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Research Article

Stability Indicating Liquid Chromatographic Method for Estimation of Trihexyphenidyl Hydrochloride and Risperidone in Tablet Formulation: Development and Validation Consideration

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This paper describes validated reverse phase high-performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of trihexyphenidyl hydrochloride (THP) and risperidone (RSP) in the pure powder form and in combined tablet dosage form. The HPLC separation was achieved on a core shell C18 (100 mm length × 4.6 mm, 2.6 μm particle size) using methanol : ammonium acetate buffer 1% (85 : 15 v/v; pH-6.5) as mobile phase and delivered at flow rate of 0.8 mL/min. The calibration plot showed good linear relationship with $r^2 = 0.997 \pm 0.001$ for THP and $r^2 = 0.998 \pm 0.001$ for RSP in concentration range of 50–175 μg/mL and 50–175 μg/mL, respectively. LOD and LOQ were found to be 0.40 and 1.29 μg/mL for THP and 1.24 and 3.92 μg/mL for RSP. Assay of THP and RSP was found to be 100.16 ± 0.03% and 99.83 ± 0.02%, respectively. THP and RSP were subjected to different stress conditions (acidic, basic, oxidative, thermal, and photolytic degradation). The degraded product peaks were well resolved from the pure drug peak. The method was successfully validated as per the ICH guidelines. The developed RP-HPLC method was successfully applied for the estimation of THP and RSP in tablet dosage form.

1. Introduction

Trihexyphenidyl hydrochloride, chemically known as 1-cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol, is one of the centrally acting muscarinic antagonists used for the treatment of parkinsonian disorders and drug-induced extrapyramidal movement disorders and as an antispasmodic. Risperidone (RSP), chemically known as 3-{2-[4-(6-fluoro-1,2-benzoxazol-3-yl) piperidin-1-yl]ethyl}-2-methyl-4H, 6H, 7H, 8H, 9H-pyrido [1, 2-a] pyrimidin-4-one, is benzisoxazole derivative and an atypical antipsychotic drug with high affinity for 5-hydrotryptamine (5-HT) and dopamine D2 receptors. It is used primarily in the management of schizophrenia, inappropriate behaviour in severe dementia, and manic episodes associated with bipolar I disorder. RSP is effective in treating the positive and negative symptoms of schizophrenia owing to its affinity for its "loose" binding affinity for dopamine D2 receptors and additional 5-HT antagonism compared to first generation antipsychotics, which are strong, nonspecific dopamine D2 receptor antagonists.

Both trihexyphenidyl hydrochloride (THP) and RSP are official in pharmacopoeia. A literature survey revealed that high-performance liquid chromatography (HPLC) for determination of THP and RSP in tablet [1–3] was reported. Further, HPLC method for estimation of RSP in plasma [4], bulk drug and pharmaceutical formulation [5], tablet [6–8], HPLC-MS/MS in plasma [9], and TLC densitometry method [10, 11] were reported. However, to the best of our knowledge, no article related to stability indicating method for estimation of THP and RSP in tablet formulation was reported. The present study describes the development and validation of a stability indicating RP-HPLC method for the simultaneous

quantitative estimation of THP and RSP in pure powder and marketed tablet formulation. The developed method was successfully applied for the routine analysis of THP and RSP in tablet formulation.

2. Experimental

2.1. Materials. The pure THP and RSP were gratis from AUM Research Laboratories (Gandhinagar, India). HPLC-grade methanol, water, and ammonium acetate were purchased from E. Merck Ltd. (Mumbai, India).

2.2. Apparatus. The method was developed using UltiMate 3000 HPLC series (Thermo Scientific) equipped with SPD 20A detector, isocratic pump system, autoinjector, and core shell C-18 SunShell Technology column (100 cm × 4.6 mm id, 2.6 μ particle size).

