Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 5 Issue 8 August 2021

Research Article

Simultaneous Estimation of Antipsychotic Drugs (Risperidone and Olanzapine) by RPHPLC

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Received: June 18, 2021

Published: July 22, 2021

Abstract

A rapid, specific RPHPLC technique has been developed for simultaneous resolve of risperidone and olanzapine. Drugs were subjected to stress conditions such as acidic, alkaline and oxidative hydrolysis. Chromatographic separation of these pure drugs was carried with a 50:50 (v:v) mixture of acetonitrile and Potassium Di Hydrogen Phosphate and 40:60 (v:v) mixture of acetonitrile and Potassium Di Hydrogen Phosphate Buffer as mobile stage. The current rate was 1.0 mL min-1 and the analysis was monitored at 235 nm by UV detection.

Keywords: Risperidone; Olanzapine; RP-HPLC; Acetonitrile; Potassium

Introduction

Olanzapine and risk phrenic medications for successful therapy for schizophrenia and similar disorders are deemed universally beneficial. In recent years, standard antipsychotic medications in schizophrenia have been substituted by greater effectiveness and lower side effects as part of the therapy of schizophrenia [1,2]. "Atypical" drug combinations are well tolerated and may be useful for schizophrenia treatment [3]. V. Currently, five atypical antipsychotic medications, including clozapine, elanpine, risperidone, ziprasidone and quetiapine, have been licensed for use in the United States (Borison., *et al*. 1986, Wetzel., *et al*. 1995, Bymaster., *et al*. 1996, Janssen., *et al*. 1988, Daniel., *et al*. However, no direct way to simultaneously assess risperidone and olanzapine in their combination form was identified in literature surveys. Chemically recognized as 3-(2-(4-(6-fluoro-1,2,2-benzisoxazol-3–yl)-1-piperidinyl) ethyl)-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a] is risperidone and olanzapine 2-methyl-4-pyrimidine 4-one (4-methyl-1-piperazinyl) -10H-thieno, respectively [2, 3-b][1,5]benzodiazepine.

The objective of the current research is to establish a quick, special and verified RP-HPLC process in combination pure form for the assessment of risperidone and olanzapine. The technique was evaluated for specificity, accuracy, linearity and stability of the solution. Drugs also suffer from acid, alkaline and oxidation hydrolysis conditions stressful Drugs (30% v/v H_2O_2) [3].

Method Development

TRAIL-1

Preparation of buffer

Take 295mg potassium dihydrogen phosphate of 0.02 M was melted in 100ml of water and regulate the pH-3 using diluted O-phosphoric acid [4].

Preparation of mobile phase

Clean and degassed combination of acetonitrile: bumper in the ratio of 50:50 and filter through 0.45 micron membrane filter [4].

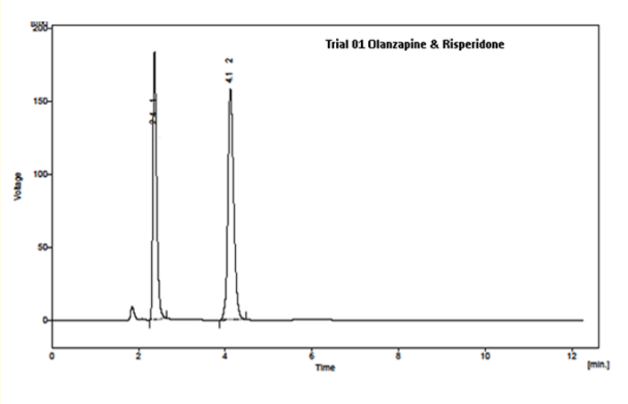

Figure 1: Olanzapine and Risperidone (Acetonitrile: Potassium Di Hydrogen Phosphate Buffer (50:50 V/ V)).

TRAIL-2

Preparation of buffer

Take 295 mg of 0.02 M potassium dihydrogen orthophosphate was melted in 100ml of water and adjust the pH-4.5 using diluted O-phosphoric acid [5].

Preparation of mobile phase

Filtered and vented mixture of acetonitrile: buffer in the ratio of 40:60 and filter through 0.45 micron membrane filter [5].

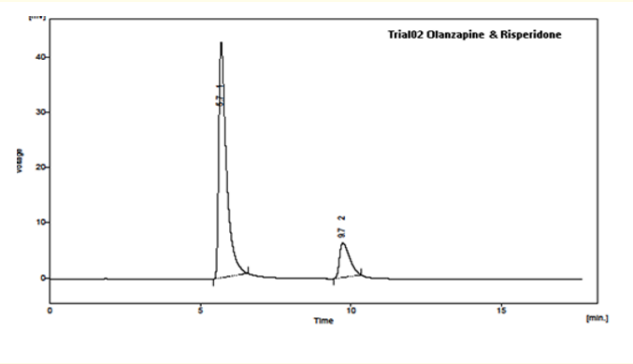

Figure 2: Olanzapine and Risperidone (Acetonitrile: Potassium Di Hydrogen Phosphate Buffer (40:60 V/V)).

