

A RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HALOPERIDOL AND TRIHEXYPHENIDYL HYDROCHLORIDE IN TABLET DOSAGE FORM

SRIKANTHA DAMMALAPATI, RAMESH RAJU RUDRA RAJU*

Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, Guntur, Andhra Pradesh

Email: rrraju1@gmail.com

Received: 4 March 2014, Revised and Accepted: 15 April 2014

ABSTRACT

Objective: To develop a simple, fast and precise reversed phase-high performance liquid chromatographic (RP-HPLC) for simultaneous determination of the binary mixture of haloperidol and tri-hexyphenidyl hydrochloride in pharmaceutical formulations.

Methods: The RP- HPLC method uses a mobile phase consisting of methanol:acetonitrile:water (50:40:10% v/v/v), Zodiac C₁₈ column in isocratic mode, detection wavelength of 221 nm and a flow rate of 1.2 mL/minutes.

Results: The measured retention times for haloperidol and trihexyphenidyl hydrochloride are 6.12±0.02 and 8.06±0.02 minutes, respectively. The resolution of the two chromatographic peaks is 8.23. The validation of the method showed good linearity in the range 5-50 µg/mL for haloperidol and in the range 2-20 µg/mL for trihexyphenidyl hydrochloride. Further, satisfactory results are also established in terms of mean percent- age recovery (99.01-99.77% for haloperidol and 99.08-100.33% for trihexyphenidyl hydrochloride), intra-day and inter-day precision (<2%) and robustness.

Conclusion: The advantages of this method are good resolution with sharper peaks and sufficient precision. This could be used for the determination of the above drugs in dosage forms in combination or individually.

Keywords: haloperidol, hallucination,

INTRODUCTION

Haloperidol (HP) is an antipsychotic drug that possesses a strong activity against delusions and hallucinations. It is most likely linked to an effective dopaminergic receptor blockage in the mesocortex and the limbic system of the brain [1]. The molecular formula of haloperidol is C₂₁H₂₃ClFNO₂ and the molar mass is 375.9 g/mol. The chemical structure of haloperidol is shown in Fig. 1(a) and its IUPAC name is 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one [2]. It is officially recognized in Indian Pharmacopoeia [2], British Pharmacopoeia [3].

Trihexyphenidyl hydrochloride (TXH) belongs to anti-parkinsonian category [3]. It exerts a direct inhibitory effect upon the parasympathetic nervous system. It also has a relaxing effect on smooth musculature; exerted both directly upon the muscle tissue itself and indirectly through an inhibitory effect upon the parasympathetic nervous system. Trihexyphenidyl hydrochloride tablets are indicated as an adjunct in the treatment of all forms of parkinsonism (postencephalitic, arteriosclerotic, and idiopathic). The molecular formula of TXH is C₂₀H₃₁NO.HCl and 337.93 g/mol is the molar mass. Its chemical name is (±)-α-Cyclohexyl-α-phenyl-1-piperidinepropanol hydrochloride. Its structure is shown in Fig.1(b) [3]. It is also officially recognized in British Pharmacopoeia [3].

Haloperidol was studied individually [4 -10] and in combination with other drugs [11,12]. It was studied in pharmaceutical form, in human serum [12], human plasma and urine [13]. HP was also determined along with its metabolites and its degradation [7, 8]. These studies are carried out using analytical methods such as high performance liquid chromatography (HPLC) [5-13] and liquid chromatography (LC) - mass spectrometry (MS) [14] and spectrophotometry [19].

Trihexyphenidylhydrochloride was studied separately [15] and in combination with other drugs [16]. It was studied in human serum by liquid chromatography (LC)-Electron sputter Ionization (ESI)-Mass spectrometry (MS) [17] and in plasma by electron impact ionization using mass selective detector [18]. Spectrophotometry and HPLC methods were used for determination of HP in the

presence of TXH [19, 20]. However, there was less information available for HP and TXH combination to the best of our knowledge.

In this paper we report simultaneous determination of haloperidol and trihexyphenidyl hydrochloride drugs quantitatively. The proposed method has faster retention time, good resolution, good recovery of the two drugs compared to the earlier reported works. Further, the method was validated based on the ICH guidelines [21].

