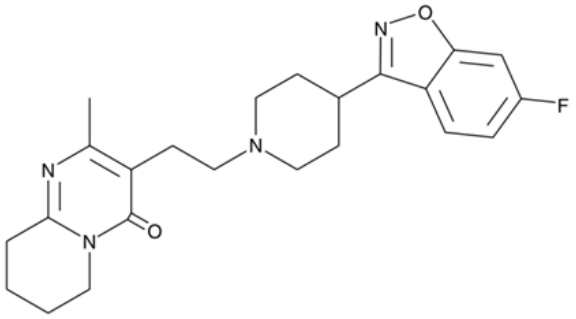

Figure 1: Structural formula of risperidone.

Pharmaceuticals (Titusville, NJ). The PLGA microspheres placebo was produced by R&D division of TTY Biopharm Company Ltd.

Instrumental and chromatographic conditions

For HPLC analysis, the study was employed using a BDS HYPERSIL C18 column (3 μm, 4.6 × 100 mm). The injection volume was 10 μL. An isocratic elution with a mixture of acetonitrile and 50 mM ammonium acetate solution (22:78, v/v). The flow rate was set at 1.5 mL/min.

For UPLC analysis, the test was performed using an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) coupled with a VanGuard pre-column (1.7 μm, 2.1 × 5 mm). The injection volume was 3 μL. UPLC separation of related substances used isocratic elution with a mixture of acetonitrile and 10 mM ammonium acetate solution (26:74, v/v). The flow rate of the mobile phase was 0.3 mL/min.

The instrument used was a Waters ACQUITY H-Class UPLC system (Milford, MA) coupled with a PDA (Photo Diode Array) detector. The column temperature for both separation methods was maintained at 30°C and the eluted analytes were monitored at the wavelength of 275 nm. All mobile phase was filtered through 0.22 μm PVDF membrane filters and degassed by an ultrasonicator at least 30 min. The output signal was processed using Empower® 3 software.

Standard solutions preparation

Accurately weighed risperidone reference standard (10 mg) was transferred to a 50 mL volumetric flask, dissolved in and diluted to the mark with 0.01 N HCl. Standard solutions were prepared by diluting the stock solution to obtain suitable concentration for chromatographic measurements. All solutions were filtered by a 0.22 μm PTFE syringe filter before analysis.

The solution for system suitability

Ten milligram of USP Risperidone Related Compounds Mixture RS was transferred to a 10 mL volumetric flask, dissolved in and diluted to the mark with diluent (10 mM ammonium acetate: water: methanol=1:9:10, v/v/v). The chemicals contained in it are risperidone cis N-oxide, bicyclorisperidone, z-oxime and risperidone. All solutions were filtered by a 0.22 μm PTFE syringe filter before analysis.

Sample preparation

Fifty milligram of Risperdal® Consta® microspheres were accurately weighed and transferred to a 100 mL volumetric flask. 10 mL of acetonitrile was added into the flask, followed by 10 min sonication to dissolve PLGA polymer. 90 mL of 0.01 N HCl was slowly added and resulted in the precipitation of PLGA polymer. After well-mixed the suspension, 2 mL of sample solution was centrifuged at 8,000 × g for 10 min to remove most of the polymer. For the remaining polymer depletion, the supernatant was filtered through a Microcon-YM10 filter with a 10,000-Da molecular mass cutoff. All solutions were filtered by a 0.22 μm PTFE syringe filter before analysis. Store under refrigeration at 4°C, the sample solution containing drug substance or impurities is stable over one week.

The similar procedure was utilized to investigate the PLGA precipitation study. After dissolving the 50 mg of Risperdal® Consta® microspheres in the 10 mL of acetonitrile, various composition of ACN-HCl (0.01N) solutions have been prepared. 1 mL of the sample was transferred to the cuvette and the transmission at 600 nm was recorded using a UV-vis spectrophotometer (DU-640, Beckman).