Trial	Mobile	Result	Wave-length	pH	Flow
1	ACN:KH_2PO_4 (50:50)	Good result	235	5.5	1ml/1min
2	ACN:KH_2PO_4 (40:60)	Less retention time	235	4.5	1ml/1min

Table 1

Initialization of the instrument

The column has originally been put on the instrument and the instruments have been switched on, cleaned with acetonitrile for 30 minutes: water (20:80). The system was then designed to operate for column saturation in the mobile phase for 30 minutes [6].

Standard preparation of olanzapine and risperidone

Standard-A: Precise weighed amounts of 10 mg Olanzapine into a 100 ml volumetric bottle, and diluent to the capacity. A volumetric flask of 5 ml was pipelined into the same diluent in the volume and then produced [6].

Standard-B: Accurately weighed amount of 5 mg of risk peri done in a 100 ml volumetric flask and thinned to the capacity. This 5 ml was then piped into a flask of 50 mL and composed with same diluents up to the capacity [6].

Mode of operation	Isocratic
Parameters	Description
Diluents	Water
Column	C18, 250x4.6mm,5µ SS column
Mobile phase	Acetonitrile: Potassium Di Hydrogen Phosphate (50:50)
Flow rate	1.0 ml/min
Detection of Olanzapine and Risperidone	235 nm
Temperature	25^0 C
Injection Volume	20 µl
Run time	20 min
Detector	UV detector

Table 2: Chromatographic conditions.

Parameters	Conditions
Column (Stationary Phase)	WATERS C18 Symmetry (4.6 x 250mm, 5 µm)
Mobile Phase	Acetonitrile: Potassium Di Hydrogen Phosphate (50:50)
Flow rate (ml/min)	1ml/mm.
Run time (min)	20
Column temperature(°C)	25^0 C
Volume of injection loop (µl)	20 µl
Detection wavelength (nm)	235
Drug RT (min)	2.2, 4.1

Table 3: Optimized method parameters.

Validation parameters

The technique has been validated using numerous parameters since the HPLC technique was created in order to guarantee that the method's performance characteristic satisfies the criteria for the intended analysis applications [7].

System suitability

Preparation of standard solution

Accurately weighed in 50 ml volumetric flask 10 milligrams of olanzapine and 5 mg Risperidone to mobile phase volume. Dilute volumetric flask to the mobile phase volume [8].

Specificity

Specificity is the capacity to evaluate an analyte definitively when components are present that may be predicted to exist. The specificity specifies that the procedure must not be impacted by the attendance of other mechanisms in the concurrent assessment of olanzapine and riskperidone. The specialty would usually be carried out by allowing the sample under stressful circumstances [9].

Heating

1 mL from the stock answer in a 10 mL bottle up to mobile phase volume should be collected for the specificity investigation. For a time period of 30 minutes the answer should be heated at 40 °C. Note that any breakdown takes place or not [10].

Treating with acids

In the 10 mL flask take 1 mL of the packaging solution. Add 1 mL hydrochloric acid to this bottle. To it. Notice of any changes in the preservation of the summit [11].

Treating with base

Add 1 mL of 0.1 M of hydroxide sodium from a solution containing stocks into a 10 ml flask. Notice any degradation [12].

Linearity

Preparation of standard stock solution

10 milligrams of olanzapine and 5 mg of riskperidone diluted to volume with mobile phase in 50 ml volumetric fiber Dilute volumetric flask to the mobile stage volume [13].

Preparation of linearity solution-I: Transfer 1ml with mobile phase from stock to 10 ml (the solution becomes 2 mcg of olanzapine and 1mcg of Risperidone) [13]

Preparation of linearity solution-II: Transfer 2ml containing the moving phase from standard solution to 10 ml (the solution becomes 4mcg of olanzapine and 2mcg of Risperidone) [13]

Determination: The linearity of the analysis technique is evaluated by the mathematical treatment of analysis findings from samples having analyte levels over the specified range. The area is graphed according to the concentration of the analyte. Curve fits are determined by percentage [13].

Acceptance criteria: The coefficient of correlation and regression for Olanzapine and Risperidone must not be less than 0.99 [13].

System precision

Preparation of stock solution

Weigh 10 mg Olanzapine and 5 mg Risperidone in 50 ml Volumetric Flask Dilute To Volume with Moveable Phase [14].

Dilution

Transfer 5 ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 5mcg of Risperidone) [14]

Accuracy

Preparation of stock solution

Weigh 10 mg olanzapine and 5 mg Risperidone in 50 ml volumetric flask dilute to volume with mobile phase [15].

