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STABILITY INDICATING HPLC METHOD FOR QUANTIFICATION OF RISPERIDONE IN TABLETS

SIGRID MENNICKENT[1]; MARTA DE DIEGO[1]; BERND LISER[3]; LINA TRUJILLO[2]*

[1]Department of Pharmacy, Faculty of Pharmacy, University of Concepción, P.O. Box 237, Concepción, Chile

ABSTRACT

A stability- indicating LC method was developed and validated for the determination of risperidone in tablets.

Quantitation was achieved by LC/DAD at 294 nm over the concentration range of 25.00 µg/mL to 250.00 µg/mL. Mobile phase was a mixture of water: glacial acetic acid 0.50 %: triethylamine 0.80 %: acetonitrile (65.00: 0.32: 0.52: 34.16, v/v), using a Purosphere STAR RP-18e 250 x 4.5 mm (5µ) column, and paroxetine as internal standard.

The method exhibited an adequate linearity (r = 0.999), selectivity, precision (RSD ≤ 0.847 %) and accuracy (recoveries from 99.55 % to 101.35 %).

Risperidone was subjected to the stress conditions of oxidative, acid, base, thermal and photolytic degradation. Risperidone was found no degrade in basic or acid stress conditions, neither in thermal stress exposition (50, 70 and 100 ºC) no at visible or UV stress conditions, during the time of the study. Only two degradation products were observed with peroxide oxidation, well resolved from analyte peak, proving the stability-indicating power of the method.

The proposed method was found to be suitable for quantitative determination and the stability studies of risperidone in tablets.

Key words: risperidone, stability- indicating method, LC, tablets, stress testing.

1. INTRODUCTION

Stress testing of the drug substance is a step very important during the drug development process. It can help to establish the degradation pathways and the intrinsic stability of the molecule and is very useful to validate the stability indicating power of the analytical procedures used. Chemical degradation usually results in a loss of potency or an increase in drugs toxicity, so that clinical use of a therapeutic agent must be unacceptable if the degradation is relatively great[1]. Stress testing helps to evaluate the behaviour of the drug substance. Stress testing assays depends on the individual drug substance and the type of drug product involved. Stress testing includes the effect of temperatures and other appropriate conditions such as humidity, light exposition, an others [1-4].

Risperidone is a benzisoxazole- derivative second-generation antipsychotic agent and is chemically unrelated to other antipsychotic drugs. It is used for the treatment of schizophrenia, bipolar disorder, and irritability in children with autism [5-7]. The major impurities products in the dosage forms, including degradation products, described in USP are: Z-oxime, biciclorisperidone, cis risperidone--N-oxide [2]. Some degradation products founded in work investigations had been 9-hydroxy risperidone and N-oxide of risperidone [8]; however, in most of works, degradation products were not identified and /or no forced degradation was performed.

Official USP method to quantify impurities in tablets is by comparison of chromatographic peak response of any impurity and chromatographic peak response of risperidone in a sample solution, using relative signal factor (F): (chromatographic peak response impurity/ chromatographic peak response risperidone) x (1/F) x 100 [2].

Diode Array HPLC Detectors are most commonly used to record the ultraviolet and visible (UV-Vis) absorption spectra of samples that are passing through a high-pressure liquid chromatograph. In view of the clinical and analytical challenger, the present study describes the development of environment friendly, simple, rapid, sensitive, accurate and precise stability indicator LC/DAD method. Among all different detectors used to analyze any drugs (evaporative light scattering detector or ELSD, refractive index detector or RID, ultraviolet detector or UV, mass spectrometry detector or MS, etc), UV-diode array detector or UV-DAD is simplest and suitable for the purpose of this work.

The reported methods for quantitative determination of risperidone in bulk drug and in pharmaceutical samples, include liquid chromatography with UV detection [8-14], spectrophotometry [15-17], capillary zone electrophoresis [18-19], and thin layer chromatography [20-22]. One LC method and the TLC methods were stability indicating. Other methods for quantitative determination of risperidone used biological fluid as matrix, instead dosage forms. Therefore, this paper deals with develop and validate of a simple stability-indicating LC-DAD method for risperidone, and with the determination of its chemical stability under various ICH stress testing.

2. EXPERIMENTAL

2.1. Instrumentation and Reagents

2.1.1. Instrumentation

The present work was carried out on isocratic high pressure liquid chromatography Flexar LC System, Perkin Elmer (Massachusetts, USA), equipped with a diode-array detector (DAD), a Purosphere STAR RP-18e (5 µm size, 250 mm × 4.6 mm i.d.) column, and a pump, (Cyberlab TM, USA) with universal loop injector (Rheodyne) of injection capacity 20 μL.