MATERIALS AND METHODS

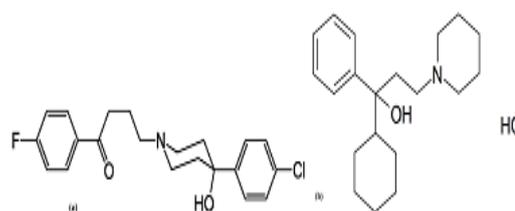


Fig.1: Chemical structure: (a) haloperidol and (b) Trihexyphenidyl hydrochloride.

Chemical and reagents

The chemicals and reagents used for the present study are HPLC grade. Methanol, acetonitrile and water (pH between 5 and 8) are procured from Merck Specialties private Ltd., Mumbai, India. For filtering the prepared solutions 0.45 µm Millipore filter paper is used.

Instrumentation

A PEAK HPLC system operated in isocratic mode was used for the present work. It was equipped with a LC 20AT pump and programmable variable wavelength ultraviolet (UV)-Visible detector (SPD-10AVP). A Zodiac C₁₈ column (250 x 4.6 mm, 5 µm particles) was used as stationary phase. A 20 µL Hamilton syringe was used for

injecting the samples. De-gassing of the mobile phase was done by using an ultrasonic bath sonicator (Loba). A Denver (SI234) balance was used for weighing the materials. Chromatograms were recorded and integrated using PEAK software. For analyzing the obtained data Microsoft Excel software was used. A UV-Visible spectrophotometer (Techcomp UV 230D6) with HITACHI software was used for determining the detection wavelength of HP and TXH.

Optimal chromatographic conditions

The optimal chromatographic conditions used for HP and TXH were: the mobile phase containing methanol: acetonitrile: water in 50:40:10% v/v/v; 221 nm detection wavelength; Zodiac C₁₈ column (250 mm x 4.6 mm, 5 µm) in isocratic mode; 1.2 mL/minute flow rate; 4.3 pH of the mobile phase and the operating pump pressure of 11.5 ± 5 MPascals. The active pharmaceutical ingredient (API) concentrations used for HP and TXH were 40 µg/mL and 16 µg/mL, respectively. The runtime was 12 minutes and the samples were injected using a 20 µL injector.

Preparation of the standard stock solution

To prepare the standard stock solution, 10 mg of HP and TXH were weighed accurately and transferred to two separate 100 mL volumetric flasks in 10 mL of methanol separately. They were sonicated for 2 minutes to dissolve completely. Then stock solutions were filtered through a 0.45 µm nylon membrane ultipore filter paper. A 1000 µg/mL solution of each drug was prepared. From this 2 mL was further diluted to 20 mL to get a stock concentration of 100 µg/mL solution for the two drugs. Required concentrations of the solutions were prepared by selective dilution.

METHOD DEVELOPMENT

The development of RP-HPLC method started with mobile phase composition and its volume ratio. The pure drugs of haloperidol (HP) and trihexyphenidyl hydrochloride (TXH) were injected into the HPLC system and run using standard organic solvents commonly used for HPLC studies. Water, acetonitrile and methanol were tested separately and in combination in order to find the best conditions for the separation of HP and TXH. It was observed that methanol, acetonitrile and water gave satisfactory results compared to two solvent combinations. This mobile phase system was tried with different proportions and with different flow rates. Finally, the optimal condition of the mobile phase was chosen as methanol, acetonitrile and water in the ratio 50 : 40 : 10% v/v/v. This composition of the mobile phase resolved the two drugs very well. The mobile phase and samples were degassed by ultra sonification for few minutes and then filtered with 0.45 µm Millipore filter paper. All measurements were carried at ambient temperature of the column. To optimize the flow rate various flow rates were used. The flow rate was also chosen keeping in mind, the recommended flow rate for the used column with a given internal diameter. The optimal flow rate was 1.2 mL/minute for the presented work. pH of the solution was determined after optimization of the mobile and it was 4.3 without adding buffer. Similarly, the pump pressure was also noted for different flow rates and mobile phase compositions and finally it was set as 11.5 ± 5 MPascals.

RESULTS AND DISCUSSION

The standard stock solution for HP and TXH was prepared with appropriate dilution. It was scanned in the wavelength region 200 nm to 400 nm using an ultraviolet (UV) - Visible spectrophotometer. The absorbance spectrum obtained was analyzed. From the spectrum of HP and TXH, 221 nm was selected as the optimum wavelength for the analysis of the binary mixture using RP-HPLC method. The absorption spectrum was shown in Figure 2 with wavelength (nm) as X-axis and absorbance (%) as Y-axis. The measured wavelength of 221 nm was in good agreement with other reported values of 220 nm [19] within a percent.