Preparation of spiking standard

Transfer 5 ml from stock solution to 100 ml with mobile phase.

Preparation of accuracy solution 1: Transfer 4ml (8mcg olanzapine and 4mcg riskperidone) from stock solution to 100 ml with motive phase and add 1ml Spiking Standard [15].

Preparation of accuracy solution 2: Transfer to 100 ml (10% Olanzapine and 5% Risperidon) and add 1 ml of Spiking Standard (5 ml of stock solution with mobile phase) and add to the package [15].

Preparation of accuracy solution 3: Transfer to 100 ml (12 mcg olanzapine and 6 mcg risk peridone), moving from stock solution, and add to the standard 1 ml [15].

Method precision

Stock solution

Weigh 10 mg olanzapine and 5 mg Risperidone in 50 ml Volumetric Flask, Dilute to Volume with Mobile Phase [16].

Dilution

Transfer 5ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 5mcg of Risperidone) [16].

Assay

Preparation of stock solution

Weigh 10 mg olanzapine and 5 mg Risperidone in 50 ml volumetric flask dilute to volume with mobile phase [15].

Preparation of standard solution

Transfer 5 ml from stock solution to 100 ml with mobile phase (The solution becomes 10mcg of olanzapine and 5mcg of Risperidone) [15].

Ruggedness

Ruggedness study was approved out by repeating the complete experimentation with different analysts, on dissimilar days in same workroom as per the following preparation [15].

Blank solution

Purity water was used as diluents.

Stock solution

Weigh 10 mg Olanzapine and 5 mg Risperidone in 50 ml Volumetric Flask Dilute to Volume With Mobile Phase [14].

Dilution

Transfer to 100 ml of mobile phase 5ml of stock solution (the solution becomes 10mcg of olanzapine and 5mcg of Risperidone) [13].

Determination

A sufficient number of samples shall be evaluated and a relative default shall be calculated by means of the test method and tool.

Acceptance criteria

The relative standard deviation should not be less than 2%.

Robustness

Stock solution

Weigh 10 mg Olanzapine and 5 mg Risperidone in 50 ml volumetric flask dilute to volume with mobile phase [11].

Dilution

Transfer 5ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 5mcg of Risperidone) [15].

S. No	Chromatographic condition	Low	High
1.	Flow rate	0.9 ml	1.1 ml
2.	Wavelength	235 nm	237nm

Table

Determination

An analysis of aliquots of homogeneous lot, using different physical parameters that may be different but are yet within the specified parameters of the assay, shall determine the robustness of an analytical approach [13].

Acceptance criteria

The percentage assay of drugs should be within the limit of 90-110%.

Results and Discussion

TRAIL-1

System suitability results

Olanzapine and Risperidone

- Tailing factor obtained from trail- 1 was 1.700 and 1.412
- Theoretical plates obtained from trail-1 was 3562 and 4798
- Resolution obtained from trail-1 was 8.843
- Retention time obtained from trail-1 was 2.367 and 4.120 [11].

TRAIL-2

System suitability results

Olanzapine and Risperidone

- Tailing factor obtained from trail- 2 was 2.909 and 2.458
- Theoretical plates obtained from trail-2 was 2795 and 3635
- Resolution obtained from trail-2 was 7.513
- Retention time obtained from trail-2 was 5.690 and 9.733 [12].

Trails result

On the evaluation of above system suitability results,

- System suitability parameters of trail-I were within the satisfactory limits.

Hence trail II shows variation in system suitability results and affected the method significantly. Trail-I shows good system suitability results and also within in the limit so the trail – I was adopted [14].

Validation results

System suitability

S No.	Parameter	Olenzapine
1	RT (min)	2.2
2	Tailing Factor	1.5
3	No. of theoretical plates	2557.000

Table 4: System suitability results for olanzapine.

S No.	Parameter	Risperidone
1	RT (min)	4.1
2	Tailing Factor	1.4
3	No. of theoretical plates	7051.000

Table 5: System suitability results for Risperidone.

Result

On the evaluation of above results it was found that all the system suitability parameters were within the satisfactory limit.

Specificity

Diluents, standard preparation and assay were prepared as per the method and the solutions were vaccinated into the chromatograph and the chromatograms logged. The retention time given in the following table [7].

S.no	Solution	Retention time (min)
1.	Olanzapine Standard preparation	2.23
2.	Olanzapine assay preparation	2.01
3.	Risperidone standard preparation	5.05
4.	Risperidone assay preparation	5.02

Table 6: Specificity results for Olanzapine and Risperidone.

S.no	Stress conditions	Observed result
1.	Heated on water bath	No degradation occurred
2.	Treated with acids	No change in retention of the peak
3.	Treated with base	No degradants formed

Table 7: Specificity results for Olanzapine and Risperidone under stress conditions.

