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Simple HPLC Method for Simultaneous Estimation of Risperidone and Trihexyphenidyl HCl in Combined Dose Tablet

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Abstract : A rapid and sensitive reverse phase high performance liquid chromatographic method is developed for simultaneous estimation of Risperidone and Trihexyphenidyl HCl in combine dose tablet. Chromatographic condition was on X bridge C18 column using phosphate buffer :Acetonitrile 0.5 ml TEA, 4.5 ± 0.5 pH adjusted by using 0.1N HCl (70:30%) as the mobile phase at a flow rate 0.8 ml/min with detection at 210nm.total run time of less then 20 min. the calibration curve were liner in the range 3-9µg/ml for Risperidone and 2-6 µg/ml for Trihexyphenidyl HCl. The method validated with respect to linearity, precision, accuracy and specificity. The mean recovery for Risperidone and Trihexyphenidyl HCl is 100.14±0.107 and 99.86±0.211, respectively. The utility of procedure is verified by application to the market formulation that was subjected tovarious stress condition. The method separated the two target drug. No chromatographic interference is observed.
Keywords : Risperidone, Trihexyphenidyl hydrochloride, Validation, RP-HPLC.

1. Introduction

Risperidone (RIS) is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives. Chemically it is 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. It is indicated for the acute and maintenance treatment of schizophrenia in adolescents aged 13-17 years and also it is indicated for the short- term treatment of acute manic or mixed episodes associated with Bipolar Disorder in adults and in children and adolescents aged 10-17 years. Trihexyphenidyl (THP) is an antidyskinetic and antiparkinson drug whose IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl)-1-propanol. THP is official in IP. IP suggest a titrimetric assay method for THP. Literature survey revealed that HPLC, UV and HPTLC methods5-28 have been reported for the estimation of RIS and THP individually and with other drugs in pharmaceutical dosage forms. RIS and THP are formulated together in the form of a tablet. Literature survey revealed no method reported for simultaneous determination of the two drugs. The present RP-HPLC method uses simple mobile phase ratio, higher sensitivity and analysis will complete before 20min. Therefore the present study was to determine both drugs concurrently by sensitive, accurate, linearity and range, robustness and precise RP-HPLC method for routine analysis.

2. Materials and Methods

2.1 Chemicals

Acetonitrile (HPLC grade), TEA (AR grade), Phosphate buffer, 0.1N HCl were purchased from Emcure pharmaceutical limited, Ahmedabad, Gujarat, India. All active pharmaceutical ingredients(API) RP reference standard obtained from Emcure pharmaceutical, Ahmedabad, Gujarat, India(99.5-100% purity).

2.2 Equipment

The chromatography was performed on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010 CHT) equipped with PDA detector, Phenomenex (Torrance, CA) Column:X-Bridge C18 (150 mm x 4.6mm,5 µm)) was used as stationary phase.

2.3 Selection of Wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. Standard solution of Risperidone (6 µg/ml) and Trihexyphenidyl HCL (4 µg/ml) were prepared using their working standard solution using mobile phase as a solvent. Each solution was scanned between 200-400 nm in a Shimadzu UV-1800, UV-Visible double beam Spectrophotometer at a fast scanning speed. The spectra were derivatized to first order and overlay spectra of both the drugs shows optimum absorbance at 210 nm. So we have selected the wavelength 210 nm for separation of both the drugs.

2.4 Selection of mobile phase

Depending on the solubility of the drugs the trials for selection of mobile phase was done.

2.5 Sample preparation

2.5.1 Preparation of solutions:

2.5.1.1 Preparation of standard stock and diluted solutions of Risperidone:

An accurately weighed quantity of Risperidone 30 mg was transferred to the 100 mL volumetric flask and dissolved in 70 ml of 0.1N HCl and sonicate for 5 min. The volume was made up to the mark with 0.1N HCl mix well. (300 µg/mL).

Diluted standard –

To prepare diluted standard solution, 2 ml above stock solution diluted to 100 ml with 0.1 N HCl to get 6 µg/mL.

2.5.1.2 Preparation of standard stock and diluted solutions of Trihexyphenidyl HCl:

An accurately weighed quantity of Trihexyphenidyl HCl 20mg was transferred to the 100mL volumetric flask and dissolved in 70 ml of 0.1N HCl and sonicate for 5 min . The volume was made up to the mark with the 0.1N HCl (200 µg/mL).

Diluted standard –

To prepare diluted standard solution, 2 ml above stock solution diluted to 100 ml with 0.1 N HCL to get 4 µg/mL.

2.5.1.3 Preparation of combined standard stock solution of Risperidone and Trihexyphenidyl HCl:

An accurately weighed quantity of Risperidone 30mg and Trihexyphenidyl HCl 20 mg was transferred to the 100 mL volumetric flask and dissolved in 70 ml of 0.1N HCl and sonicate for 5 min. The volume was made up to the mark with 0.1N HCl mix well.