Other instrumentations used were WLW Centrifuge (Germany), Heidolph shaker Metrohm (USA), and Reacti-Vap evaporator Thermo Fisher Scientific (Waltham, MA, USA).

2.1.2. Reagents and chemicals

Standards of risperidone (98.21 % purity), and paroxetine (98.00 % purity), were purchased from Sigma- Aldrich, St.Louis, MO. Methanol, glacial acetic acid, triethylamine, acetonitrile, were obtained from Merck, Darmstadt, Germany. All of the reagents were pro-analysis quality.

2.2. Methods

2.2.1. Standard Solution preparation

Stock solution of risperidone and stock solution of paroxetine were prepared by dissolving an appropriate amount in HPLC grade methanol to a concentration 500.00 µg/mL each.

Separate solutions were prepared for the calibration standards and quality control samples. These solutions were diluted immediately before with mobile phase, to obtain working solutions of 25.00, 50.00 100.00, 150.00, 200.00 and 250.00 µg/mL. Paroxetine was added to each solution at concentration of 100.00 µg/mL. All solutions were stored at 4 ºC until analysis.

Each 20 μL standard solution was injected into the column after filtration using 0.2 micron membrane filter.

2.2.2. Sample preparation

Pharmaceutical preparations were tablets, nominally containing 1.00 mg of risperidone. Twenty tablets were weighed, crushed, and mixed in a mortar and pestle. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred into 50 mL volumetric flask and made up to the volume with mobile phase and mixed well. Then it was stirred in vortex for 15 s, filtered through a 0.45 micron nylon filter, and centrifuged for 4 min. Suitable aliquots of filtered solutions were transferred to a 10 mL volumetric flask and made up to the volume with mobile phase to yield concentration of risperidone 200.00 µg/mL.

2.2.3. Chromatographic conditions

HPLC analyses were carried out on a Purosphere STAR RP-18e 250 x 4.5 mm column (5µ) (Darmstad, Germany).

Separation was achieved using a mobile phase consisting of water: glacial

e-mail: smennick@udec.cl

acetic acid 0.50 %: triethylamine 0.80 %: acetonitrile (65.00: 0.32: 0.52: 34.16, v/v), at pH= 5.5 and a flow rate of 1 mL/min. The eluted compounds were monitored at 294 nm. The column was maintained at ambient temperature, and an injection volume of 20 μL was used. Chromatographic run was 10 minutes.

The mobile phase was filtered through 0.45 micron membrane filter and ultrasonicated for 10 minutes prior to use. For analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 200–400 nm. Peak homogeneity was expressed in terms of peak purity values and was obtained directly from spectral analysis report obtained using the instrument software.

2.2.4. Validation

The parameters validated were linearity, detection limit, quantitation limit, repeatability, intermediate precision, accuracy and selectivity.

Validation was performed according to International Conference on Harmonization (ICH) guidelines [23].

2.2.5. Stability-indicating capability of the LC assay/Stress testing

In order to determine the stability indication of the analytical method and assay, risperidone tablets were stressed to conduct forced degradation studies, in concordance with Regulatory guidance in ICH and FDA [23-25].

Solutions of risperidone used in forced degradation studies were prepared with final concentrations of 1.00 mg/mL (hydrolysis assay), and 2.00 mg/mL (for all other assays). Each assay was performed in triplicate.

2.2.5.1. Hydrolysis:

It was established by forced decomposition of 5 mL of a solution of risperidone 1.00 mg/mL, with 10 mL of 0.1 N hydrochloric acid or 10 mL of 0.1 N sodium hydroxide. Samples were heated on a hot plate at 60 ºC for 60 minutes, cooled to room temperature, diluted with methanol to 100.00 µg/mL, and analyzed.

2.2.5.2: Oxidation:

Oxidation of risperidone was carried out in 2 mL of 3.00 % H_2O_2 at a concentration of 2.00 mg/mL, using 0.5 mL of solution, at room temperature (25 ± 2ºC) and room light for 8 days, and also to 80 ºC for 6 hours.

2.2.5.3: Temperature:

Risperidone sample 1.00 mg/mL was kept in oven at 50 ºC, 70º C, and 100 ºC for 8 hours, taking samples each 2 hours. Each sample was transferred to a volumetric flask, and diluted with methanol to volume (final concentration of risperidone: 2.00 mg/mL).

2.2.5.4: Photostability:

1.00 g of risperidone sample was taken in to a petri dish and exposed to UV (UV lamp) and VIS radiation (daylight) for 3 hours. 200.00 mg of this sample was taken in to a 100 mL volumetric flask, and diluted to volume with methanol.

