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Der Pharmacia Lettre, 2016, 8 (6):275-280
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ISSN 0975-5071
USA CODEN: DPLEB4

Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Estimation of trihexyphenidyl and trifluoperazine in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, precise and accurate isocratic RP-HPLC stability-indicating assay method has been developed to determine trihexyphenidyl and trifluoperazine in their combined dosage form. Isocratic separation was achieved on a Kromasil -C18, column at room temperature in isocratic mode, the mobile phase consists of Methanol: Sodium Acetate in water (80:20, v/v) at a flow rate of 1.0 ml/min, the injection volume was 20µl and UV detection was carried out at 232nm.The drug was subjected to acid and alkali hydrolysis, oxidation, photolysis, UV light and heat as stress conditions. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The method was linear in the drug concentration range of 2–12 µg/ml and 5-30 µg/ml for trihexyphenidyl and trifluoperazine respectively. The precision (RSD) of six samples was 0.868 and 0.1.191% for repeatability, and the intermediate precision (RSD) among six-sample preparation was 1.212 and 0.803% for trihexyphenidyl and trifluoperazine, respectively. The mean recoveries were between 98.19–101.38% and 98.76–100.50% for trihexyphenidyl and trifluoperazine respectively. The proposed method can be used successfully for routine analysis of the drug in bulk and combined pharmaceutical dosage forms.

Key words: trihexyphenidyl and trifluoperazine, RP-HPLC, method development and validation, stability studies.

INTRODUCTION

Trihexyphenidyl: Trihexyphenidyl also known as benzhexol and trihex, is an antiparkinsonian agent belongs to phenylpropylamines class [1-5]. Trihexyphenidyl possesses both anticholinergic and antihistaminic effects. It is used for the symptomatic treatment of Parkinson's disease in mono and combination therapy also commonly used to treat extrapyramidal side effects occurring during antipsychotic treatment. It reduces the frequency and duration of oculogyric crises as well as of dyskinetic movements and spastic contractions [6, 7]. The drug may improve psychotic depression and mental inertia frequently associated with Parkinson's disease and symptomatic problems caused by antipsychotic treatment. It has also been prescribed for essential tremor and akathisia. The drug is available in various forms like Solution, Elixir, Syrup and tablet obtained by oral administration [8-12].

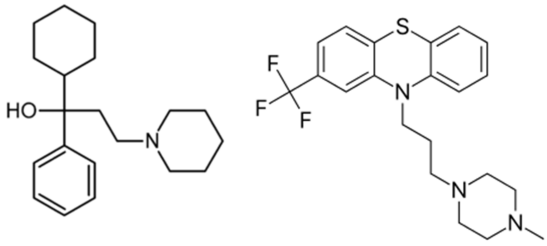

Figure A: chemical structure of Trihexyphenidyl and Trifluoperazine

Trifluoperazine: Trifluoperazine is a typical antipsychotic belongs to phenothiazines class. These are polycyclic aromatic compounds containing a phenothiazine linear tricyclic system that consists of a two benzene rings joined by a parathiazine [13-18]. The primary application of the drug is for schizophrenia. The drug is prescribed for the treatment of anxiety disorders, depressive symptoms secondary to anxiety and agitation [19]. Other official indications may vary country by country. But it is also indicated for use in agitation and patients with behavioural problems, severe nausea and vomiting as well as severe anxiety, borderline personality disorder [20, 21], tardive dyskinesia, to reverse addiction to opioids[22-24]. The drug is sold as tablet, liquid and injectable USP. Though few analytical techniques have reported for analysis of Trihexyphenidyl and Trifluoperazine in combined or individual [25-33] but very few methods have been reported for stability indicating studies of the above mentioned combined drugs.

MATERIALS AND METHODS

Instruments and chemicals used:
Working standards of Trihexyphenidyl and Trifluoperazine were provided by reputed laboratories. Methanol, water are HPLC grade of Merck chemicals, Mumbai, india. Sodium Acetate, acetic acid, HCl, NaOH and H2O2 used were of analytical reagent grade of finar chemicals purchased from local chemical store. For stability indicating HPLC method development a isocratic PEAKHPLC instrument with kromasil C18 column (250 mm x 4.6 mm, 5µ) and all the weighing was done on Electronic balance-DENVER (SI234). The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC –7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Preparation of standard solution:
Standard stock solutions of trihexyphenidyl and trifluoperazine were prepared separately by dissolving 25 mg of drug in 25 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, mixed working standard solution was prepared to contain 100 µg/ml of trihexyphenidyl and 100 µg/ml of trifluoperazine. Further dilutions were prepared from the above concentrated solution.