Diluted standard –

To prepare diluted standard solution, 2 ml above stock solution diluted to 100 ml with 0.1 N HCl to get 6 µg/mL and 4 µg/mL for Risperidone and Trihexyphenidyl HCl respectively.

2.6 Preparation of calibration curve

An accurately weighed quantity of Risperidone 30mg and Trihexyphenidyl HCl 20 mg was transferred to the 100 mL volumetric flask and dissolved in 70 ml of 0.1N HCl and sonicate for 5 min. The volume

was made up to the mark with 0.1N HCl mix well to get 300 µg/mL and 200 µg/mL for Risperidone and Trihexyphenidyl HCl respectively.

From the above stock, aliquot of 1, 1.5, 2, 2.5, and 3ml were transferred in a series of 100 ml volumetric flask and the volume was adjusted up to the mark with 0.1N HCl to give the concentration of 3, 4.5, 6,7.5 and 9 µg/ml for Risperidone and 2, 3, 4, 5 and 6 µg/ml for Trihexyphenidyl HCl.

2.7 System suitability testing (n=6)

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. For system suitability test prepared the mixture solution 6 µg/ml for Risperidone and 4 µg/ml for Trihexyphenidyl HCl and taken the six chromatogram and observe the Area, Retention time, Resolution, Theoretical plate and Tailing factor. % RSD was Calculated.

2.8 Method Validation:

As per ICH guidelines Q2R1, the method validation parameters studied were linearity and range, accuracy, precision and specificity.

2.8.1 Linearity and range (n=2):

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range 3-9 µg/ml (3, 4.5, 6, 7.5 and 9 µg/ml) for Risperidone and in the range of 2-6 µg/ml (2, 3, 4, 5 and 6 µg/ml). The plot of peak area against concentration was plotted.

Linearity range was established through consideration of required practical range and according to each drug concentration present in the pharmaceutical product, to give accurate, precise and linear results.

2.8.2Accuracy (% Recovery) (n=3):

Accuracy studies were carried out to determine suitability and reliability of proposed method. It was carried out by the standard addition method in which, known amounts of standards samples of Risperidone and Trihexyphenidyl HCl at 50 %, 100 % and 150 % levels were added to the pre-analysed samples. Known amount of standard solutions of Risperidone (0, 3, 6 and 9 µg/ml) and Trihexyphenidyl HCl (0, 2, 4 and 6 µg/ml) were added to a pre-quantified sample solution of Risperidone and Trihexyphenidyl HCl (6 and 4 µg/ml, respectively) the recovered amounts of Risperidone and Trihexyphenidyl HCl were calculated at each level and % Recovery was reported.

2.8.3Precision:

2.8.1.1Repeatability (n = 6):

From the combine stock solution , an aliquot of 2 ml was transferred to a separate 100 ml volumetric flask and diluted up to mark with 0.1 N HCl such that it gives the concentration of 6 µg/ml of Risperidone and 4 µg/ml of Trihexyphenidyl HCl. The solution was injected into the system. The peak areas of Risperidone and Trihexyphenidyl HCl were observed. The procedure was repeated six times and % RSD was reported.

2.8.4Specificity (n=3):

In the case of assay, demonstration of specificity is required to show that the procedure is unaffected by the presence of impurities or excipients. Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparison of the chromatograms of -

i. Standard sample solutions of Risperidone and Trihexyphenidyl HCl.
ii. Blank (0.1 N HCl)
iii. Sample solution of Risperidone and Trihexyphenidyl HCl.

2.8.5 Robustness (n=3)

The robustness of method was established by introducing small changes in various parameters like detection wavelength and flow rate. The changes made in wavelength and flow rate were ± 2 nm (208, 210 and 212 nm), ± 0.2 ml/min (0.8, 1.0 and 1.2 ml/min) respectively. The robustness of the method was evaluated by calculating % RSD.

3. Results and Discussion

3.1 Selection of wavelength:

The mode of detection was absorption in the UV region and wavelength selected for detection was 210 nm. The wavelength was selected on the bases that the solutions of Risperidone and Trihexyphenidyl HCl show considerable absorbance in UV-visible spectrophotometer at this wavelength . Both the drugs showed typical peak nature and peak symmetry at 210 nm in HPLC.