Method Validation

There were nine parameters that have to be validated for the developed method in accordance with the ICH guidelines [20]. They were specificity, linearity, precision, accuracy, recovery, limit of

detection, limit of quantification, robustness and ruggedness. In the present work eight out of nine parameters have been validated for both the drugs.

Selectivity and specificity

By complete separation of HP and TXH with the optimized chromatographic conditions, the specificity of the developed RP-HPLC method was validated with retention time, tailing factor (*t_r*) and resolution. The measured chromatographic peaks for both the drugs were sharper, have good signal to noise ratio and were well separated in a runtime of 12 minutes. They were shown in Figure 3 and a resolution of 8.23 was measured. The measured retention times were 6.12 ± 0.02 minutes for HP and 8.06 ± 0.02 minutes for TXH, respectively. The retention time reported for HP in this work was a factor of 2 more than the reported value of 2.99 minutes [19]. Similarly, the retention time reported for TXH in this work was a factor of 0.12 more than the reported value of 5.01 minutes [19]. The main difference was from the choice of mobile phase composition and the flow rate. The tailing factors of HP and TXH peaks were 0.89 and 1.06, respectively. These values fall in the recommended ranges of ICH guidelines (see Table 1). Similarly, the theoretical plates for HP and TXH were very well above the recommended values.

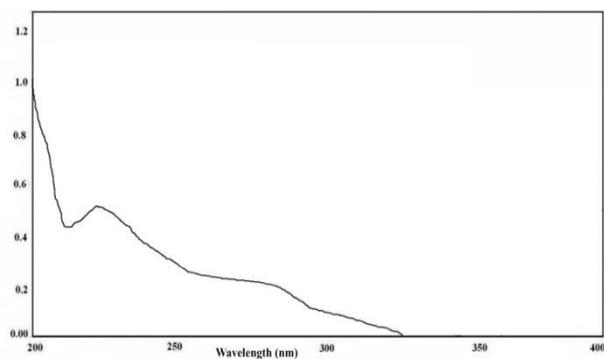


Fig.2: Absorption spectrum of haloperidol (HP) and trihexyphenidyl hydrochloride (TXH) (Maximum absorption at 221 nm).

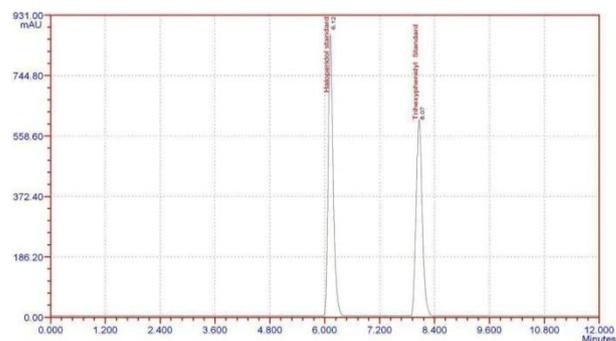


Fig.3: Chromatogram of haloperidol (HP) and trihexyphenidyl hydrochloride (TXH) standards. The retention time of 6.12 minutes for HP and 8.07 minutes for TXH were also shown.

Linearity

In the present work, ten different concentrations of each drug were prepared for linearity studies. The ten concentrations covered the range 5-50 µg/mL (5 µg/mL steps) for HP and 2-20 µg/mL (2 µg/mL) steps for TXH, respectively. The calibration curve was linear

in the range 5-50 µg/mL for the peak areas of HP. Similarly, the calibration curve was linear for the peak areas in the range 2-20 µg/mL for TXH. For HP, the values of regression parameters for the curve, described by the equation: $y = ax + b$, were calculated as: $a = 57143 \pm 5052$, $b = 21279 \pm 163$ and $r^2 = 0.9995$. For TXH, $a = -3999 \pm 3456$, $b = 25229 \pm 279$, $r^2 = 0.999$. All the regression parameters are statistically significant at 95% confidence interval.

Table 1: Summary of some validation parameters, system suitability parameters and ICH guide lines for haloperidol (HP) and trihexyphenidyl hydrochloride (TXH).