Stress degradation studies

To prove the specificity of the stability-indicating method, the stress degradation studies were conducted to check the possible degradation of the active pharmaceutical ingredient or PLGA under various states such as alkaline, heating, and oxidative conditions. The forced degradation conditions have been referred to the USP 36th official monograph of the risperidone tablet (peak identification solution). Briefly, 20 mg of Risperdal® Consta®/PLGA microspheres or 10 mg of risperidone API were suspended in the 100 mL volumetric flask with 10 mL of 0.1 N NaOH solution (pH 8.5) at 90°C for 24 h. Subsequently, 10 mL of 0.1% aqueous hydrogen peroxide was added to the flask and kept at 90°C for additional 2 hr. For the reaction work-up, the solution was cooled to room temperature and diluted with methanol to volume. The degraded sample solutions were filtered by a 0.22 μm PTFE syringe filter before analysis.

Validation procedures

Validation of the newly developed method was constructed of linearity, range, precision, accuracy, and robustness, limit of quantitation (LOQ) and limit of detection (LOD) as per ICH guidelines [10]. Since the lack of the qualified purity impurities, the diluted risperidone solution was used for all studies in terms of quantitation e.g., linearity, sensitivity, precision and accuracy. The risperidone related compounds mixture solution was subjected to prove system suitability. The resolution between bicyclorisperidone and z-oxime under various conditions should be evaluated and greater than 1.5. The USP tailing factor of a peak should less than 2.0. For linearity, the peak response for risperidone was carried out at five concentration levels from LOQ to 150% of the specification (0.10%). The calibration curves were generated and regression parameters were calculated by Excel. The precision of the method was determined by checking the intra-day and inter-day precision for five independent preparations. The %RSD should not more than 5%. Accuracy of the method was evaluated by calculating the percentage recoveries by adding risperidone reference standard to the placebo. LOD and LOQ were estimated at a signal-to-noise ratio was 3:1, 10:1, respectively. To determine the robustness of the developed method, different experimental conditions including mobile phase composition (± 2%), column temperature (± 5°C) and flow rate (± 0.02 mL/min) were altered. All specificity of the UPLC assay method was evaluated by stress test. Risperdal® Consta® and placebo were subjected to various forced degradation conditions. The peak purity was calculated by Empower® 3 to ensure the analyte chromatographic peak is not attributable to more than one component.

Results and Discussion

Method development

The developed and validated method was aimed to establish chromatographic conditions, capable of qualitative and quantitative of risperidone impurities. The chromatographic condition was initially based on the pharmacopoeial provided method. Figure 2a shows the conventional HPLC chromatogram for risperidone impurities assay. Although the resolution of bicyclorisperidone and z-oxime is qualified (Rs: 3.5>1.5), but the suggested flow rate (1.5 mL/min) often caused the relatively high backpressure to the system and resulted in poor peak shape. Based on our past experience, the tailing factor got worse after replicate injections. In order to overcome this problem, we transferred the method from HPLC to UPLC and varied the conditions until met the acceptance criteria. Various mobile phase compositions and flow rates were adjusted. An isocratic RP-UPLC method was developed for screening all contents with UV detection at 275 nm. In Figure 2b, a smooth and flat baseline and a symmetry peak of risperidone at 5.4 min were observed in the UV chromatogram. The compared results of UPLC and HPLC method are given in Table 1. The improved USP tailing factor (from 1.75 to 1.01) and reduced analyzing time demonstrated that the UPLC-based method is more efficient than HPLC. The system suitability results are given in Table 2 and developed UPLC method was found to be a well-separated approach for risperidone and its main impurities.