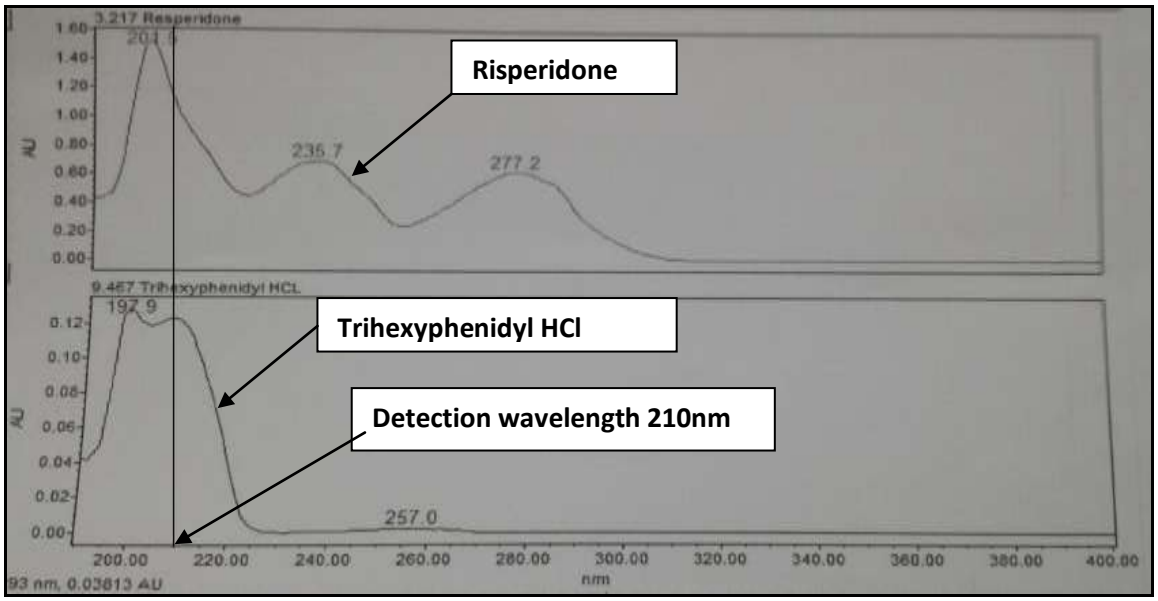

Fig. 1 Overlain UV spectra showing detection wavelength

3.2 Selection of mobile phase:

For the selection of the mobile phase, trials were done to find the best condition for the separation of Risperidone and Trihexyphenidyl HCl with an effective resolution and peak symmetry.

The trials for selection of mobile phase are shown in table.

Table 1 Trials for selection of mobile phase (C18 Column)

Sr. No.	Mobile phase	Ratio	RT		Remarks
			Risperidone	Trihexyphenidyl HCl	
1	Buffer: ACN Flow rate-0.8ml/min	50:50	1.990	12.709	Intensity of solvent peaks are high
2.	Buffer: ACN Flow rate-0.8ml/min	60:40	2.923	15.480	Interference of solvent peak
3.	Buffer: ACN Flow rate-0.8ml/min	80:20	6.280	21.430	High retention time and high resolution
4.	Buffer: ACN Flow rate-1.0ml/min	70:30	3.101	13.720	Fronting is observed and peaks of solvent are observed.

5	Buffer: ACN Flow rate-0.6ml/min	70:30	5.720	19.830	Fronting is observed and high retention time
6	Buffer: ACN Flow rate-0.8ml/min	70:30	4.693	17.075	Resolution, theoretical plates and all are good

The combination of ACN and phosphate Buffer was selected as mobile phase as final mobile phase as it was found to be ideal to resolve Risperidone (RT 4.963) and Trihexyphenidyl HCl (RT 17.075) optimum resolution and good symmetry. The chromatographic conditions kept as follows:

3.3 Chromatographic conditions

Table 2 Chromatographic conditions

Sr. No.	Parameters	
1	Pumps	i)Mode of chromatography:Reversed phase
		ii) Mode of elution:Isocratic
		iii) Flow rate:0.8 ml/min
2	Detector	i) Type:PDA detector
		ii) Lamp:D2 lamp
		iii) Wavelength:210 nm
3	Other Parameters	i)Column:X-Bridge C18 (150 mm x 4.6mm,5 µm)
		ii) Sample volume:50 µL
		iii) Column Temperature:27℃
		iv) Run time:20 min
		v) Mobile phase**:**Buffer : Acetonitrile 0.5 ml TEA, 4.5 ±0.5 pH adjusted by using 0.1N HCl (70:30 v/v).
		vi)Retention Time: 4.8 Min Risperidone & 16.2 Min Trihexyphenidyl HCL
		vii) Resolution: 17.075

