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Development and Validation of Analytical Method for the Estimation of Trihexphenidyl and Trifluoperazin in Bulk and Combination Dosage form by RP-HPLC

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Abstract

Developed an accurate, precise and reproducible high performance liquid chromatographic method for simultaneous estimation of Trihexphenidyl and Trifluoperazin in bulk and tablet dosage forms. Chromatographic separations of the drugs were achieved on a Symmetry ODS C18 (4.6×150mm, 5.0 µm) using a mobile phase consisting of Methanol: TEA Buffer pH-4.8 (35:65) v/v at a flow rate of 1.0 ml/min. The drugs elute were monitored at 276 nm. The retention time obtained for the Trihexphenidyl was 2.090 min and for the Trifluoperazin was 5.289 min. The calibration curves were linear over the range of 20- 60µg/ml and 25-75µg/ml for Trihexphenidyl and Trifluoperazin respectively. The method is validated as per ICH guideline by determining its specificity, accuracy, precision, linearity & range, ruggedness, robustness and system suitability. The results of the study show that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of Trihexphenidyl and Trifluoperazin in bulk and tablet dosage forms. The method could be applied for determination of in its tablet dosage forms without any interference from excipients or endogenous substances. The proposed method is suitable for routine quality control analysis.

Keywords: Trihexphenidyl and Trifluoperazin, RP-HPLC, Accuracy, ICH Guidelines

Introduction

Trihexyphenidyl hydrochloride is a selective M1 muscarinic acetylcholine receptor antagonist. It is able to discriminate between the M1 (cortical or neuronal) and the peripheral muscarinic subtypes (cardiac and glandular). Trihexyphenidyl hydrochloride partially blocks cholinergic activity in the CNS, which is responsible for the symptoms of Parkinson's disease. It is also thought to increase the availability of dopamine, a brain chemical that is critical in the initiation and smooth control of voluntary muscle movement. It is rapidly absorbed from the gastrointestinal tract.

Trihexyphenidyl is an anticholinergic used in the symptomatic treatment of all etiologic groups of parkinsonism and drug induced extrapyramidal reactions (except tardive dyskinesia). Trihexyphenidyl possesses both anticholinergic and antihistaminic effects, although only the former has been established as therapeutically significant in the management of parkinsonism.

Minor side effects, such as dryness of the mouth, blurring of vision, dizziness, mild nausea or nervousness. Isolated instances of suppurative parotitis secondary to excessive dryness of the mouth, skin rashes, dilatation of the colon, paralytic ileus, and certain psychiatric manifestations such as delusions and hallucinations, plus one doubtful case of paranoia all of which may occur with any of the atropine-like drugs, have been rarely reported with Trihexyphenidyl hydrochloride. Trihexyphenidyl has been reported as a drug of abuse, and while this is uncommon it may be prudent to be cautious in prescribing this drug to patients with a history of drug addiction. The drug has euphoriant and aphrodisiac properties and is smoked, insufflated, swallowed, or dissolved under the tongue and has enhanced activity when injected.

It works by blocking the action of acetylcholine in the central nervous system, helping to balance the cholinergic and dopaminergic activity in the brain. This relaxes muscles and reduces stiffness, tremors, and poor muscle control.

It is an adjunct therapy for all forms of parkinsonism (idiopathic, arteriosclerotic, and postencephalitic) and for controlling extrapyramidal symptoms caused by antipsychotic medications.

Common Side Effects

Most side effects are minor and tend to decrease with continued treatment.

- Dry mouth
- Blurred vision
- Dizziness, drowsiness, or weakness
- Nausea and vomiting
- Constipation or decreased urination
- Headache
- Nervousness or restlessness

Limited data from animal studies suggest that Trifluoperazin may undergo first-pass metabolism may occur via the liver and/or lungs. Trifluoperazin appears to be extensively metabolized, likely in the liver, to nor Trifluoperazin and other metabolites. Nor Trifluoperazin, the principal active metabolite, is formed via N-demethylation of Trifluoperazin. Nor Trifluoperazin appears to be comparable pharmacologic potency as Trifluoperazin. Trifluoperazin and nor Trifluoperazin both undergo phase II glucuronidation reactions in the liver. It is also thought that Trifluoperazin and nor Trifluoperazin undergo O-dealkylation to form p-trifluoro methylphenol, which is then subsequently metabolized to hippuric acid.

