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Development and Validation ofRPHPLC Method for Estimation of Methohexital ina Tablet Dosage Form

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Abstract:*An isocratic reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the determination of Methohexital in API, dosage formulations and human serum. Chromatographic separation was achieved on SYMMETRY C_{18} 150X4.6mm,3.7µmand columns using mobile phase, methanol: water (70:30 v/v) adjusted to pH 3.0 via phosphoric acid 85% having flow rate of 1.0 mL min -1 at ambient temperature with detector set at 272 nm. Calibration curves were linear over range of 5-25 µgmL -1 with a correlation coefficient ± 0.999. LOD and LOQ were in the ranges of 0.4-2.3 µgmL -1 . Intra and inter-run precision and accuracy results were 98.0 to 102%.*
Keywords: *Methohexital Barbiturate; Rp-Hplc*

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I. Introduction

The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicatinganalytical methods. Methohexital or methohexitone (marketed under the brand names Brevital and Brietal) is a drug which is a barbiturate derivative. It is classified as short-acting, and has a rapid onset of action. It is similar in its effects to sodium thiopental, a drug with which it competed in the market for anaesthetics.Methohexital binds to a distinct site which is associated with Cl^- ionophores at $GABA_A$ receptors. This increases the length of time which the Cl^- ionopores are open, thus causing an inhibitory effect.Metabolism of methohexital is primarily hepatic (i.e., taking place in the liver) via demethylation and oxidation Side-chain oxidation is the primary means of metabolism involved in the termination of the drug's biological activity.The chemical name is5-Allyl-1-methyl-5-(1-methyl-2-pentynyl)barbituric acid.

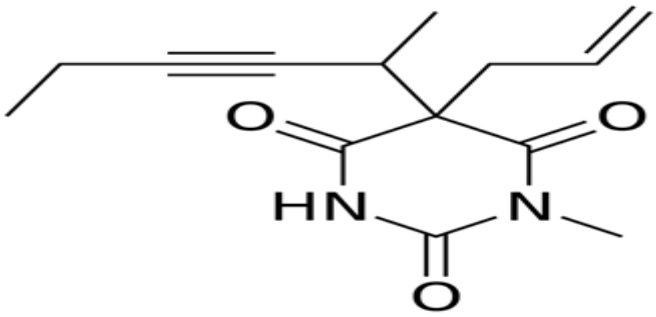

Fig no: 1 Structure of Methohexital

Experimental Conditions:

Reference substances, chemicals, reagents and samples The entire experiment was performed using "class A" volumetric glassware. Pharmaceutical grade methohexitalactive pharmaceutical ingredient (API) and tablets were procured from Bio - Leo labs, Hyderabad. The chemicals like tetrahydrofuran, acetonitrile and perchloric acid were purchased from Merck, Mumbai. Millipore water was generated from TK water system. The analytical column used was Inertsil ODS-3.0 X 50 mm column with a particle size of 2 µm.

Instrumentation

Methohexitalassay analysis was performed by using waters UPLC (Milford, MA, USA) PDA system consisting of a quaternary solvent manager, a sample manager, column-heating compartment and photodiode array detector. This system was controlled and the output signal was monitored by waters empower software. Inertsil ODS-3, 50 X 4.6 mm with 3µm column was employed as stationary phase for chromatographic separation. Sartorius semi micro balance was used for all weighing's and Thermo Orion pH meter was used for buffer pH adjustment. Sonication was carried out with Bandelinsonicator and rotary shaker was used for shaking of samples during preparation.

Optimised Chromatographic conditions of Methohexital

Column: Inertsil ODS-3, 50 X 3.0 mm, 2 µm Mobile phase: Mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio of 10:220:770:1 (v/v) Flow rate: 1.0 mL minute-1 Injection volume: 5 µL Run time: 2 minutes Detection: Ultra violet detection at 245 nm.

Standard preparation

Weighed accurately 25.0 mg of methohexitalhydrochloride working standard and transferred into a 250 mL volumetric flask. 50 mL of acetonitrile and 100 mL of water were added and sonicated to dissolve. The contents were diluted to the volume with water and mixed thoroughly.