2.2.6. Stability study of stored solutions

To establishment the stability of stored risperidone samples, in normal storage conditions, the study was performed as follows: six solutions with analyte and internal standard were used at three different concentrations: 25.00 µg/mL, 150.00 µg/mL and 250.00 µg/mL. These solutions were stored at three different conditions: freezer temperature, room temperature with light protection, and room temperature without light protection. Concentration determination was evaluated at 0, 1, 4, 7, 11 and 15 days of storage. Each sample was determined by duplicated.

2.2.7 Application of the method

The method was used for quantitative determination of risperidone in tablets.

3. RESULTS

3.1. Method validation

3.1.1 Linearity

Calibration curves were constructed over the concentration range of 25.00 to 250.00 µg/mL for risperidone, and 100.00 µg/mL of internal standard. Each solution was injected three times. Standard curves were constructed by plotting the peak area ratio of the analyte to the internal standard as a function of the concentration added.

The mean equation (curve coefficients ± standard deviation) for the calibration curve (n=5), obtained from five points, was $y = 0.0074x + 0.067$, with a correlation coefficient, r =0.999.

Resultant HPLC chromatograms following the analysis of a standard solution of risperidone can be observed in Fig.1.

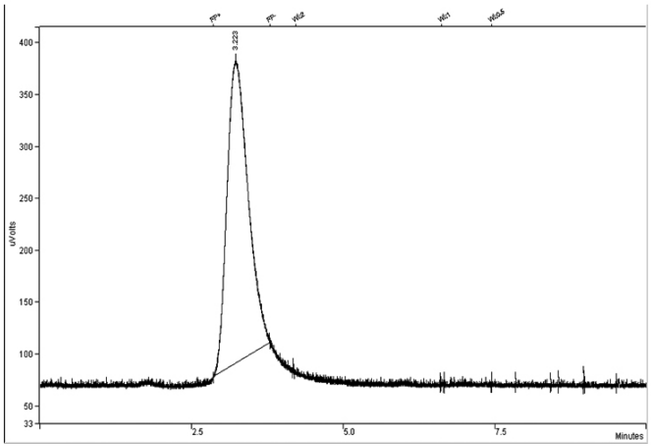

Figure 1: Resultant HPLC chromatograms following the analysis of a standard solution of risperidone 500.00 µg/mL.

3.1.2. Detection and quantitation limits

The limit of detection (LOD) and limit of quantification (LOQ) were calculated preparing solutions at three concentrations (10.00- 20.00- 30.00 µg/mL) in the lower range of linear regression curve, including a concentration under the curve. LOD was 4.40 µg/mL and LOQ was 13.33 µg/mL, determined using the equations: LOD = 3.3 σ/b; LOQ = 10 σ/b, where σ is the standard deviation of the responses, and "b" corresponds to the slope obtained from the curve peak area ratio of the analyte to the internal standard versus concentration of the analyte. These values were experimentally verified applying the method to prepared solution with these analyte concentrations.

3.1.3. Precision

The intra-assay precision was determined by analysis of solutions with concentrations of 25.00, 150.00 and 250.00 µg/mL on the same day, each of which was independently prepared and each of them being applied three times [23,25]. Intermediate precision (inter-assay precision) included analyses at the same three points; each solution was analyzed three times on the same day for three different days. Precision analysis studies showed an intra-assay variation between 0.039% and 0.140 % and an inter-assay variation between 0.039 % and 0.847%. (Table I).

Table I. Precision of the method.

Relative standard deviation (RSD), %		
Concentration (µg/mL)	Intra-assay[a]	Inter-assay[b]
25..00	0.039	0.039
150.00	0.123	0.545
250.00	0.140	0.847

[a] n= 3; analyzed on the same day (three solutions of each concentration)

[b] n= 9; analyzed on three different days (three solutions of each concentration prepared for 3 days).

3.1.4. Accuracy

The accuracy was determined by standard addition, applying the method to pharmaceuticals preparation (200.00 µg/mL) to which known amounts of standard substance corresponding to 80.00 %, 100.00 % and 120.00 % of the concentration expected in the samples were added. Three solutions of each concentration were prepared and three replicates were measured for each one. The accuracy was then calculated from the test results as the percentage of analyte recovered by the assay. The recovery percentage was between 99.55 % and 101.35 %, and is independent of the concentration with a coefficient of variation between 0.094 % and 0.860 % as shown in Table II.

Table II. Method accuracy.