Parameter	HP	TXH	Recommended values
Linearity range ($\mu\text{g/mL}$)	5-50	2-20	---
Correlation coefficient (r^2)	0.9995	0.999	>0.999
Resolution		8.23	>2
Theoretical plates	7907	26929	>2500
Tailing factor (t_t)	0.89	1.06	$0.8 \leq t_t \leq 1.5$

LOD and LOQ

LOD and LOQ The parameters limit of detection (LOD) and limit of quantification (LOQ) were calculated for the HP and TXH drugs. These were determined from the sensitivity during linearity

measurements. These were calculated on the criteria $LOQ=3.3LOD$. For HP, LOD was $0.03 \mu\text{g/mL}$ and LOQ was $0.1 \mu\text{g/mL}$ whereas for TXH, LOD was $0.02 \mu\text{g/mL}$ and LOQ was $0.07 \mu\text{g/mL}$.

Recovery

The recovery studies for both the drugs were carried out using standard addition technique to know the accuracy of the proposed method. Three different percentage determinations (50%, 100%, 150%) were used to study the recovery of the drugs. The analysis of each percentage level was repeated three times ($n = 3$) for HP and TXH drugs. Finally, the percentage recovery of HP and TXH was compared with the actual amounts. The results were presented in Table 2. Shown in the table were the mean concentrations obtained and their standard deviation (SD). The coefficient of variation (CV) or the relative standard deviation (RSD) in percentage was also given for the two drugs. The mean recovery of the drug and its corresponding SD, RSD along with the calculated percentage error (last column) was also given in the table for the two studied drugs. The good recovery in the range of 99.01% (lower limit) to 99.77% (upper limit) for HP and 99.08% (lower limit) and 100.33% (upper limit) for TXH suggests the high accuracy of the method. This was within the statistical limits of the ICH guidelines [20].

Table 2: Recovery study results of haloperidol (HP) and trihexyphenidyl hydrochloride (TXH) by standard addition method.

Analyte	Analyte added	Target ($\mu\text{g/mL}$)	Spiked ($\mu\text{g/mL}$)	Total ($\mu\text{g/mL}$)	Concentration Obtained ($\mu\text{g/mL}$) Mean \pm SD	RSD or CV (%)	% of Recovery Mean \pm SD; RSD	%Error
HP	50%	20	10	30	29.70 \pm 0.16	0.53	99.01 \pm 0.52; 0.53	0.31
	100%	20	20	40	39.91 \pm 0.26	0.66	99.77 \pm 0.64; 0.64	0.37
	150%	20	30	50	49.74 \pm 0.075	0.15	99.52 \pm 0.14; 0.14	0.08
TXH	50%	8	4	12	11.91 \pm 0.06	0.5	99.25 \pm 0.51; 0.51	0.295
	100%	8	8	16	15.85 \pm 0.02	0.1	99.08 \pm 0.085; 0.08	0.05
	150%	8	12	20	20.06 \pm 0.23	1.1	100.33 \pm 1.14; 1.1	0.64

Precision

Another parameter that was commonly validated for drugs under study was precision through intra-day and inter-day measurements. The repeatability of the two drugs for the application was measured. Repeatability application was evaluated for six samples by measurement of the peak area for each sample and by comparing the relative standard deviation (RSD). Intra-day precision was studied at $40 \mu\text{g/mL}$ of HP and $16 \mu\text{g/mL}$ of TXH for all the six samples on the same day. The same concentration was used for the two drugs for inter-day precision studies. The variation in the peak area was studied for three successive days in a week. The data obtained for HP and TXH from intra-day and inter-day measurements were given in Table 3. Given were the mean normalized peak areas of the two drugs for six ($n=6$) different samples. They were normalized with the peak area of one of the samples. The corresponding standard deviation (SD) and the relative standard deviation (RSD) were also given. The RSD of all the samples of HP from intra-day measurements was 0.31 and from inter-day measurements was 0.85, which was less than 2%, respectively. The RSD of all the TXH samples from intra-day measurements was 0.61 and for inter-day measurements was 0.99, which was less than 2%. This satisfies the ICH guidelines and hence the method can be said precise. The method was also validated for ruggedness of HP and TXH drugs by two analysts separately with six samples each. These results were also given in Table 3 as a mean normalized peak area, SD and %RSD for HP and TXH. The RSD for both the drugs was less than 2%.