Sample preparation

One tablet of alfoo (Dr.Reddys Laboratory, Hyderabad) was taken in a 100 mL dry volumetric flask. 20 mL of acetonitrile were added, sonicated with intermediate shaking until the tablets disintegrate and kept on a cyclomixer for 5 minutes. 50 mL of water were added and sonicated for 30 minutes with intermediate shaking. The solution was finally made up to the volume with water and mixed. A portion of the sample solution was centrifuged at 3500 RPM for 15 minutes and filtered through 0.45µm filter.

Blank preparation

A mixture of acetonitrile and water in the ratio 1:4 (v/v) was used as the blank.

Placebo preparation

Weighed accurately microcrystalline cellulose USP-NF (704 mg) and hypromellose 3 cps USP-NF (36 mg) (Dr.Reddys Laboratory, Hyderabad) and taken in a 100 mL dry volumetric flask. 20 mL of acetonitrile were added, sonicated with intermediate shaking until the tablets disintegrated and kept on a cyclomixer for 5 minutes. 50 mL of water were added and sonicated for 30 minutes with intermediate shaking. The solution was finally made up to the volume with water and mixed. A portion of this solution was centrifuged at 3500 RPM for 15 minutes and filtered through 0.45µm filter.

Mobile phase

A mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio 10:220:770:1 (v/v) was used as the mobile phase.

Chromatographic conditions

The chromatographic column used was Inertsil ODS-3 with dimensions of 50 X 4.6 mm with 3 µm particle size. The isocratic method was employed with the mobile phase. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 245 nm. Injection volume was maintained as 5 µL and the mobile phase flow was set at 1.0 mL min-1 .

Evaluation of system suitability

From the chromatogram obtained for the standard preparation, the tailing factor was found as 1.5 which is less than permitted limit (2.0) and the relative standard deviation of replicate injections was calculated as 0.1%. These values indicate the suitability of the proposed system for the determination of alfuzosin.

II. Results and discussion

Method development and optimization Method development was initiated by the review of literature survey and studies on methohexitalhydrochloride physical and chemical characteristics. The solubility of

methohexitalwas tested in different solvents and identified that mixture of water and acetonitrile solution was suitable for extraction of methohexitalfrom its tablets. Based on spectral profile and absorption characteristics of alfuzosin, UV detector at 245 nm wavelength was selected for its detection. Preliminary experiments were carried out under various chromatographic conditions as follows.

Method Validation

The proposed test method was validated to include requirements of International conference on Harmonization (ICH) guidelines in terms of specificity, linearity, precision, accuracy, range, robustness and ruggedness. The stability of mobile phase, standard, sample solutions and system suitability were also examined.

Specificity

The specificity parameter of the method was evaluated by injecting the blank, placebo, standard preparation and sample preparation into the chromatographic system and the retention times were measured. The recorded chromatograms are shown.

No peak was observed at retention time of methohexitalhydrochloride for blank and placebo. Specificity results of methohexitalhydrochloride.

Linearity:

Different aliquots of standard methohexitalhydrochloride solutions containing variable amounts of the analyte were injected into the chromatographic column and the chromatograms were recorded. The peak areas of the resultant chromatograms were recorded and tabulated. The calibration plot drawn between the peak areas and concentration of the analyte showed that the method is suitable for the determination of methohexitalin the range 10 - 60 µg mL-1.

Precision

To evaluate system precision, six sample preparations of methohexitalsolution were injected into an ultra-performance liquid chromatographic system under optimal conditions and the chromatograms were recorded. The relative standard deviation was calculated for methohexitaland obtained as 0.24% and 0.56 % which was found to be satisfactory against the prescribed limits.

Accuracy

Different known aliquots of methohexitalstandard solution containing different known amounts, each one in triplicate, except lower and maximum concentrations were injected into the chromatographic column and the chromatograms were recorded under established experimental conditions. From the peak area values the mean recoveries of methohexitalwere evaluated .