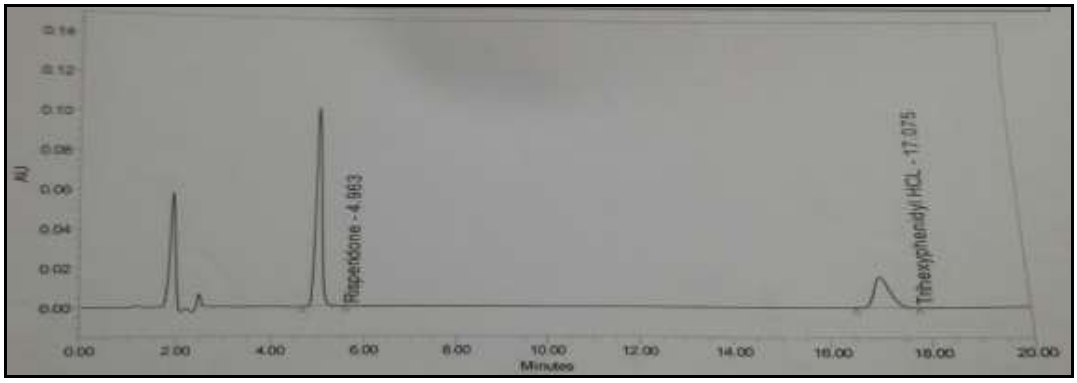

Fig. 2 Chromatogram of mixture of Risperidone and Trihexyphenidyl HCl

3.4 System suitability Tasting

System suitability testing is the checking of system to ensure system performance before or during the analysis. Parameter were checked. In this, solution of Risperidone (6 µg/ml) and Trihexyphenidyl HCl (4 µg/ml) was prepared as per dosage ratio. Parameter such as theoretical plate, tailing factor, resolution and reproducibility (%RSD, retention time and area for six replication) were determine and compared against the specification set for the method result show in table.

Table 3 Results of System suitability

System suitability parameters	Risperidone Mean ± SD	Trihexyphenidyl HCl Mean ± SD
Area	885814.7±1864.32	342213.5±1673.08
Retention time	4.960±0.0285	17.071±0.0379
Theoretical plates	7738±68.27	13092±87.6
Tailing factor	1.200±0.012	1.320±0.061
Resolution	16.907±0.0619	-

***(n=6)**

3.5 Method validation

3.5.1 Linearity and range (n=2):

Linear relation was obtained between mean peak area and concentration of the drug in the range of 3-9 µg/ml for Risperidone and 2-6 µg/ml for Trihexyphenidyl HCl. The data of the peak areas obtained with the respective concentrations in µg/ml are shown in table and for Risperidone and Trihexyphenidyl HCl respectively. The linearity curves, the straight line equation and correlation coefficient for Risperidone and Trihexyphenidyl HCl are shown in Fig. and Fig. respectively.

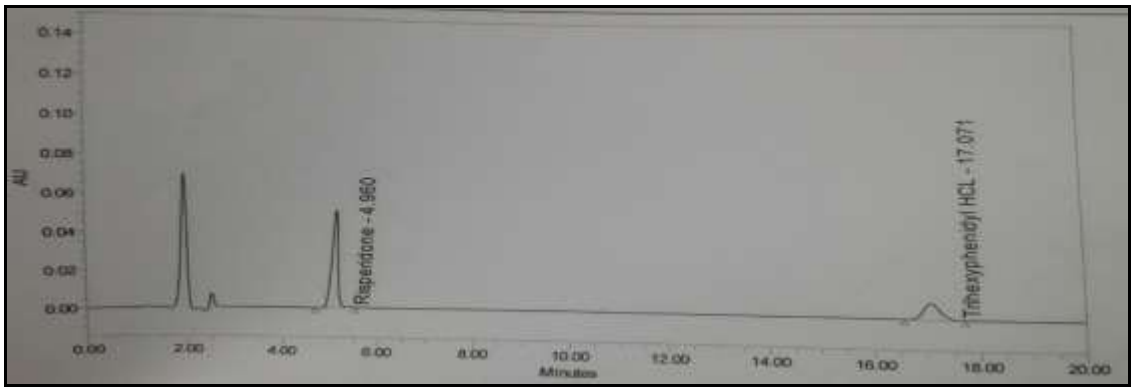

Fig.3 Chromatogram for standard solution of Risperidone (3 µg/ml) and Trihexyphenidyl HCl(2 µg/ml)

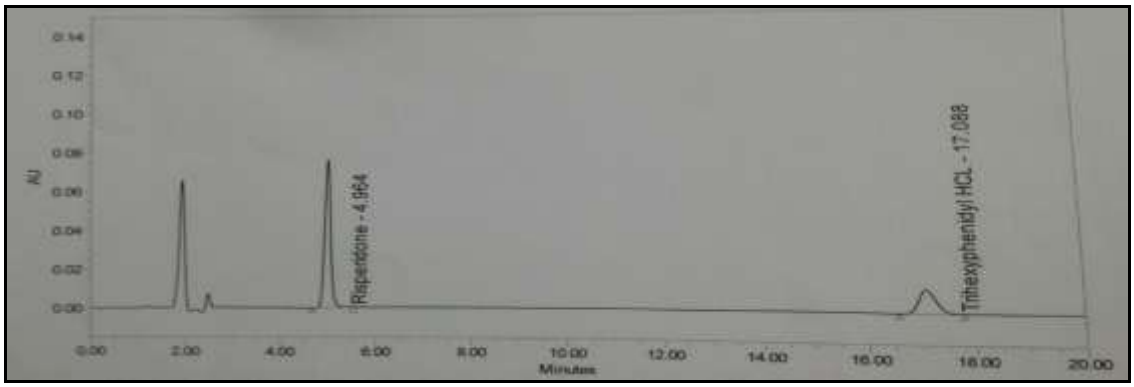

Fig.4 Chromatogram for sstandard solution of Risperidone (4.5 µg/ml) and Trihexyphenidyl HCl (3 µg/ml)