Robustness

The robustness of the method was verified by deliberate changes made to mobile phase volume ratio, pH of the solution and detection

wavelength. The change in peak area was checked by consciously changing the mobile phase volume ratio ($\sim 10\%$), pH value ($<10\%$) and wavelength ($\sim 1\%$) from the optimized conditions of the above parameters. They were given in Table 4 along with percentage change in peak areas with respect to the standard peak area. HP concentration of $40 \mu\text{g/mL}$ and TXH concentration of $16 \mu\text{g/mL}$ was used for these studies. The calculated percentage change in peak area for each parameter was found to be less than 2% satisfying the ICH guide lines. The values shown in Table 4 indicate robustness of the method. Though the method was robust at 10% variation of the chosen parameters; mobile phase composition variation was found to be less sensitive from the standard peak area. The influence of the pH was more from the standard peak area. About a five percent (5%) increase in pH resulted about two percent increase in the peak area where as five percent decrease in pH value resulted in almost no change in peak area. A less than one percent change in wavelength from the central value showed less than 1% peak area change.

Formulation assay

The proposed RP-HPLC method was validated by simultaneously determining HP and TXH in combined pharmaceutical mixture using Mindol Fort tablets. From $100 \mu\text{g/mL}$ solution, $40 \mu\text{g/mL}$ solution of HP and $16 \mu\text{g/mL}$ of TXH were prepared and used for formulation assay studies. The two drugs assay results were expressed as percentage of label claims. For HP and TXH the recovery percentages were 98.46% and 98.78%, respectively. These were in good agreement within the 90 to 100% of the label claim (Table 5). Chromatogram peaks of the two drugs were dominant in the drug sample with negligible interference from excipients that were normally present in the tablets. The demonstrated method could be used for routine analysis of the drugs in tablet dosage form.

Table 3: Results of intra-day, inter-day precision and ruggedness for HP (40 µg/mL) and TXH (16 µg/mL).

Parameter	Normalized Area Mean±SD (n=6)	% RSD	Normalized Area Mean±SD (n=6)	% RSD	Recommended Values of RSD
	HP		TXH		
Intra-day precision	0.998±0.003	0.31	1.007±0.006	0.61	<2%
Inter-day precision	0.995±0.009	0.85	1.011±0.01	0.99	<2%
Ruggedness	1.001±0.005	0.53	1.003±0.01	1.01	<2%

Table 4: Robustness results of haloperidol (HP) and trihexyphenidyl hydrochloride (TXH) for variation of mobile phase volume ratio, pH and detection wavelength.

Parameter	Value	HP ^a		TXH ^b	
		Area (mAU)	% Change	Area (mAU)	%Change
Standard		901198	---	393686	---
Mobile Phase (Methanol: Acetonitrile: Water)	48:42:10 (v/v/v)	910107	0.99	396002	0.59
	52:38:10 (v/v/v)	894853	0.70	391375	0.59
pH	4.1	885393	1.75	388910	1.21
	4.5	901638	0.05	391903	0.45
Wavelength	219 nm	896759	0.49	391350	0.59
	223 nm	896178	0.56	396568	0.73

^a At 40 µg/mL, ^b At 16 µg/mL

Table 5: Formulation assay of commercial tablet.

Brand Name	Form	Label Claim	Concentration Prepared	Amount Found	% Assay
Mindol Fort	Tablet	HP : 5 mg	40 µg/mL	39.38 µg/mL	98.46
		TXH : 2 mg	16 µg/mL	15.80 µg/mL	98.78

CONCLUSION

In summary, the described RP-HPLC method in this paper was simple, fast and specific to carry out. The proposed method demonstrates simultaneous determination of haloperidol and trihexyphenidyl hydrochloride with good resolution and sharper chromatographic peaks in a runtime of 12 minutes. The presented validation parameters results for intra-day and inter-day were precise and recovery results were accurate, which were established by statistical parameters and also satisfy the ICH guide lines. Hence, this method could be used for the determination of these drugs in pure and pharmaceutical preparations in laboratories as well as in industry.

ACKNOWLEDGEMENTS

One of the authors (D. S.) wishes to thank the principal and the management of Loyola Institute of Technology and Management (LITAM), Sattenapalli, Guntur, Andhra Pradesh for their encouragement during the course of this work.