2.3. Chromatographic Condition. The mobile phase consisted of methanol : ammonium acetate buffer (pH 6.5) in ratio of 85 : 15 which was filtered through a Nylon 0.45 μm membrane filter and degassed before use. The mobile phase was pumped at a flow rate of 0.8 mL/min. The amount of drug solution injected was 20 μL. The UV light absorption of analyte in elution was detected at a wavelength of 215 nm.

2.3.1. Preparation of Ammonium Acetate Buffer. Accurately, weighed 38.5 gm of ammonium acetate was transferred into a 500 mL beaker and dissolved in 400 mL HPLC grade water. The pH of the solution was adjusted to 6.5 by dropwise addition of glacial acetic acid. This solution was transferred into a 500 mL volumetric flask and diluted up to the mark using HPLC-grade water.

2.4. Preparation of THP Standard Stock Solution. An accurately weighed quantity of pure THP 50 mg was transferred into 50 mL volumetric flask and made up the volume with mobile phase to obtain the final concentration of 1000 μg/mL.

2.5. Preparation of RSP Standard Stock Solution. An accurately weighed quantity of pure RSP 50 mg was transferred into 50 mL volumetric flask and made up the volume with mobile phase to obtain the final concentration of 1000 μg/mL.

2.6. Sample Preparation for Determination of THP and RSP in Tablet Formulation. Tablet was powdered and weight equivalent to 25 mg of RSP and 25 mg of THP was transferred into 25 mL volumetric flask. About 10 mL of mobile phase was added and sonicated for 10 minutes. The solution was cooled to the room temperature and made up the volume with mobile phase. The solution was filtered through Whatman filter paper (grade 42); filtrate was collected after discarding the first few mL. One mL of this filtrate was transferred to 10 mL volumetric flask and diluted to 10 mL with mobile phase to obtain the final concentration of 100 μg/mL of both of the drugs.

2.7. Method Validation

2.7.1. System Suitability Test. The system suitability test was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using six-replicate injection of a reference solution of THP and RSP. The parameters measured were retention time, number of theoretical plates, and tailing factor.

2.7.2. Specificity. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used formulation excipients were spiked into a preweighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined [12–16].

2.7.3. Linearity Study. Calibration curves were constructed by plotting peak area versus concentrations [12–16] of THP and RSP, respectively, and regression equations were calculated. The calibration curves were plotted over the five different concentrations in range 50–175 μg/mL of THP and 50–175 μg/mL of RSP.

2.7.4. Precision. Precision of the analytical method was studied by analysis of three replicates of standard solution in three different concentrations. It was demonstrated by repeatability (intraday precision) and intermediate precision (interday precision) of the solutions [12–16].

2.7.5. Detection Limit and Quantitation Limit. The limit of detection (LOD) is the lowest concentration of an analyte that can be reliably differentiated from background levels; limit of quantification (LOQ) of an individual procedure is the lowest amount of analyte that can be quantitatively determined [12–16].

2.7.6. Accuracy (% Recovery). The accuracy of the method was determined by calculating the recovery of THP and RSP by the standard addition method. Three different solutions were prepared with a known addition of pure THP and RSP to give a concentration range of 50%–175% of that in a test preparation.

2.7.7. Robustness. Robustness of the method was studied by changing the temperature, flow rate of mobile phase, and the pH of mobile phase [12–16].

3. Forced Degradation Study

3.1. Acid Hydrolysis. Standards of THP (100 mg) and RSP (100 mg) were accurately weighed and transferred into three sets of 250 mL round bottom flasks. About 20 mL of 2 N HCl was added to all flasks and refluxed on heated mantle for 45 min at 80°C.