In general, for determination of the encapsulation efficiency or drug-loading, the sustained released PLGA microspheres would be dissolved in organic solvent and directly subjected for HPLC analysis [11,12]. However, for the measurement of the impurities content, a large amount of drug products should be injected into the column to satisfy the limit of quantitation with trace substances. One of the key issues in sample preparation is to avoid injecting PLGA polymer into the UPLC column. It may easily lead to physical clogging of the column even though the pre-column has been installed. To determine whether the PLGA precipitation depend on organic solvent composition, the transmission of the PLGA solution under different ACN concentrations were measured (Figure 3). At high volume of organic phase (>90% ACN), a clear sample solution and high transmission were observed. However, at the high volume of aqueous phase, measured low transmission means large amount of PLGA polymer has been precipitated. By these features, PLGA polymer could be depleted by organic-aqueous phase transfer. Meanwhile, the contained drug substances were dissolved and recovered under both solution systems for further analysis.

Specificity

The diluent sample, stress degraded Risperdal® Consta® and placebo (without API) were analyzed under the proposed chromatographic condition to prove the selectivity. Figure 4a displays the UV spectrum of stress degraded Risperdal® Consta®. Figure 4b, c shows the UV spectrum obtained from direct analysis of sample diluent (10% ACN in 0.01 N HCl) and forced degradation placebo. No signal of solvent or hydrolyzed PLGA monomers was presented in the spectrum. Fortunately, all generated impurity peaks do not overlap with the remaining signal of risperidone. The purity angle of risperidone is less than purity threshold which proves the specificity of this method was verified.

Linearity and range

Linearity was conducted by preparing diluted standard solutions at five concentration levels including from 0.1, 0.25, 0.5, 1.0 and 1.5 µg/mL. The regression equation of the linearity plot of concentration

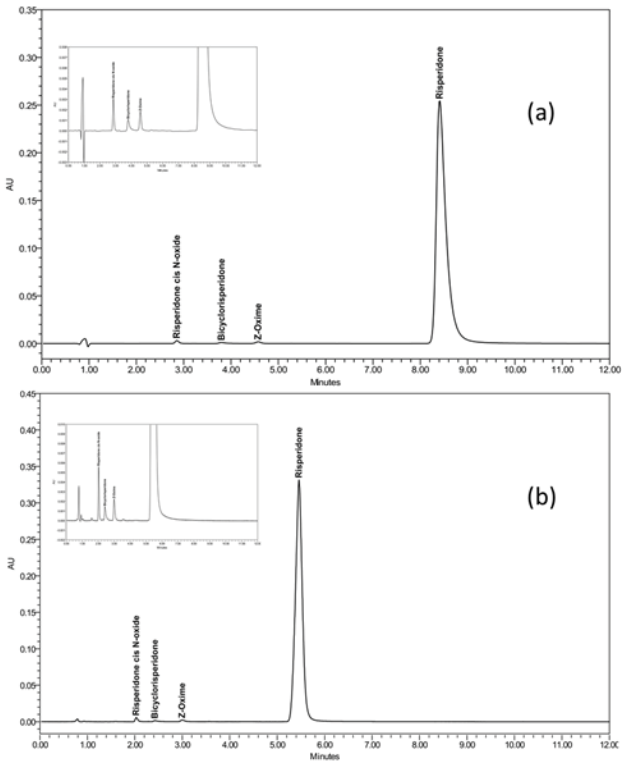

Figure 2: (a) HPLC and (b) UPLC UV chromatograms of the system suitability solution.

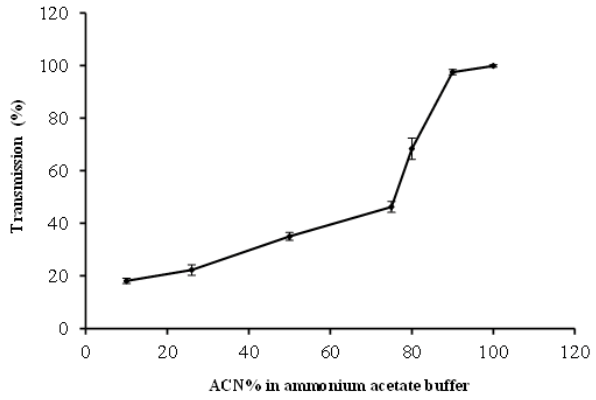

Figure 3: Effect of ACN percentages on precipitation of PLGA.

of risperidone over its peak area was found to be peak area=35380 × (conc.)–323.8. The correlation coefficient was found to be least R^2=0.9993. It indicates that the anayte gives a linear response for all ranges of concentrations.