Trifluoperazin, an antidepressant agent belonging to the selective serotonin reuptake inhibitors (SSRIs), is used to treat depression, bulimia nervosa, premenstrual dysphoric disorder, panic disorder and post-traumatic stress. According to the amines hypothesis, a functional decrease in the activity of amines, such as serotonin and norepinephrine, would result in depression; a functional increase of the activity of these amines would result in mood elevation. Trifluoperazin's effects are thought to be associated with the inhibition of 5HT receptor, which leads to an increase of serotonin level. Antagonism of muscarinic, histaminergic, and α1–adrenergic receptors has been hypothesized to be associated with various anticholinergic, sedative, and cardio vascular effects of classical tricyclic anti depressant (TCA) drugs. Trifluoperazin binds to these and other membrane receptors from brain tissue much less potently *in vitro* than do the tricyclic drugs.

Metabolized to nor Trifluoperazin, Trifluoperazin is a selective serotonin- reuptake inhibitor (SSRI), it blocks the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on 5HT1A auto receptors. SSRIs bind with significantly less affinity to histamine, acetylcholine, and norepinephrine receptors than tricyclic antidepressant drugs.

Labeled indication include: major depressive disorder (MDD), moderate to severe bulimia nervosa, obsessive-compulsive disorder (OCD), premenstrual dysphoric disorder (PMDD), panic disorder with or without agoraphobia, and combination treatment with Trihexphenidyl for treatment- resistant or bipolar I depression. Unlabeled indications include: selective mutism, mildde mentia-associated agitation in non psychotic patients, post-traumatic stress disorder (PTSD), social anxiety disorder, chronic neuropathic pain, fibromyalgia, and Raynaud's phenomenon.

Materials and Methods

Chemicals and Reagents

Samples of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride were gifted by Sura labs, Hyderabad. Water for HPLC, Methanol for HPLC, Acetonitrile for HPLC, Tri ethyl Amine was procured from Merck.

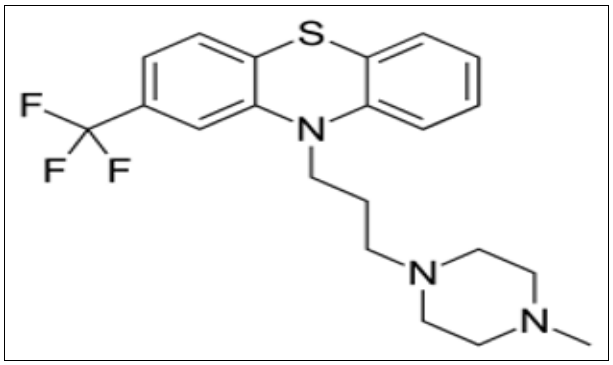

Fig 1: Trifluoperazin hydrochloride

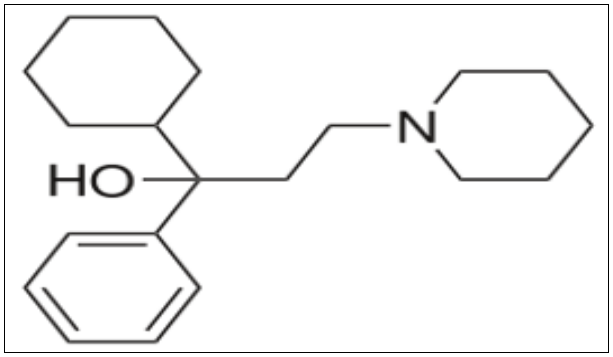

Fig 2: Trihexyphenidyl hydrochloride

Instrumentation

Chromatographic system consisted of a LC-10AT VP Shimadzu liquid chromatography with SPD-10A Shimadzu UV-Vis detector equipped with auto sampler Photodiode array detector. The data recorded using LC Solutions software. The column used was C-18 column, Inertsil ODS C18 (4.6×150mm, 5.0 µm)., particle size 5 µm with flow rate of 1 ml / min using PDA, the drugs elute were monitored at 276 nm.

Selection of mobile phase

The pure drug of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride were injected into the HPLC system and run in different solvent systems. Different mobile phases were tried in order to find the best conditions for the separation Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride. Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer pH-4.8 (35:65) v/v respectively.