3.2. Alkali Hydrolysis. Standards of THP (100 mg) and RSP (100 mg) were accurately weighed and transferred into three

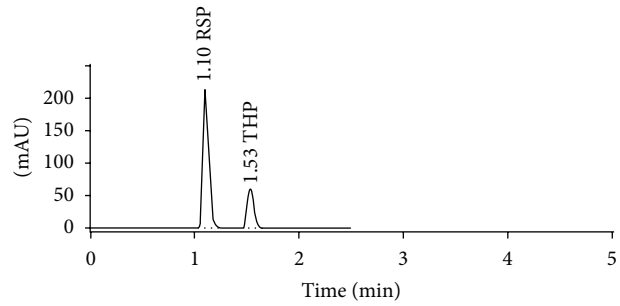

Figure 1: Chromatogram of THP and RSP in methanol : ammonium acetate (85 : 15 v/v, pH 6.5) at flow rate 0.8 mL/min, at 215 nm.

sets of 250 mL round bottom flasks. About 20 mL of 1 N NaOH was added to all flasks and refluxed on heated mantle for 60 min at 80°C.

3.3. Oxidative Degradation. Standards of THP (100 mg) and RSP (100 mg) were accurately weighed and transferred into three sets of 250 mL round bottom flasks. About 20 mL of 6% H_2O_2 was added to all flasks and refluxed on heated mantle for 2 hr at 80°C.

3.4. Thermal Degradation. Standard of THP (100 mg) and RSP (100 mg) was accurately weighed and transferred into Petri dish individually and spread into thin layer with spatula; this Petri dish was placed in the hot air oven for 1 hour at 80°C.

Heated drug samples of THP (100 mg) and RSP (100 mg) were taken into 100 mL volumetric flask and dissolved in mobile phase. Volume was made up to the mark using mobile phase. 1 mL of above sample was transferred into 10 mL volumetric flask and diluted up to the mark using mobile phase. It was filtered through 0.45 μ cellulose acetate filter and filtrate was used for chromatographic analysis.

3.5. Photolytic Degradation. Standard of THP (100 mg) and RSP (100 mg) was accurately weighed and transferred into Petri dish individually and spread into thin layer with spatula; this Petri dish was put inside the UV Chamber for 1 hour.

UV-exposed drugs samples of THP (100 mg) and RSP (100 mg) were taken into 100 mL volumetric flask, and dissolved in mobile phase. Volume was made up to the mark using mobile. 1 mL of above sample was taken into 10 mL volumetric flask in a few mL of mobile phase and sonicated for 10 min; this solution was cooled to the room temperature and made up the volume up to the mark using mobile phase. It was filtered through 0.45 μ cellulose acetate filter and filtrate was used for chromatographic analysis.

4. Results and Discussion

4.1. HPLC Method Development and Optimization. To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of THP and RSP with good peak symmetry and steady baseline was obtained with the mobile phase composition of methanol : ammonium acetate buffer (85 : 15 v/v, pH 6.5). Quantitation of analytes was carried out based on peak area obtained with UV detection at 215 nm wavelength. Complete resolution of the peaks with clear baseline separation was obtained (Figure 1).

Table 1: Summary of HPLC method development and validation.

Parameters	Data obtained	
	RSP	THP
Theoretical plate	3508 ± 230	2670 ± 190
Retention time (min)	1.10 ± 0.01	1.53 ± 0.01
Correlation coefficient	0.998 ± 0.01	0.997 ± 0.01
Linearity ($n = 6$)	50–175 (μg/mL)	50–175 (μg/mL)
Precision (% RSD)		
Intraday	0.047	0.368
Interday	0.273	0.561
Repeatability ($n = 6$)	0.181	0.656
Accuracy (% recovery)	99.42–100.26%	99.06–100.42%
LOD	0.40 (μg/mL)	1.24 (μg/mL)
LOQ	1.29 (μg/mL)	3.92 (μg/mL)