Sensitivity

LOQ and LOD are expressed as a known concentration of analyte at a specified signal-to-noise ratio, usually 10:1 for LOQ or 3:1 for LOD. It is the lowest concentration of analyte in a sample can be quantitated or detected under the stated UPLC method. The LOQ (mean S/N=11.5) and LOD (mean S/N=4.9) of risperidone were 0.1 and 0.05 µg/mL, respectively.

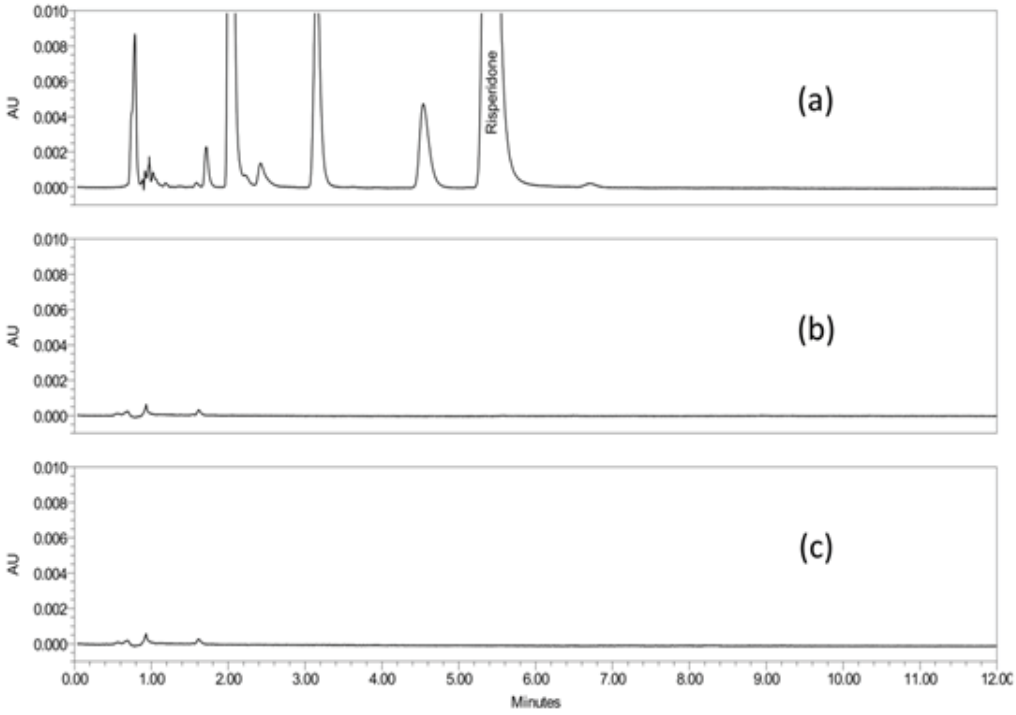

Figure 4: UPLC-UV chromatograms of (a) stress degraded Risperdal® Consta®, (b) diluent, and (c) PLGA microsphere placebo.

	K'	RT	N	T
UPLC	4.46	5.459	5974	1.01
HPLC	7.41	8.412	8257	1.75

Table 1: Comparison of developed UPLC and HPLC method. K': K prime; RT: retention time; N: theoretical plate; T: USP tailing.