Result

- No peaks should be detected at the retention time of Olanzapine and Risperidone in the chromatograms of diluents preparation
- From the stress conditions performed, various degradation products were formed and there was no change in the detection of the analyte in the presence of other components.

Accuracy

% Concentration (at specification Level)	Area	Amount Added (mcg)	Amount Found (mcg)	% Recovery	Mean Recovery
80%	154.4297	9	8.93	99.26%	
100%	189.5143	11	10.96	99.66%	
120%	223.4167	13	12.92	99.41%	99.44%

Table 8: The accuracy results for olanzapine.

Acceptance criteria

The % Recovery for each level should be between 98.0 to 102.0%.

% Concentration (at specification Level)	Area	Amount Added (mcg)	Amount Found (mcg)	% Recovery	Mean Recovery
80%	92.97033	4	3.88	99.34%	
100%	113.7897	6	5.89	99.48%	
120%	135.1153	8	7.99	99.95%	99.59%

Table 9: The accuracy results for risperidone.

Acceptance criteria

The percentage recovery should vary from 98.0 to 102.0 for each level.

Discussion

On the evaluation above results % recovery of the drug shows 99.5% hence the method is found to be accurate.

Method precision

S No.	Rt	Area
1	2.21	171.772
2	2.19	173.657
3	2.217	173.768
4	2.2	174.41
5	2.207	172.398
Avg	2.2048	173.201
St. dev	0.010281	1.081429
%RSD	0.47	0.62

Table 10: Method precision results (Olanzapine).

S No.	Rt	Area
1	5.077	104.183
2	5.053	104.585
3	5.097	105.061
4	5.053	104.819
5	5.073	104.958
Avg	5.0706	104.7212
St dev	0.018461	0.349754
%RSD	0.36	0.33

Table 11: Method precision results (Risperidone).

Acceptance criteria

The RSD percentage should not exceed 2% for the five standard injection results.

Discussion

On the evaluation above results % RSD values are within the limit hence the method is precise.

Ruggedness

Injections	Area	
	Analyst-1	Analyst-2
1	174.236	175.224
2	172.238	172.344
3	173.244	174.432
4	173.322	171.238
5	174.414	173.224
6	172.314	173.382
Avg	173.2947	173.3073
Std dev	0.918758	1.425564
% RSD	0.52	0.82

Table 12: Intermediate precision (ruggedness) for Olanzapine.

Injections	Area	
	Analyst-1	Analyst-2
1	105.715	106.734
2	104.722	105.735
3	105.744	106.765
4	103.755	106.788
5	105.711	105.734
6	104.760	105.797
Avg	105.0678	106.089
Std dev	0.80344	1.336743
% RSD	0.76	0.84

Table 13: Intermediate precision (ruggedness) for Risperidone.

Discussion

The %RSD is less than 2% for the results of two analyst indicating the ruggedness of the method.

Robustness

By making small variations to the process parameters like flow rate changes by ± 10 percent of actual flow rate, the robustness of the method is determined.

S No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3191	1.544
2	1.1	7037	1.446

Table 14: Results for Olanzapine.

S No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	7512	1.431
3	1.1	2772	1.518

Table 15: Results for Risperidone.

Summary and Conclusion

The RP-HPLC technique for the simultaneous assessment in combination dose form of olanzapine and risperidone has been tested. As the literature review showed, few techniques are available at a time, but a simple, cost-effective and correct technique for estimating the aforesaid combination in combined dose form is required.

HPLC Water The mobile phases of buffer dipotassium hydrogen phosphate: acetonitrile (50:30), which was pumped at a flow rate of 1 mL/min and measured by a UV detector, have been injected using sickrooms 21cfr software, UV detector and C18 symmetry column (250mm X 4.6 mm, 5 µ). At 2.2 and 4.1 respectively, the maxima of Olanzapine and Risperidone were well separated.

Different deteriorating products have been shown to be selective and there are no changes to analyte detection in the presence of other components.

The system adequacy investigations indicated that all system adequacy parameters were subject to the criterion of approval.

The findings shown in precise terms were that for Olanzapine 99.4% for Risperidone were the percentage recovery values of pure medication from the pre-analyzed formulations, indicating that the procedure was accurate.

Robustness findings have been shown that with the change in parameters such as flow rate and wave length, there is minimal change in findings, which indicate the robustness of the approach.

The chromatographic technique devised to determine the dose of Olanzapine And Risperidone was simple, fast, accurate, specific, robust, and cost effective. The mobile phase is easy to prepare and cost-effective, dependable and time saving.

Given that the system suitability trials and their validation tests have also been successful, it is determined to be most beneficial for analytical purposes that the simple and short recommended approach is.

Conflict of Interest

The authors have no conflicts of interest.

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Volume 5 Issue 8 August 2021