4.2. Method Validation. The linearity data described in the present study demonstrate an acceptable linearity for THP and RSP. The calibration plot showed good linear relationship with $r^2 = 0.997 \pm 0.001$ for THP and $r^2 = 0.998 \pm 0.001$ for RSP in concentration range of 50–175 μg/mL and 50–175 μg/mL, respectively. The precision, evaluated as the repeatability of the method, was studied by calculating the RSD value. The intermediate precision composed of two parameters: intraday ($n = 3$) and interday ($n = 3$) in which the RSD value for intraday precision for THP was 0.368 ± 0.112 and 0.047 ± 0.20 for RSP. In interday precision RSD value for THP was 0.273 ± 0.130 and 0.561 ± 0.212 for RSP. The RSD value for intermediate precision was found to be <2%, which indicates that the proposed method is reproducible. The LOD and LOQ were determined from slops of linear regression curves. LOD and LOQ were found to be 0.40 and 1.29 μg/mL for THP and 1.24 and 3.92 μg/mL for RSP. The accuracy was assessed by the recovery experiments that were performed by the standard addition method. The recoveries obtained were 100.22 ± 0.74 and 99.91 ± 0.34 for THP and RSP, respectively. The high value indicates that the method is accurate. Retention time variation was found to be <1%. Hence, the method was found to be robust for estimation of THP and RSP. Excipients used in the specificity studies did not interfere with the estimation of either of drugs by the proposed method. Hence, the method was found to be specific for estimation of THP and RSP. All the parameters of system suitability come within acceptable range. Results indicate that the system is suitable for the analysis intended (Table 1).

4.3. Method Application. The proposed RP-HPLC method was applied for the determination of THP and RSP in tablet formulation. Assay of THP and RSP was found to be 100.16 ± 0.03 and 99.83 ± 0.02%, respectively. The results demonstrate

Table 2: Assay results of tablet formulation by RP-HPLC method.

	Name of drug		Amount found		% Assay ± SD (n = 3)	
	RSP	THP	RSP	THP	RSP	THP
Tablet	2 mg	2 mg	2.02	1.99	99.83 ± 0.02	100.16 ± 0.03
	2 mg	2 mg	1.98	2.04		
	2 mg	2 mg	1.99	1.98		

