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Analytical Method Development and Validation for the Simultaneous Estimation of Risperidone and Trihexyphenidyl Hydrochloride by Using RP-HPLC method

Relangi Karun Kumar[1*], Gope Edward Raju[2], Kandregula Uma Maheswari[3], Doonaboyina Raghava[4], Kavala Nageswara Rao[5]

Department of Pharmaceutical Analysis, K.G.R.L College of Pharmacy, Bhimavaram-534201, A.P, India

A b s t r a c t

A new method was established for simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Risperidone and Trihexyphenidyl hydrochloride by using ThermosilC18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. HPLC instrument is Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.566mins and 3.417mins. The % purity of Risperidone and Trihexyphenidyl hydrochloride was found to be 101.04% and 99.24% respectively. The system suitability parameters for Risperidone and Trihexyphenidyl hydrochloride such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Risperidone and Trihexyphenidyl hydrochloride was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC

Keywords: ThermosilC18 column, Risperidone and Trihexyphenidyl hydrochloride, RP-HPLC

A r t i c l e I n f o

Corresponding Author
Relangi Karun Kumar
Department of Pharmaceutical Analysis,
K.G.R.L College of Pharmacy, Bhimavaram-534201, A.P, India

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C O N T E N T S

1. Introduction

Risperidone is a second-generation antipsychotic (SGA) medication used in the treatment of a number of mood and mental health conditions including schizophrenia and bipolar disorder. It is one of the most widely used SGAs. Risperidone binds with a very high affinity to 5-HT2A receptors, approximately 10-20 fold greater than the drug's binding affinity to D2 receptors, and carries lesser

activity at several off-targets which may responsible for some of its undesirable effects. Trihexyphenidyl is a centrally acting muscarinic antagonist used for treatment of Parkinsonism and drug-induced extrapyramidal disorders. Its discovery was published in 1949 in a study looking for drugs with antispasmodic activity. Trihexyphenidyl is rarely used in the treatment of Parkinsonism, and is not a first line treatment due to significant adverse effects.

2. Methodology

The instrument used was HPLC Shimadzu model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The stationary phase used was ThermosilC18 column (4.0×125mm) 5µDigital weighing balance-Model number BSA224SCW (Ascoset), Sonicator (Enertech)-SE60US, pH meter Model number
AD102U.

Materials and reagents

Risperidone and Trihexyphenidyl hydrochloride were gift samples provided by NATCO Laboratories, Hyderabad, Potassiumdihydrogen, Acetonitrile, Methanol and Water for HPLC were supplied by Merck India Ltd, Mumbai

Method development

Five trials were made by changing the mobile phase ratios and solvents MeOH: H_2O (60:40%v/v)ACN: Methanol (40:60%v/v)ACN: pH 4 buffer (70:30 % v/v) ACN: pH 3 buffer (65:35% v/v) Methanol: Sodium acetate buffer (70: 30 % v/v). Finally, the mobile phase was optimized to Methanol: Sodium acetate buffer (70: 30 % v/v).

Chromatographic conditions

The chromatographic conditions were success fully developed for the separation of Risperidone and Trihexyphenidyl hydrochloride by using ThermosilC18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm.

3. Results & Discussion

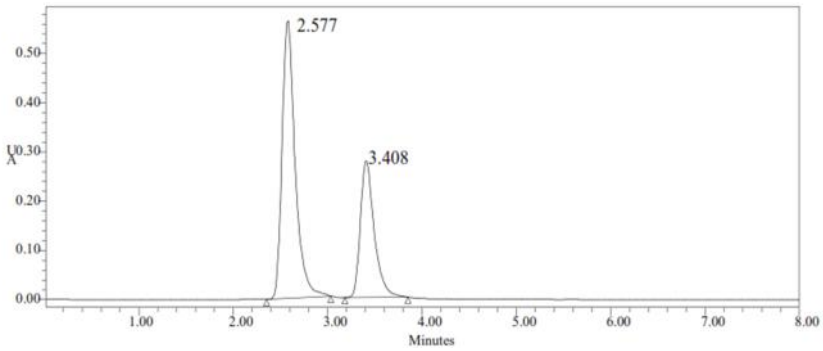


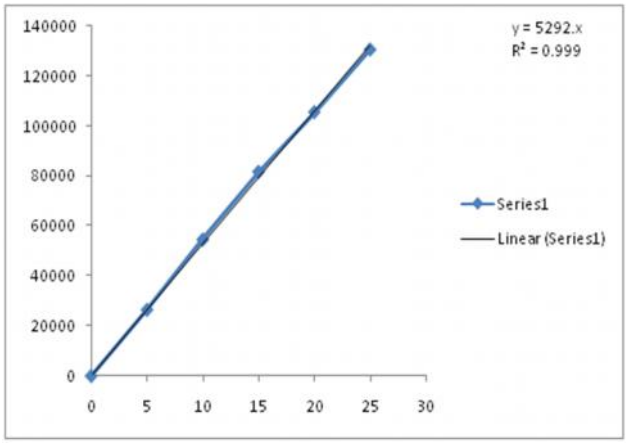
Figure 1 Chromatogram showing sample injection