Estimation of Trihexyphenidyl and Trifluoperazinin pharmaceutical dosage form

Procedure Preparation of mobile phase: Accurately measured 350 ml (350%) of HPLC Methanol and 650 ml of TEA (65%) were mixed and degassed in a digital ultra Sonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:

Accurately measured 350ml (350%) of HPLC Methanol and 650ml of TEA (65%) were mixed and degassed in a digital

ultra Sonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Assay

Preparation of the Trihexyphenidyl and Trifluoperazin standard solution: Preparation of standard solution: (Trihexyphenidyl)

Accurately weigh and transfer 10mg of Trihexyphenidyl, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluents and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Trifluoperazin)

Accurately weigh and transfer 10 mg of Trifluoperazin working standard intoa10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Further pipette 0.4 ml of Trihexyphenidyl, 0.5ml of Trifluoperazin from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:

Take average weight of 10 Tablets and crush in a mortar by using pestle and weight 10 mg equivalent weight of Trihexyphenidyl, Trifluoperazin sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume upto the mark with the same solvent.

Procedure:

Further pipette 0.4ml of Trihexyphenidyl and 0.5ml of Trifluoperazin from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%\ \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

% ASSAY was calculated by using the formula and reported in the Table: 15, 16 (7.1.1).

Development and validation of HPLC Method Present study was conducted to obtain a new, affordable, cost effective and convenient method for HPLC determination of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996, and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness.

Specificity & Selectivity:

The specificity of the RP-HPLC method was determined by complete separation of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride, with parameters like retention time (tR), resolution (RS) and tailing factor (Tf). Here tailing factor for peaks of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride was less than 2% and resolution was also more than 1%.

Linearity:

Linearity of the method was determined by constructing calibration curves. Standard solutions of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride at different concentrations level were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy:

Recovery studies were carried out by applying the method to drug sample present in tablet dosage form to which known amount of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride, corresponding to 80%, 100% and 120% of label claim was added (standard addition method). The concentration of the sample mixture was determined as per the procedure given for the tablet formulation by determining AUC at selected analytical wavelength 276 nm. The variation of the results within the same day was analyzed and statistically validated.

Precision:

The precision of the method was determined by repeatability (intraday) and intermediate precision (interday) study. Repeatability was determined by performing four repeated analysis of the standard solutions of Trihexyphenidyl hydrochloride (40µg/ml) and Trifluoperazine hydrochloride (100µg/ml) on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis of previous standard solutions on three different days (inter-day) in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method. The procedure for the preparation of solution for the determination of precision was same as explained in the analysis of tablet formulation.

Reproducibility:

Reproducibility expresses the precision between laboratories. It is assessed by means of inter laboratory trial. It should be considered in case of standardization of an analytical procedure. The area under curve of the sample mixture was measured by another analyst at selected analytical wavelength 276nm under the same chromatographic condition as described above. The results obtained were evaluated using t-test to verify their reproducibility.

Robustness:

The evaluation of robustness should be considered during the development phase and depends upon the type of

procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing Trifluoperazine hydrochloride 100µ g/ml and 40µ g/ml Trihexyphenidyl hydrochloride was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate, percentage of acetonitrile in the mobile phase and column temperature.

System suitability:

The system suitability was assessed by six replicate analysis of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride at a 100% level to verify the resolution and reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for both Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride.

Table 1: Peak Results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Trihexyphenidyl	2.090	327989	39785		1.72	5657
2	Trifluoperazin	5.289	3576856	232354	9.80	1.77	5869

Optimized Chromatogram (Sample)

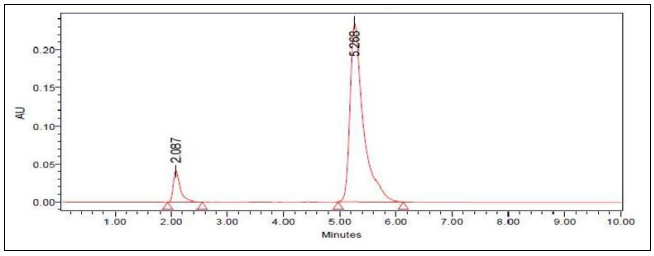

Fig 3: Optimized Chromatogram (Sample)

Linearity

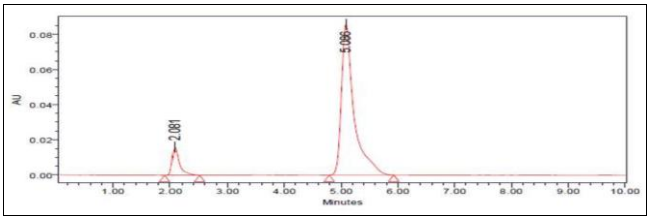

Fig 4: Chromatogram for linearity concentration-20µg/ml of Trihexyphenidyl &25 µg/ml of Trifluoperazin