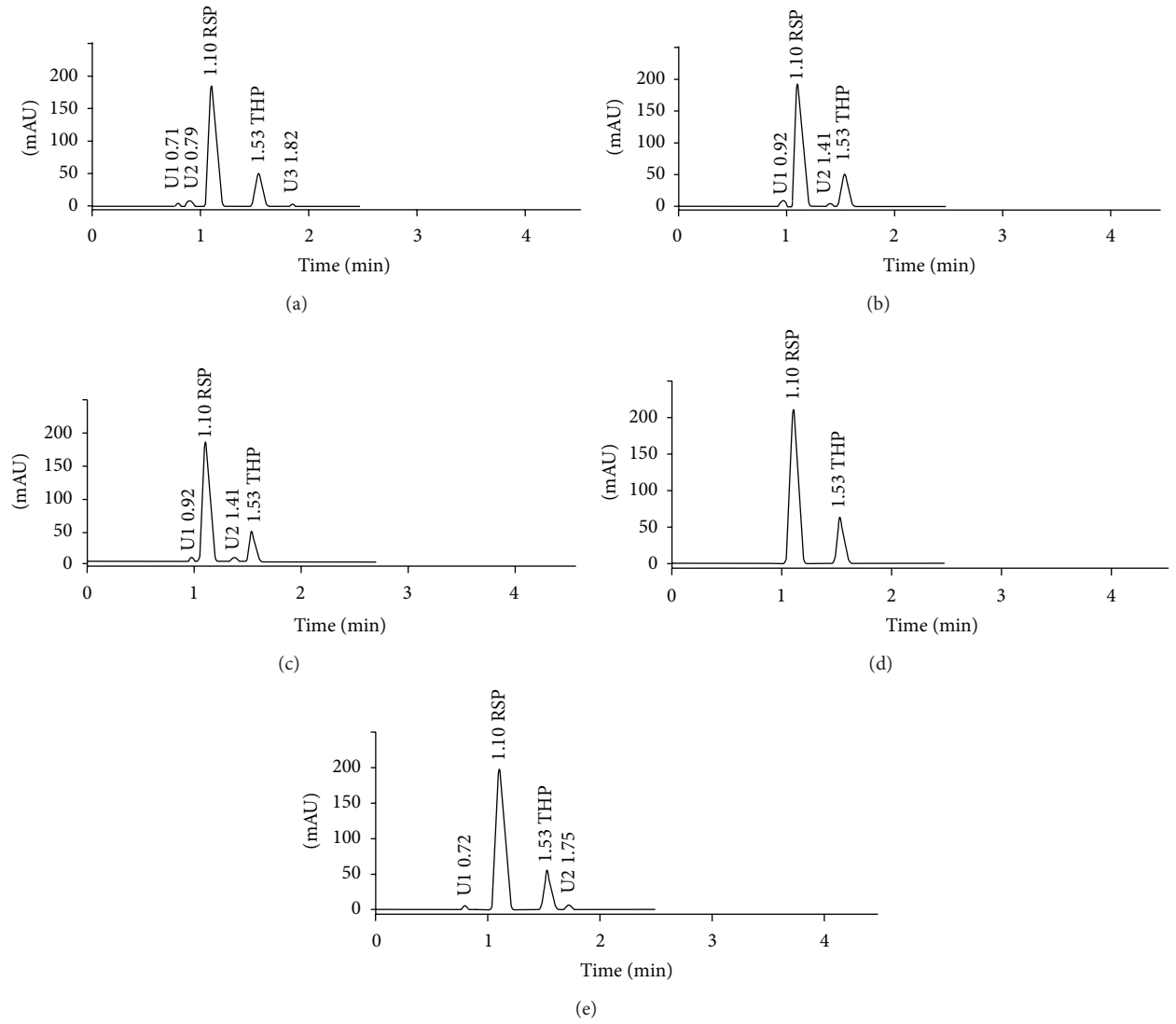

Figure 2: Chromatogram of THP and RSP degradation. (a) Acidic degradation. (b) Basic degradation. (c) Oxidative degradation. (d) Thermal degradation. (e) Photolytic degradation.

the quality of the analyzed pharmaceutical samples and the applicability of the method for QC analysis (Table 2).

4.4. Forced Degradation Study. Forced degradation is performed to provide stability indicating properties of an analytical method. The result from stress testing studies indicated that the method was specific for THP and RSP. Both drugs were found to be unstable for thermal degradation. Chromatograms of mixture drugs after degradation under various force conditions are shown in Figure 2. Percentage of degradation of pure drugs is mentioned in Table 3.

5. Conclusion

RP-HPLC method for estimation and quantification of THP and RSP was successfully developed and validated according to the ICH guidelines. The validation results showed that this method was specific, sensitive, linear, precise, accurate,

Table 3: Degradation of THP and RSP at different stress conditions.

Stress condition/strength/duration	Drugs	Peak area	% Assay of drug after degradation	% Degradation
Acidic/2 N HCl/45 min	RSP	68539	79.09%	20.90%
	THP	20852	82.46%	17.53%
Basic/1 N NaOH/60 min	RSP	75677	87.29%	12.70%
	THP	20710	89.79%	18.09%
Oxidative/6% H_2O_2/2 hr	RSP	74820	86.33%	13.66%
	THP	19901	75.53%	24.46%
Thermal/dry heating/80°C/60 min	RSP	86689	99.99%	No degradation
	THP	25286	99.99%	No degradation
Photolytic/UV light/60 min	RSP	76385	91.60%	11.88%
	THP	22528	95.02%	10.90%

and robust. It can be concluded that there is no other coeluting peak with main peaks and the method is specific for the determination of THP and RSP in combined tablet formulation. So it can be concluded that the developed RP-HPLC method can be successfully applied to the combination of THP and RSP in tablet dosage form.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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