Accuracy study found that the mean recovery of methohexitalwas between 99.06% and 100.56% at different concentration levels (10 – 60 µg mL-1).The small variation in the recovery percentages of methohexitalobtained at different concentration levels indicate that the obtained results are accurate.

Results for Detection and Quantitation limits:

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. From the standard stock solution 0.04 ml was pipetted out into 10 ml volumetric flask and the volume was made up to the mark with distilled water. The Limit of detection was found to be 0.084 µg/ml.

Limit of quantitation (LOQ):

Based on the LOD strength (0.15 mcg / ml, standard solution), the LOQ values were calculated by multiplication with three times. From the standard stock solution 0.15 ml was pipetted out was placed into 10 ml volumetric flask and volume was made up to the mark with distilled water. The Limit of quantitation was found to be 0.280 µg/ml.

Robustness

The robustness study of the proposed method was carried out with respect to organic solvent, flow rate and column oven temperature. The chromatographic conditions were maintained same as per test method in each case. The results are shown in Table. From the obtained results, it was observed that there was no much variation in retention time, theoretical plates and asymmetry of methohexitalpeak, obtained at different wavelength varied conditions from the test method. Hence the method is robust for all the varied conditions.

III. Conclusion

The literature survey revealed that almost all the reported chromatographic methods for the validation of methohexitalare based on RP-HPLC principle. The present proposed method is the Robustness Condition Tailing factor % RSD Normal Condition 1.6 0.5 Organic solvent (Acetonitrile) -10% 1.6 0.4 Organic solvent (Acetonitrile) +10% 1.7 0.8 Flow changed to 1.2 mL minute-1 1.1 0.2 Flow changed to 0.8 mL minute-1 1.5 0.2 Column Temperature changed to 30°C 1.6 0.3 Column Temperature changed to 25°C 1.6 0.5 first novel UPLC method.