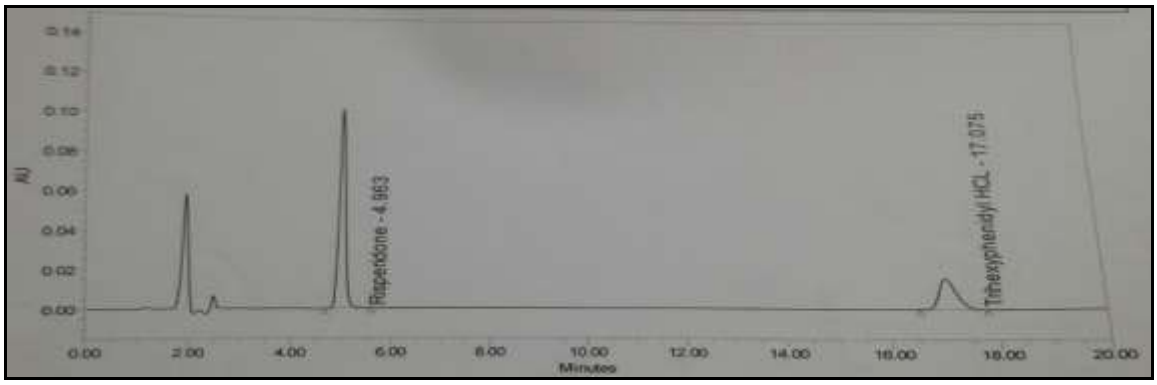

Fig.5 Chromatogram for standard solution of Risperidone (6 µg/ml) and Trihexyphenidyl HCl (4 µg/ml)

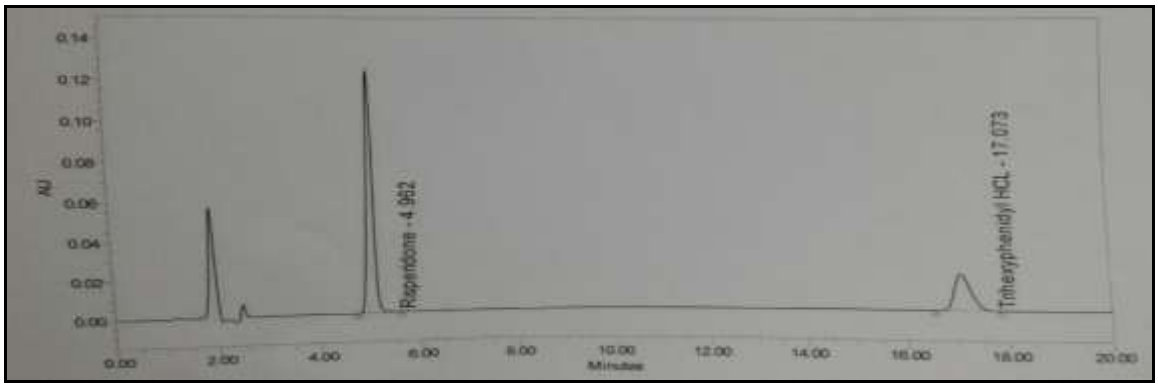

Fig.6 Chromatogram for standard solution of Risperidone (7.5 µg/ml) and trihexyphenidyl HCl (5 µg/ml)

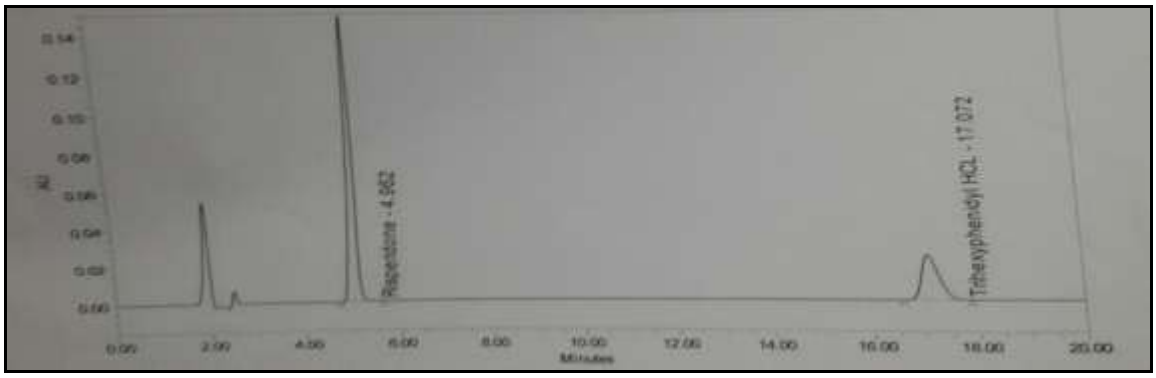

Fig.7 Chromatogram for standard solution of Risperidone (9 µg/ml) and Trihexyphenidyl HCl (6 µg/ml)