Table 1 Linearity Results for Risperidone

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	231543
2	II	10 ppm	456277
3	III	15 ppm	684999
4	IV	20 ppm	896124
5	V	25 ppm	1102139
Correlation Coefficient			0.999

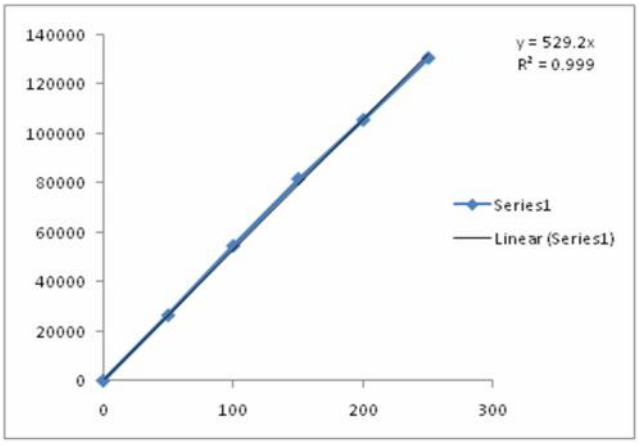
Table 2 Linearity Results for Trihexyphenidyl hydrochloride

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	26472
2	II	100 ppm	54541
3	III	150ppm	81655
4	IV	200 ppm	105541
5	V	250 ppm	130567
Correlation Coefficient			0.999



Risperidone r2 = 0.999

Figure 2 Showing calibration graph for Risperidone



Trihexyphenidyl hydrochloride r^2 = 0.999

Figure 3 Showing calibration graph for Trihexyphenidyl hydrochloride

Table 3 Showing accuracy results for Risperidone

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table 4 Showing accuracy results for Trihexyphenidyl hydrochloride

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	

Table 5: Showing% RSD results for Risperidone

Peak Name: Risperidone

	Peak Name	RT	Area	Height (µV) 1
	Risperidone	2.755	5223559	541538.3
2	Risperidone	2.687	5208511	485548.5
3	Risperidone	2.632	5323569	574440.4
4	Risperidone	2.612	5259147	557413.5
5	Risperidone	2.616	5273463	565020.1
Mean			5257650	
Std. Dev.			45206.4	
% RSD			0.86	

Table 6: Showing %RSD results for Trihexyphenidyl hydrochloride

Peak Name: Trihexyphenidyl hydrochloride

	Peak Name	RT	Area	Height (µV) 1
	Trihexyphe	3.616	2742453	238643.4
2	Trihexyphe	3.634	2762750	271543.5
3	Trihexyphe	3.460	2797670	281711.6
4	Trihexyphe	3.446	2793578	274499.8
5	Trihexyphe	3.437	2778483	276713.0
Mean			2774987	
Std. Dev.			22806.9	
% RSD			0.82	

Intermediate precision/Ruggedness: The intermediate precision study was performed for five injections of Risperidone and Trihexyphenidyl hydrochloride. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

Table 7 Showing results for intermediate precision of Risperidone

Peak Name: Risperidone

	Peak Name	RT	Area	Height (µV)
1	Risperidone	2.756	5698542	539568.1
2	Risperidone	2.688	5682534	536985.4
3	Risperidone	2.633	5695846	539584.1
4	Risperidone	2.613	5689452	534569.8
5	Risperidone	2.617	5636591	534985.5
Mean			5600593	
Std. Dev.			203577.3	
% RSD			0.44	

Table 8 Showing results for intermediate precision of Trihexyphenidyl hydrochloride

Peak Name: Trihexyphenidyl hydrochloride.

	Peak Name	RT	Area	Height (µV) 1
	Trihexyphe	3.617	2624315	231325.6
2	Trihexyphe	3.635	2623598	231315.4
3	Trihexyphe	3.461	2623541	231250.1
4	Trihexyphe	3.447	2624987	231342.6
5	Trihexyphe	3.438	2635698	231765.2
Mean			2626428	
Std. Dev.			5215.78	
% RSD			0.19	

Table 9 Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Risperidone	373625.50	581075863	3.17
Trihexyphenidyl hydrochloride	5772.40	476579210	0.0172

Table 10 Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Risperidone	372727.80	574265980	5.80
Trihexyphenidyl hydrochloride	5761.30	478828490	0.212

Table 11 Showing system suitability results for Risperidone

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	**1**	**4668**	**1.3**
3	1.2	5216	1.4

Table 12 Showing system suitability results for Trihexyphenidyl hydrochloride

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1. 3

13 Showing system suitability results for Risperidone

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	***Actual**	**4668**	**1.3**
3	5 % more	6387	1.4

Table 14 Showing system suitability results for Trihexyphenidyl hydrochloride

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3

2	***Actual**	**6089**	**1.2**
3	5 % more	4817	1.2

4. Conclusion

A new method was established for simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Risperidone and Trihexyphenidyl hydrochloride by using ThermosilC18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. HPLC instrument is Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Risperidone and Trihexyphenidyl hydrochloride was found to be 101.04% and 99.24% respectively. The system suitability parameters for Risperidone and Trihexyphenidyl hydrochloride such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Risperidone, Trihexyphenidyl hydrochloride was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Risperidone and Trihexyphenidyl hydrochloride in API and Pharmaceutical dosage form.

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