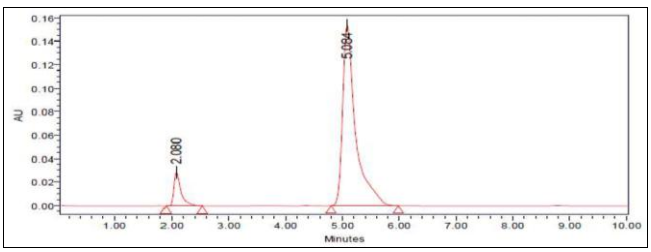

Fig 5: Chromatogram for linearity concentration-30 µg/ml of Trihexyphenidyl & 37.5µg/ml of Trifluoperazin

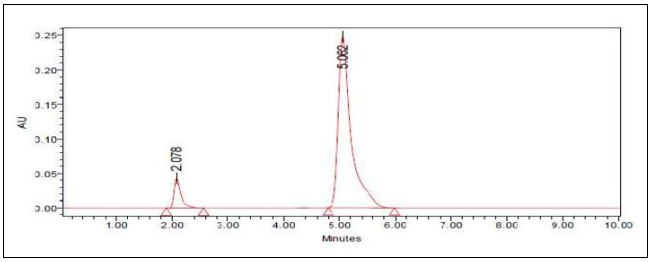

Fig 6: Chromatogram for linearity concentration-40 µg/ml of Trihexyphenidyl & 50µg/ml of Trifluoperazin

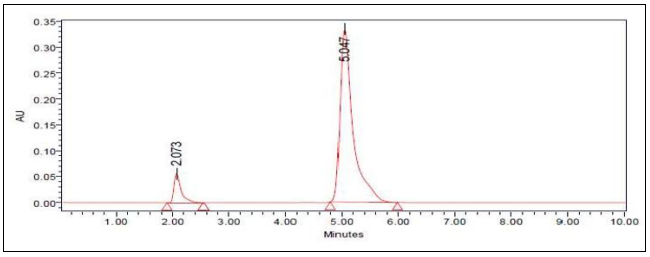

Fig 7: Chromatogram for linearity concentration-50 µg/ml of Trihexyphenidyl & 62.5µg/ml of Trifluoperazin

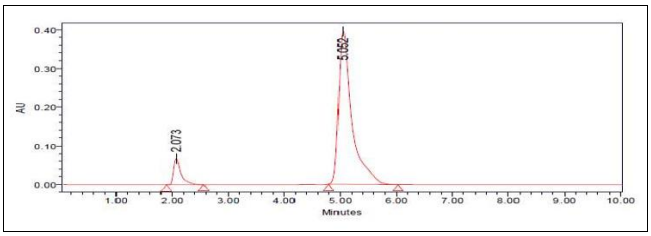

Fig 8: Chromatogram for linearity concentration-60 µg/ml of Trihexyphenidyl &75 µg/ml of Trifluoperazin

Table 2: Chromatographic data for linearity study: Trihexyphenidyl

Concentration µg/ml	Average Peak Area
20	164436
40	348687
50	439024
60	534830

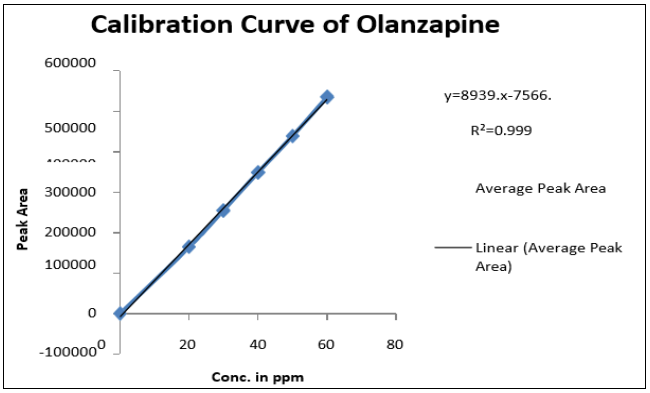

Fig 9: Calibration graph for Trihexyphenidyl

Table 3: Trifluoperazin

Concentration µg/ml	Average Peak Area
25	1782454
37.5	2728974
50	3688678
62.5	4658022
75	5592695