Table 4 Linearity data for Risperidone

Conc.(µg/ml)	Mean Area ± SD	%RSD
0	0 ± 0	0
3	442403 ± 0061.0	0.013
4.5	653710 ± 0502.5	0.076
6	882840 ± 1216.0	0.137
7.5	1086997 ± 0159.0	0.014
9	1302184 ± 1470.5	0.112

***(n=2)**

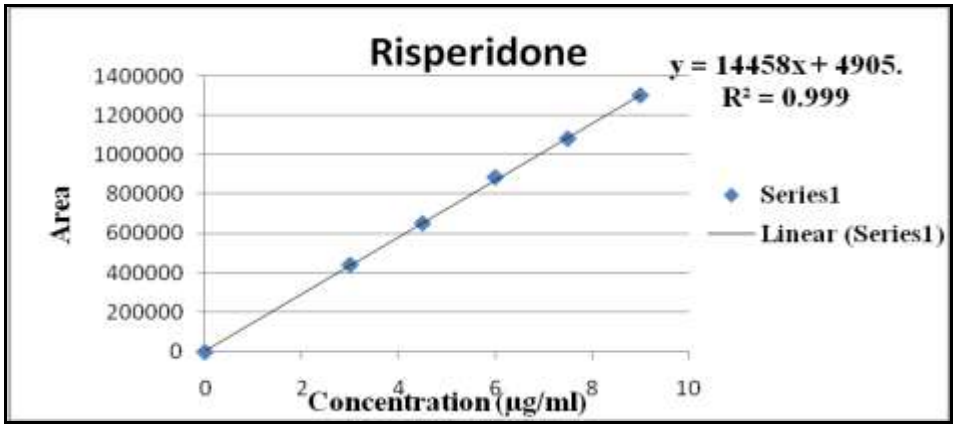

Fig. 8 Calibration curve for Risperidone

Table 5 Linearity data for Trihexyphenidyl HCl

Conc. (µg/ml)	Mean Area ± SD	%RSD
0	0 ± 0	0
2	172968 ± 126.0	0.072
3	253039 ± 109.5	0.043
4	342265 ± 203.5	0.059
5	417274 ± 059.5	0.014
6	502265 ± 802.5	0.159

***(n=2)**

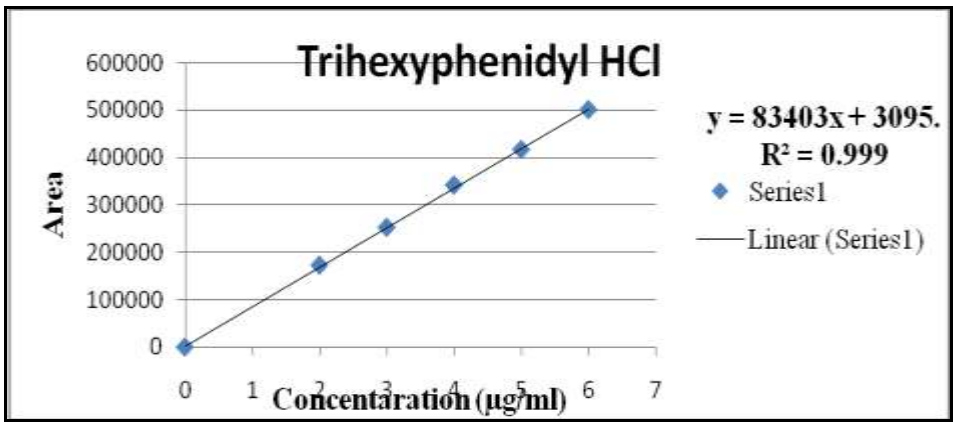

Fig.9 Calibration curve for Trihexyphenidyl HCl

3.5.2 Accuracy:

The results of the accuracy study are shown below table. The result showed that the % recoveries for Risperidone and Trihexyphenidyl HCl were found to be 99.55- 101.16 % and 99.66 – 100.25 % respectively.

Table 6 Accuracy data for Risperidone

Level (%)	Target conc. (µg/ml)	Std. added (µg/ml)	Total amount (µg/ml)	Amount found mean ± SD	% Recovery ± SD	% RSD
50	3	1.5	4.5	4.48±0.04	99.55±0.109	0.109
100		3	6	6.07±0.01	101.16±0.272	0.266
150		4.5	7.5	7.48±0.01	99.73±0.238	0.239

***(n=3)**

Table 7 Accuracy data for Trihexyphenidyl HCl

Level (%)	Target conc. (µg/ml)	Std. added (µg/ml)	Total amount (µg/ml)	Amount found mean ± SD	% Recovery ± SD	% RSD
50	2	1	3	2.99±0.01	99.66±0.596	0.62
100		2	4	4.06±0.02	100.25±0.670	0.66
150		4	6	5.98±0.02	99.68±0.447	0.44

***(n=3)**

3.5.3 Precision:

3.5.3.1 Repeatability:

The repeatability data for Risperidone and Trihexyphenidyl HCl were shown in table. The % RSD for Risperidone and Trihexyphenidyl HCl were found to be 0.21 and 0.48 respectively.