Actual concentration (µg/mL)	Measured concentration (µg/mL) [a]	Accuracy, % [b]	RSD, %
160.00	159.20 ± 0.15	99.55	0.094
200.00	200.70 ± 0.51	100.35	0.254
240.00	243.24 ± 2.09	101.35	0.860

3.1.5. Selectivity

The selectivity of the method was evaluated through possible interference due to degradation products.

Also, the selectivity between risperidone and paroxetine (internal standard), was studied. The tR was 3.5 for risperidone, and 7.5 for paroxetine (Fig.2).

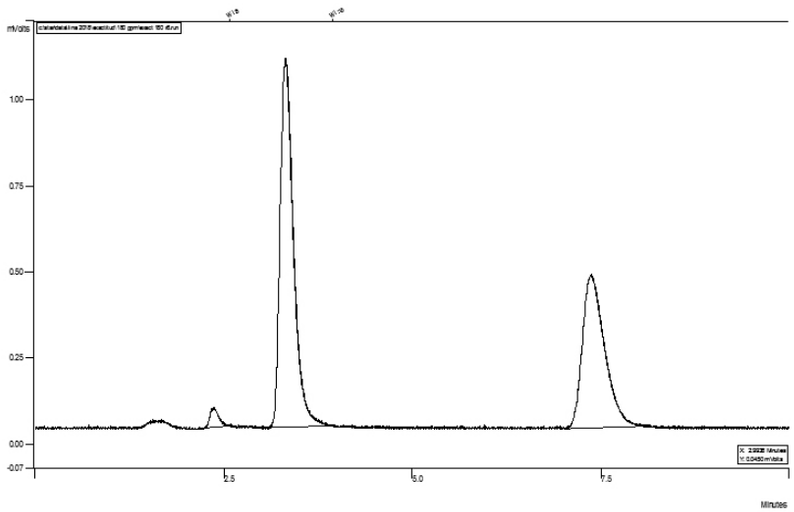

Figure 2: Selectivity of the method. Study with internal standard. Peaks: tR= 3.5: risperidone; tR= 7.5: paroxetine.

3.2. Stability-inidicating capability of the LC assay

Acidic and Basic conditions

The drug was found to comparatively stable to acid and to alkaline hydrolysis, on refluxing the drug in 0.1 M HCl or in 0.1 M NaOH for 8 hours.

Photolytic condition

When the drug was exposed to forced degradation with UV or sunlight, no degradation product was observed after exposure of solid drug.

Oxidative Conditions

The drug was highly labile to hydrogen peroxide (3.00 %) at room temperature. After 6 hours, steep fall in the drug peak area was observed. Major degradation products were appeared at tR= 2.0 and tR = 5.3. These peaks were resolved from risperidone (tR= 3.5) (Fig. 3). Risperidone was degraded to 35.00 % when it was exposed to room light for 8 days, and it was degraded to 17.00 % when it was exposed to 80 ºC for 6 hours (Fig. 4). One degradation products founded in work investigations had been N-oxide of risperidone [2, 8].

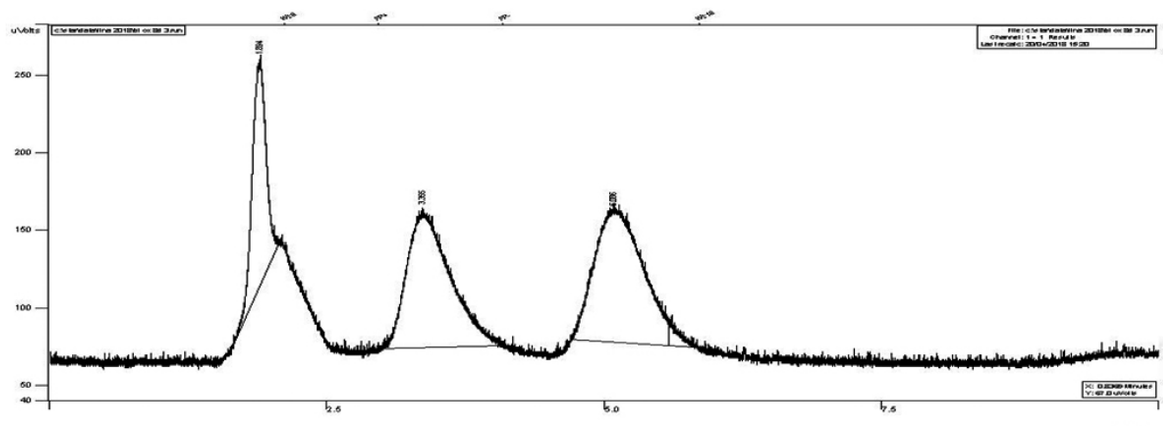

Figure 3: Study at forced degradation of risperidone with 3.00 % H2O2 at room light for 8 days, and with 3.00 % H2O2 at 80 ºC for 6 hours. Peaks: tR= 2.0: degradation product.; tR= 3.5: risperIdone; tR= 5.3: degradation product.