Method development:
The UV spectra of the both trihexyphenidyl and trifluoperazine were showed the balanced wavelength at 232 nm on methanol solvent. To effect ideal separation of the drug under isocratic conditions, mixture of solvents like Methanol, Acetonitrile and water with or without different buffers in the different combinations were tested has mobile phases on RP-C-18 stationary phase. Finally find that a mixture of Methanol: Sodium Acetate in water 80:20 (v/v) in the ratio of 80:20 (v/v) was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined & resolved and almost tree from tailing. The pH of the mobile phase was adjusted to 5.0 by using acetic acid. 1.0 ml/min mobilphase flow rate was found as optimum flow rate. No interference in blank [figure B] and placebo solutions for both drug peaks in the trail injections with a runtime of 10min. The above optimized chromatographic conditions were followed for the simultaneous determination of trihexyphenidyl and trifluoperazine Hydrochloride in bulk samples and its combined tablet formulations.

Method Validation:
After the method conditions were established as described above, method was validated as per ICH guidelines. The accuracy, precision, Linearity, limit of detection (LOD) and quantification (LOQ) were determined. The linearity was studied by analyzing six concentrations of each drug and process was repeated six times. Precision of the

system was evaluated by analyzing six independent standard preparations and % RSD value was calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day variation. Recovery studies were carried out by addition of standard drug to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Mean percentage recovery was determined. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Assay procedure (Preparation of sample solution):
Ten tablets (Doxogem-TZ) each containing 2mg of trihexyphenidyl and 5mg of trifluoperazine was weighed and powdered. Powder equivalent to 2 mg of trihexyphenidyl and 5 mg of trifluoperazine was transferred to 25ml volumetric flask and was diluted with methanol to 25 ml (2000 µg/ml of trihexyphenidyl and 5000µg/ml of trifluoperazine). Further dilutions were made with methanol to get the final concentration of 8µg/ml of trihexyphenidyl and 20 µg/ml of trifluoperazine. The prepared sample solution was injected under proposed condition and % assay was calculated.

Stress degradation studies:
Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and mixed working standard solution subjected to stress conditions. Mixed working standard solution was mixed with equivalent volumes with 0.1N hydrochloric acid, 0.1 N sodium hydroxide, aqueous solution, 3% Hydrogen peroxide individually in a volumetric flask. Test solutions were stored in ambient temperature for 48 hrs. After 48 hrs all samples were neutralized and subjected for HPLC analysis. Dry heat, Ultraviolet and photolytic degradation were carried out in solid state. After stress conditions working standard concentration solutions were prepared and injected into the system.

RESULTS AND DISSCUSION

Development of the optimum conditions:
Chromatographic separation studies were carried out on the working standard solutions of trihexyphenidyl and trifluoperazine. Initially, trials were carried out and after several trials, Methanol with Sodium Acetate in water 80:20 (v/v) with C18 column at 232nm UV detector wavelength was proved to be the most suitable which resulted in good resolution and acceptable peak parameters. Retention time were found as Trihexyphenidyl– 3.54min and Trifluoperazine- 5.79min [figure C]. The proposed HPLC conditions are given in table 1.

Validation of the developed stability indicating method:
Validation of the proposed method was carried under ICH guidelines. The data obtained in the linearity experiment was subjected to linear-regression analysis. A linear relationship between peak areas and concentrations was obtained in the range of 2 - 12 µg/ml for trihexyphenidyl and 5-30 µg/ml for trifluoperazine with r^2 0.999 and 0.999 respectively. Precision results indicates that the developed method was found to be precise as the % RSD value for both interday and intraday were less than 2. Recoveries were obtained at each level of added concentration. The result obtained (n = 3 for each level) indicated the mean recovery between 98% to 102% for both trihexyphenidyl and trihexyphenidyl. Limit of detection and limit of quantitation was found to be 0.10µg/ml and 0.35µg/ml for trihexyphenidyl and 0.25µg/ml and 0.80µg/ml for trifluoperazine respectively. The robustness and ruggedness results are within the acceptance limits. Summary of validation studies are presented in table no 2. Combined marketed formulation (Doxogem-TZ, 2mg-trihexyphenidyl and 5-mg trifluoperazine) was analyzed with proposed method condition and good recovery of the drug indicates that the proposed method can be useful for analysis of market formulations also. The formulation chromatogram was presented in figure D.