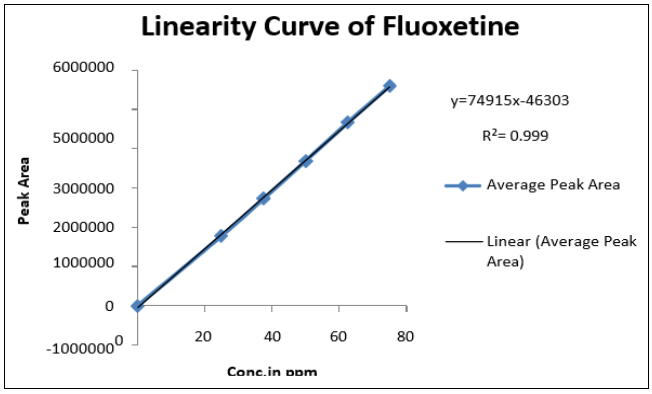

Fig 10: Calibration graph for Trifluoperazin

Table 4: Results of Repeatability for Trihexyphenidyl

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Trihexyphenidyl	2.086	327689	41697	5081.3	1.8
2	Trihexyphenidyl	2.083	327978	41402	5144.1	1.8
3	Trihexyphenidyl	2.083	327879	41540	5118.1	1.8
4	Trihexyphenidyl	2.081	327868	42256	5147.3	1.8
5	Trihexyphenidyl	2.081	327859	42143	5101.8	1.8
Mean			327854.6			
Std. Dev			104.2176			
%RSD			0.031788			

Table 5: Results of method precision for Trifluoperazin

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Trifluoperazin	5.178	3576985	241253	5969.5	2.0	9.8
2	Trifluoperazin	5.199	3578989	2365824	5865.1	2.0	9.7
3	Trifluoperazin	5.235	3576859	239568	5936.4	2.0	9.9
4	Trifluoperazin	5.202	3578458	2386547	5964.4	2.0	9.8
5	Trifluoperazin	5.206	3579864	241425	5045.6	2.0	9.5
Mean			3578231				
Std. Dev			1296.889				
%RSD			0.036244				

Table 6: The Accuracy Results for Trihexyphenidyl

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	%Recovery	Mean Recovery
50%	186584.7	20	20.026	100.13	100.435%
100%	367968.7	40	40.32	100.80	
150%	545922	60	60.225	100.375	

Table 7: The Accuracy Results for Trifluoperazin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	%Recovery	Mean Recovery
50%	1925532	25	25.084	100.336	100.284%
100%	3790965	50	49.985	99.970	
150%	5695646	75	75.410	100.546	

Table 8: Results for robustness Trihexyphenidyl

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	327989	2.090	5698	1.70
Less Flow rate of 0.9 mL/min	302986	2.736	5569	1.82
More Flow rate of 1.1mL/min	316989	1.673	5598	1.91
Less organic phase	315989	2.736	5651	1.82
More organic phase	308986	1.673	5452	1.91

Table 9: Results for robustness Trifluoperazin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3576856	5.289	5689	1.77
Less Flow rate of 0.9 mL/min	3458978	6.746	5658	1.88
More Flow rate of 1.1mL/min	3589871	4.032	5245	1.91
Less organic phase	3579124	6.746	5154	1.88
More organic phase	3578698	4.032	5652	1.91

Summary & Conclusion

The analytical approach was developed through analyzing one of kind parameters. The column used for study changed into Symmetry ODS C18 (4.6×150mm, 5.Zero µm). Mobile section is Methanol: TEA Buffer pH-four.8 (35:65) turned into constant because of right symmetrical top. So this mobile phase was used for the proposed study. The percentage healing was determined to be 98.0-102 become linear and specific over the identical range. Both gadget and method precision become found to be correct and within range. The analytical approach became found linearity over the variety 20-60µg/ml of Trihexyphenidyl and 25-75 µg/ml of Trifluoperazin of the final concentration.

This technique turned into simple, considering the fact that diluted samples are without delay used with no preliminary chemical derivatization or purification steps. Trihexyphenidyl and Trifluoperazin turned into freely soluble in ethanol, methanol and sparingly soluble in water. The % RSD values have lies within 2 and the approach changed into discovered to be precise. This technique can be used for the habitual dedication of Trihexyphenidyl and Trifluoperazin in bulk drug and in Pharmaceutical dosage bureaucracy.

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