Table 8 Repeatability data for Risperidone and Trihexyphenidyl HCl

Concentration of Risperidone (µg/ml)	Area of Risperidone	Concentration of Trihexyphenidyl HCl (µg/ml)	Area of Trihexyphenidyl HCl
6	887954	4	342339
	887127		342189
	885116		341113
	882684		340895
	884905		345415
	884963		341330
Mean response	885814.7	Mean response	342213.5
SD	1864.32	SD	1673.08
% RSD	0.21	% RSD	0.48

***(n=6)**

3.5.4 Specificity:

The specificity was determined by the comparison of the Chromatograms of

a) Standard sample solutions of Risperidone and Trihexyphenidyl HCl
b) Blank (0.1 N HCl) ,
c) Sample solution of Risperidone and Trihexyphenidyl HCl.

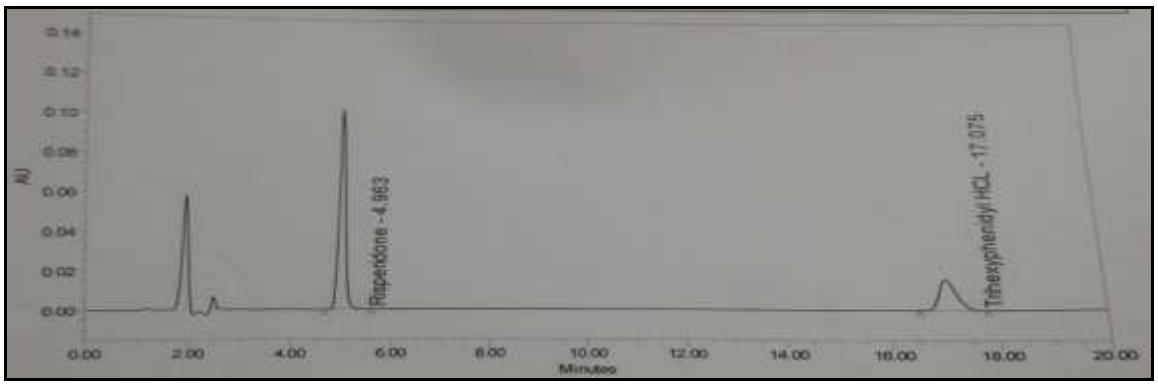

Fig.10 Chromatogram for standard sample solution of Risperidone (6 µg/ml) and Trihexyphenidyl HCl (4 µg/ml)