Stability studies:
The chromatograms of the stability studies are clearly indicate that the proposed method was capable to separate the degraded products from the drug compound and represent it as a spate peak. The results of stress studies indicates the both drugs are sensitive to the acid, base, peroxide and thermal stress condition where three degraded products have been identified. Sunlight and UV light conditions shown two additional degraded peaks, where as the drugs are stable in aqueous conditions where only one additional peak was observed. The chromatograms of the stability studies are presented in figure E.

Figure B: blank chromatogram

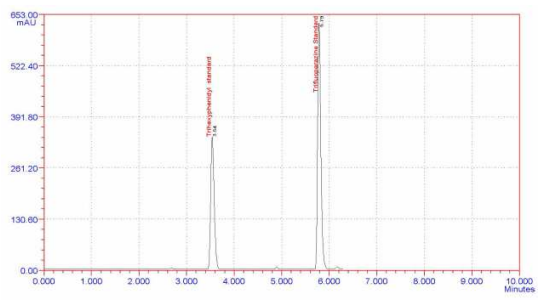

Figure C: standard chromatogram of trihexyphenidyl and trifluoperazine

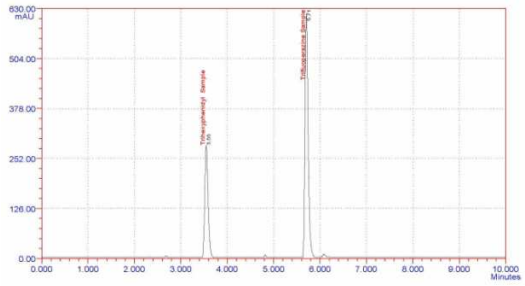

Figure D: formulation chromatogram of trihexyphenidyl and trifluoperazine

Table 1: Proposed HPLC method conditions

S.NO	Parameter	Results
1	Mobile phase	Methanol: Sodium Acetate in water 80:20 (v/v)
2	UV detector wavelength	232nm
3	Stationary Phase (column)	RP- C18 Column
4	pH of Mobile phase	5.0 with Acetic Acid
5	Flow Rate	1.0ml/min
6	Pump Mode	Isocratic
7	Pump Pressure	12.5±5MPa
8	Temperature	Ambient
9	Run time	10min
10	Retention time	Trihexyphenidyl– 3.54min Trifluoperazine- 5.79min

Table 2: summary of validation results:

S.NO	PARAMETER	TRIHEXYPHENIDYL	TRIFLUOPERAZINE
1	API Concentration	8µg/ml	20µg/ml
2	Retention time	3.54min	5.79min
3	Resolution	-----	14.83
4	Theoretical Plates	7136	29346
5	Tailing Factor	0.97	1.79
6	Linearity range	2-12µg/ml	5-30µg/ml
7	Correlation coefficient	0.999	0.999
8	Intraday Precision (in % RSD)	0.868	1.191
9	Interday Precision(in % RSD)	1.212	0.732
10	Ruggedness (in % RSD)	1.199	0.803
11	Recovery range (%)	98.19-101.38	98.76-100.50
12	Robustness (% Change)	0.60 to 1.40	-0.69 to 1.14
13	Limit of detection	0.10µg/ml	0.25µg/ml
14	limit of quantitation	0.35µg/ml	0.80µg/ml
15	Formulation assay (%)	99.538	99.664