REFERENCES

- Froemming JS, Francis Lam YW, Jann MW, Davis CM. Pharmacokinetics of haloperidol. *Clinical Pharmacokinetics* 1989; 17 Suppl 6: 396-423.
- Indian Pharmacopoeia, Vol. 1 New Delhi, The Controller of publication, Government of India 2010; p. 1435.
- British Pharmacopoeia, Vol. 1 (online version). London, The British Pharmacopoeia commission; 2012.
- Lea AR, Hailey DM, Drugged PR. Analysis of haloperidol tablets by high-performance liquid chromatography-an inter-laboratory study. *J Chromatogr A* 1982; 250: 35-42.
- Imran A, Hassan YA. Fast determination of haloperidol in pharmaceutical preparations using HPLC with a monolithic silica column. *J Liq chromatogr and Related Technologies* 2005; 28 Suppl 20: 3169-79.
- Fang J, Gorrod JW. High-performance liquid chromatographic method for the detection and quantitation of haloperidol and seven of its metabolites in microsomal preparations. *J Chromatogr B: Biomed Sci and Appl* 1993; 614 Suppl 2: 267-273.
- Trabelsi H, Bouabdallah S, Bouzouita K, Santa F. Determination and degradation study of haloperidol by high performance liquid chromatography. *J Pharm Biomed Anal* 2002; 29 Suppl 4: 649-657.
- Monser L, Trabelsi H. A rapid LC method for the determination of haloperidol and its degradation products in pharmaceuticals using a porous graphitic carbon column. *J Liq Chromatogr and Related Tech* 2003; 26 Suppl 2: 267-271.
- Xu X, Stewart JT. HPLC analysis of haloperidol and its related compound in the drug substance and a tablet dosage form using a non-porous silica octadecylsilane column. *J Liq chromatogr and Related Technologies* 1999; 22 Suppl 12: 1857-1866.
- Patela YP, Dhorda UJ, Sundaresan M. A comparative study using HPLC and packed column supercritical fluid chromatography for the assay of three anti-psychotic dosage forms. *Talanta* 1998; 47 Suppl 3: 625-630.
- Sane RT, Ghadge JK, Jain AB, Vaidya AT, Kotwal SS. RP-HPLC estimation of haloperidol and propantheline bromide in tablets or capsules. *Indian Drugs* 1992; 29: 240-4.
- Water S, Bauer S, Roots I, Brockmoller J. Quantification of the antipsychotics flupentixol and haloperidol in human serum by high - performance liquid chromatography with ultraviolet detection. *J Chromatogr B: Biomed Sci and Appl* 1998; 720 Suppl 1-2: 231-237.
- Ravisankar P, Devadasu CH, Devala Rao G, Nageswara Rao M. A validated RP-HPLC method for the determination of sparfloxacin in bulk and pharmaceutical dosage form. *Asian J Pharm and Clinical Research* 2014; 7 Suppl 1: 135-140.
- Arinobu T, Hattori H, Iwai M, It A, Kumazawa T, Suzuki O, Seno H. Liquid chromatographic-mass spectrometric determination of haloperidol and its metabolites in human plasma and urine. *J Chromatogr B: Biomed Sci and Appl* 2002; 776 Suppl 1: 107-113.
- Bargo E. Determination of trihexyphenidyl HCL dosage forms. *J Pharm Sci* 2006; 68 Suppl 4:503-5.
- Mahadik KR, Aggarwal H, Kaul N. RP-HPLC estimation of trihexyphenidyl HCL and chlorpromazine HCL in tablets. *Indian Drugs* 2002; 39: 441-5.
- Capka V, Xu Y, Chen YH. Stereoselective determination of trihexyphenidyl in human serum by LC-ESI-MS. *J Pharm Biomed Anal* 1999; 21 Suppl 3: 507-517.

18. Desage M, Rousseau-Tsangaris M, Lecompte D, Brazier JL. Quantitation of trihexyphenidyl from plasma using a mass-selective detector and electron-impact ionization. *J Chromatogr B: Biomed Sci and Appl* 1991; 571 Suppl 1-2: 250-256.
19. Wate SP, Borkar AA. Simultaneous spectrophotometric estimation of haloperidol and trihexyphenidyl HCL in tablets. *Indian J Pharm Sci* 2010; 72 Suppl 2:265-267.
20. Wate SP, Borkar AA. RP-HPLC estimation of haloperidol and trihexyphenidyl HCL in tablets. *Intern J ChemTech Res* 2009; 1 Suppl 3:675-676.
21. The International Conference on Harmonization (ICH), Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2005.