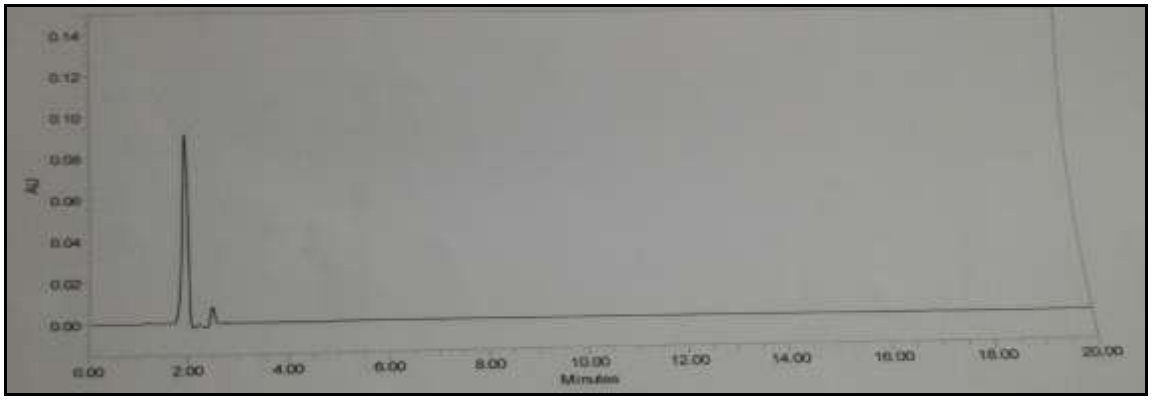

Fig.11 Chromatogram of blank

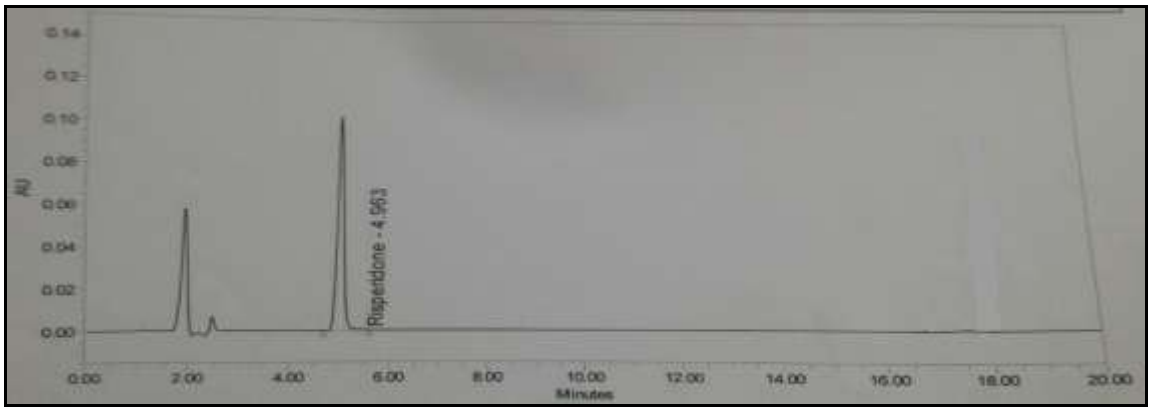

Fig.12 Chromatogram for standard solution of Risperidone (6 µg/ml)

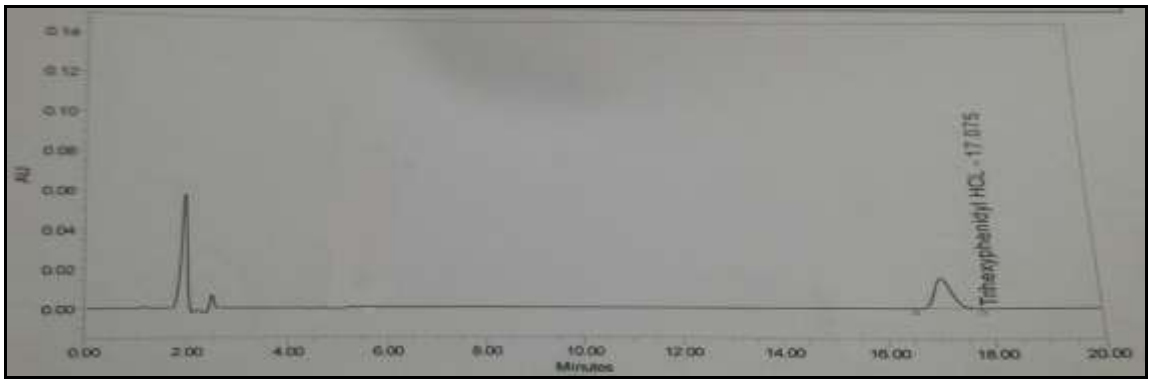

Fig. 13Chromatogram for standard solution of Trihexyphenidyl HCl (4 µg/ml)

3.5.5 Robustness:

The change was done in flow rate and pH of buffer. % RSD for area was calculated which found to be less than 2. The change in flow rate data and change in pH of buffer data are shown in table and respectively.

Table 9 Results for change in flow rate

Flow rate (ml/min)	Risperidone(6 µg/ml)		Trihexyphenidyl HCl (4 µg/ml)	
	Mean Area ± S.D.	% RSD	Mean Area ± S.D.	% RSD
0.6	885116±1205.09	0.15	341113±2279.45	0.77
`0.8	882684±1067.77		340895±2450.12	
1.0	884905±1478.19		345415±2849.36	

***(n=2)**

Table10 Results for change in wavelength

Wavelength (nm)	Risperidone(6 µg/ml)		Trihexyphenidyl HCl (4 µg/ml)	
	Mean Area ± S.D.	% RSD	Mean Area ± S.D.	% RSD
208	886478±1575.35	0.03	341245±2449.89	0.01
210	886102±1589.48		341306±2484.48	
212	886605±1622.18		341255±2519.78	

***(n=3)**

3.5.6 Summary of Validation parameters:

The summary of validation parameters shown in table

Table 11 Summary of Validation parameters

Sr No.	Parameters		Risperidone	Trihexyphenidyl HCl
1	Linearity (n=2)		3-9 µg/ml	2-6 µg/ml
2	Correlation coefficient		0.9997	0.9996
3	Accuracy (% recovery) (n=3)		99.55 - 101.16 %	99.66 - 100.25 %
	Precision (% CV)			
4	Repeatability (n=6)		0.21	0.48
5	Robustness	Change in flow rate	0.15	0.77
		Change in wavelength	0.03	0.01

4. Conclusion

Thus, it can concluded that the proposed method is sufficiently sensitive, reproducible, and specific in analysis of Risperidone and Trihexyphenidyl HCl in combine dose tablet within short analysis term (less then 20 min). The proposed HPLC method validated by evaluation of the evaluation parameter. The accuracy, precision, repeatability, linearity and range, robustness, resolution for this technique were obtained. Assay parameter used in this study showed better resolution of the target analyte with proper symmetry. The method also separate the degradation product from the target analyte and thus allow estimation in their presence.

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