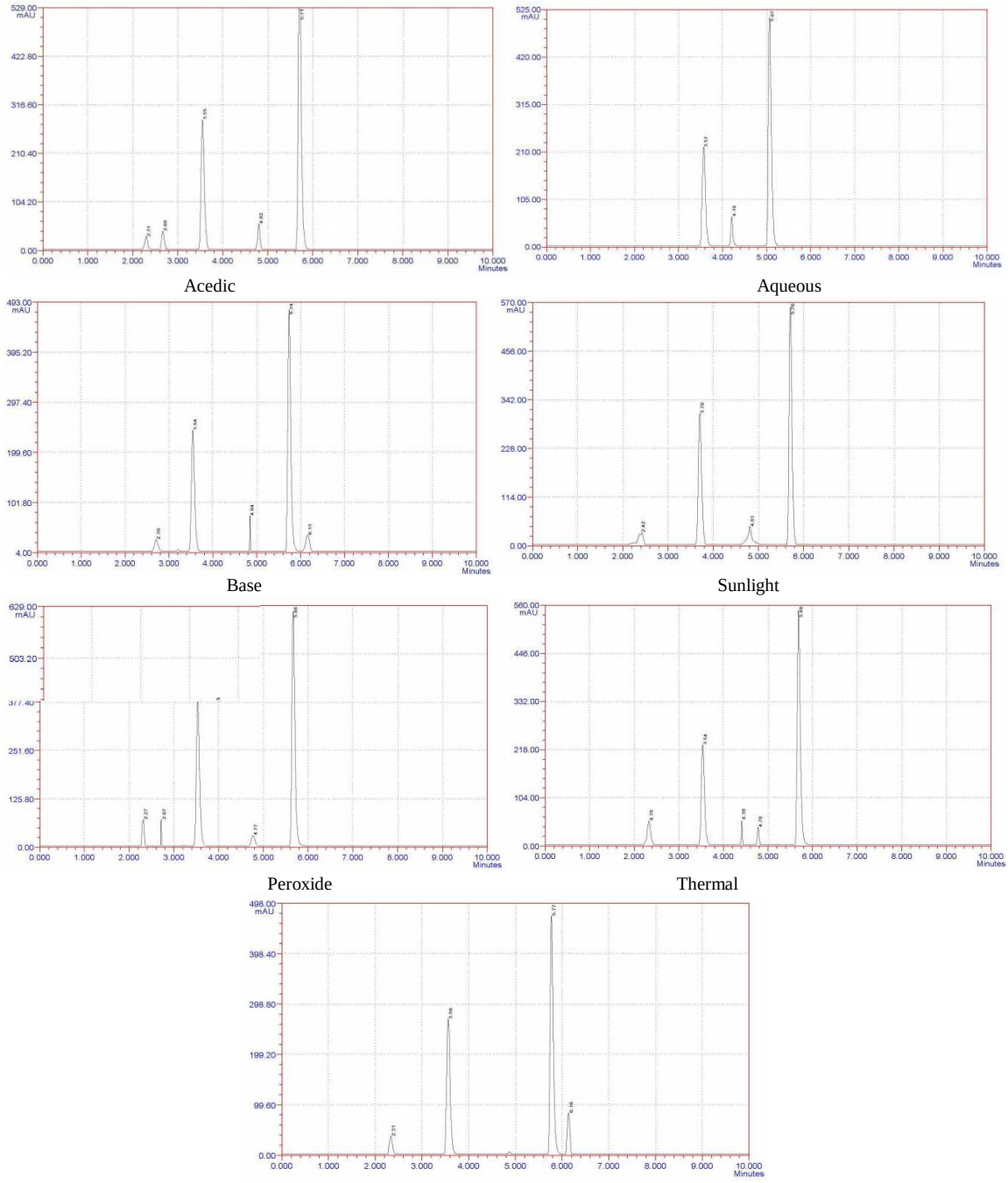

Figure E: stability chromatograms at different stress conditions

CONCLUSION

A validated stability-indicating RP-HPLC method has been developed for determination of trihexyphenidyl and trifluoperazine in their combined tablet dosage form. The results obtained by the stress degradation conditions of the drugs show that the method is specific and stability-indicating. The stability results reveal that both drugs are sensitive to the acid, base, peroxide and thermal stress condition and stable in aqueous conditions. The method was found to be simple, accurate, precise and sensitive. The method was successfully applied for the determination of

both drugs in combined tablet dosage form. In the future, this method may be applied for routine analysis of both the drugs in bulk drug, dissolution studies, bioavailability and pharmacokinetic studies.

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