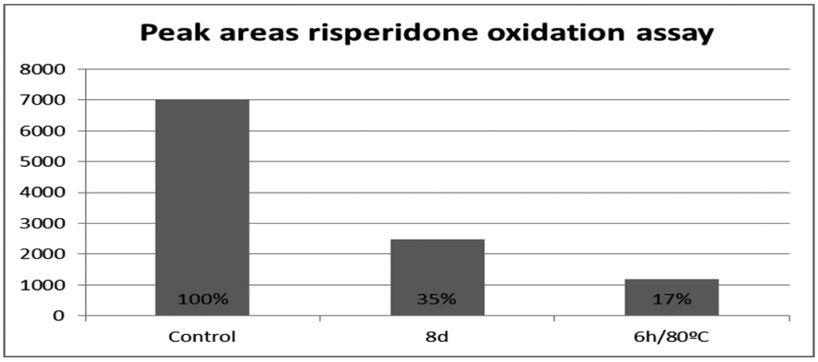

Figure 4: Risperidone degradation at 3.00 % H2O2 exposition.

Thermal degradation

When risperidone was exposed to dry heat at 50 ºC, 70 ºC and 100 ºC, for 8 hours, no degradation product was found, during the time of the study.

3.3. Stability of stored solutions

Observed concentrations of risperidone during the time of the assay ranged between 95.50 %-97.80 %, and concentrations of Paroxetine was between 96.70 % - 97.90 %.

3.4. Application of the method

The proposed method was applied to the determination of risperidone in commercial tablets .The result of these assays yielded 99.80 % (%R.S.D = 0.93 %) of label claim for the tablets.

4. DISCUSSION

During method development, different chromatographic conditions were tried to achieve optimal results. Some mobile phases were proved to obtain optimal resolution and peak shape and symmetry. Buffer solution was replaced with organic solvent to simplify the preparation of the mobile phase and to avoid precipitate formation into the column. The mobile phase water: glacial acetic acid 0.50 %: triethylamine 0.80 %: acetonitrile, (65.00: 0.32: 0.52: 34.16, v/v), at ambient temperature, resolved peaks of analyte, internal standard and degradation products. pH of mobile phase was selected according to pka of the analyte. Several wavelengths were tried, chosen 294 nm as working wavelength. Complete resolution of the peaks with clear baseline separation was obtained of this way. The developed method was linear between the concentrations range expected, precise, accurate, sensible, and selective for the quantitative determination of risperidone in tablets. About specificity for LC-DAD method, it allows obtain optimal peak purity, therefore, it is useful to show that the analyte chromatographic peak is not attributable to more than one component. A RP-C18 column (Purosphere STAR RP-18e 250 x 4.5 mm column (5µ) maintained at ambient temperature (25°C) was used for the separation and the method validation.

Risperidone was subjected to the stress conditions of oxidative, acid, base, thermal and photolytic degradation. Risperidone was found no degrade in basic or acid stress conditions, neither in heat stress exposition (50, 70, and 100 ºC) no at visible or UV stress conditions, during the time of the study. Only two degradation products were observed with peroxide oxidation, well resolved from analyte peak, proving the stability-indicating power of the method.

Only another stability indicating LC method was found in literature. The stability indicating LC method described in this manuscript has some advantages compared with the stability indicating LC method developed by Dedania et al as[11]: intra-assay variation, in our method, was between 0.039 % and 0.140 %, and inter-assay variation was between 0.039 % and 0.847 %, whereas Dedania obtained RSD of 1.04 % and 1.22 %, respectively. In our method, calibration curves were constructed over the concentration range of 25.00 µg/mL to 250.00 µg/mL for risperidone, whereas Dedania worked with a calibration curve between 10.00 µg/mL to 60.00 µg/mL. Wider range for calibration curve is especially useful to evaluate small losses of analyte due to degradation process in dosage forms.

5. CONCLUDING REMARKS

A stability-indicating LC/DAD assay method for the determination of the chemical stability of risperidone has been developed and validated, with appropriate precision, sensibility, accuracy and selectivity.

The method is stability-indicating since it allows the determination of risperidone in the presence of degradation products founded, according to the International Conference on Harmonization (ICH) guidelines. The method is simple and not expensive, and it is suitable for a wide concentrations range of analyte, compared with other methods described for quantification and degradation studies of risperidone. Therefore, the proposed method can be useful for stability studies of risperidone in tablets.

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