	Risperidone cis N-oxide	Byclorisperidone	Z-Oxime	Risperidone
RT(min)	2.03	2.42	2.99	5.46
RRT	0.37	0.44	0.55	-
R_s	-	3.17	3.82	11.91
T	1.51	1.11	1.57	1.01
N	7006	3936	5855	5974

Table 2: System suitability report. RT: retention time; RRT: relative retention time; R_s: resolution; T: USP tailing; N: theoretical plate.

Nominal Conc of Risperidone(µg/mL)	Intra-day (n=5)%RSD	Inter-day (n=5) %RSD
0.5 µg/mL	0.92%	4.79%
1.0 µg/mL	0.58%	1.86%
1.5 µg/mL	0.14%	2.14%

Table 3: Intra-day and inter-day precision data for the method.

Level	Spiked Conc. (µg/mL)	Found Conc. (mean ± SD, µg/mL)	Recovery (%)
50%	0.5	0.49 ± 0.02	97.93%
100%	1	1.00 ± 0.01	100.33%
150%	1.5	1.49 ± 0.03	99.51%

Table 4: Extraction recovery of risperidone in PLGA microspheres (n=3).

Precision

The results of intra-day and inter-day precision are reported in Table 3. %RSD values were not more than 0.92% for intra-day precision and not more than 4.79% for inter-day precision. The data demonstrated that the values met the acceptance criteria.

Accuracy

Accuracy measurements are obtained by comparison of the results with the analysis of the target concentration (1 µg/mL of risperidone). The experiments are repeated for three times at three different concentration levels. The total average recovery is 99.26% with %RSD=1.23%. The detailed results are list in Table 4. Furthermore, stress degraded risperidone API was spiked into the PLGA microsphere placebo to estimate the extraction recovery (Figure S1). The total recovery was determined by calculating the sum of peak area. The mean recovery is 98.72 ± 2.43%. It means not only drug substance could be successfully extracted but also all impurities were easily recovered using this approach.

Robustness

The robustness of the method was studied by small but deliberate changes the acetonitrile content, column temperature and flow rate. The data provided in Table 5 indicates that the analytical method was robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. As expected, a proportionate increase in retention time ratio of analytes with increasing acetonitrile content and vice versa with decreasing acetonitrile content was observed. A similar result has been obtained while deliberating the flow rate. Elevated flow rate for eluting analytes resulted in raised retention time ratio as well. Moreover, with the exception of light drift in retention time, the chromatographic properties of the method remained constant when different column temperatures were adjusted.

Retaintion time ratio of impurities to Risperidone						
Parameter altered	**Variation**	**Risperidone cis N-oxide**	**Bicyclorisperidone**	**Z-Oxime**	**Resolution between Bicyclorisperidone and Z-Oxime**	**USP tailing factor of Risperidone**
ACN Conc	24%	0.34	0.41	0.53	4.29	0.97
	26.00%	0.36	0.43	0.54	3.76	1.01
	28%	0.4	0.46	0.57	3.44	0.89
Column Temp	25°C	0.37	0.44	0.55	3.51	0.93
	30°C	0.36	0.43	0.54	3.76	1.01
	35°C	0.36	0.43	0.54	4.12	0.91
Flow rate (mL/min)	32.00%	0.41	0.45	0.54	3.54	0.97
	30.00%	0.36	0.43	0.54	3.76	1.01
	28.00%	0.32	0.4	0.52	3.86	1.13

Table 5: Summarized robustness data.

According to the resolution item, all results are still compliance with acceptance criterion.

Conclusion

A validated rapid, specific and reproducible method was developed for analyzing the relative substances in Risperdal® Consta®. The simple phase-to-phase precipitation strategy for the extraction recovery of risperidone from PLGA microspheres was to be found excellent and reproducible. The process impurities generated from forced degradation conditions are well-separated, showing satisfactory data for all the ICH guidance requirements. Hence, the proposed means can be employed in routine analysis for product or stability samples.

Acknowledgments

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