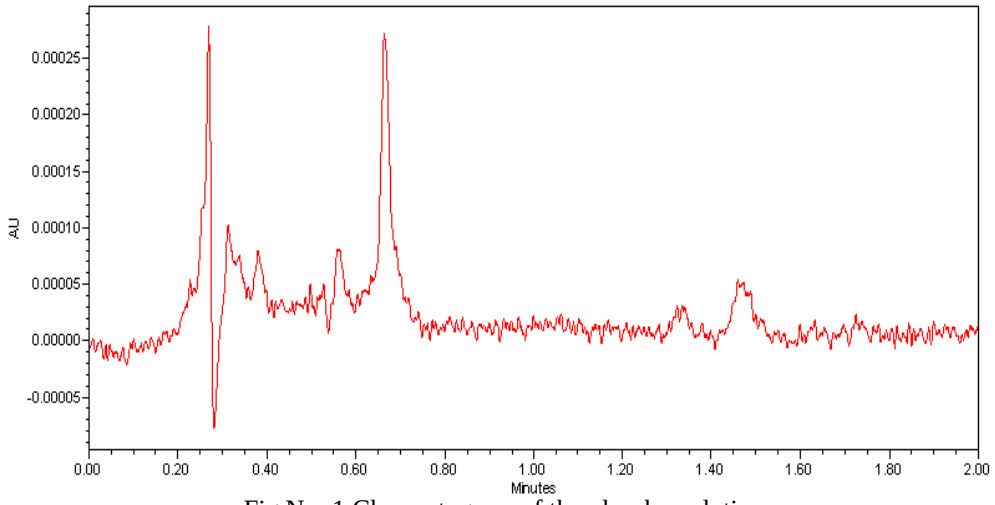

Fig No: 1 Chromatogram of the placebo solution

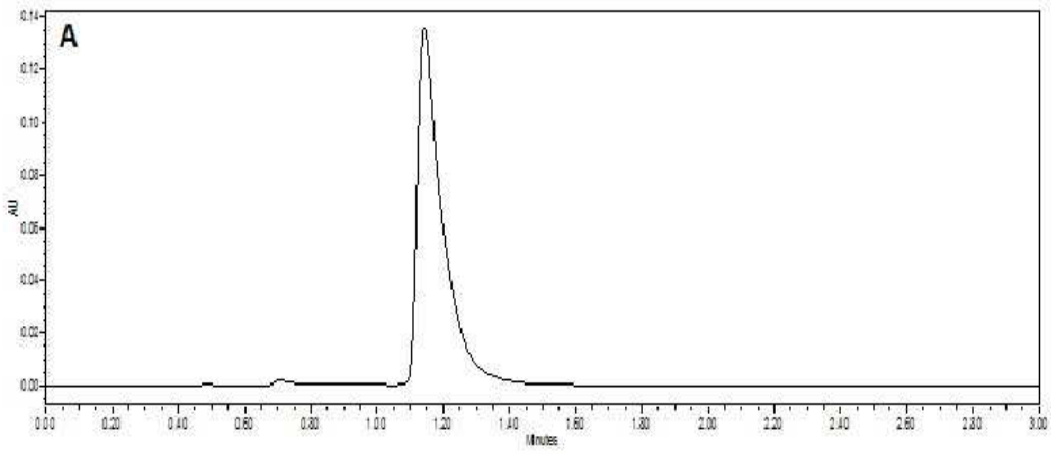

Fig No: 2 Chromatogram of the Standard solution

Sl no	Sample	Retention time
1	Blank	-
2	Placebo	
3	Standard	1.303
4	Sample	1.317

Table no:1 Specificity Results of Methohexital

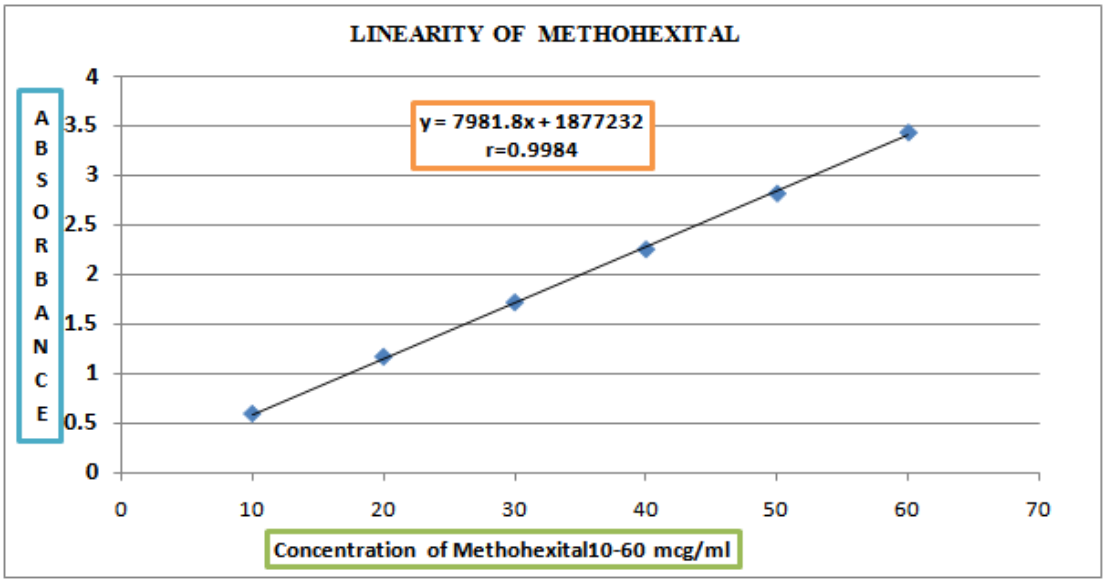

Fig No:3 Calibration curve of Methohexital 10 -60 mcg/ml

S.No	Precision Studies			
	INTRA DAY	ASSAY	INTER DAY	ASSAY
1.	0.532	100.08	0.531	99.89
2.	0.531	99.89	0.534	100.45
3.	0.53	99.70	0.535	100.64
4.	0.529	99.51	0.529	99.51
5.	0.531	99.89	0.538	101.21
6.	0.533	100.27	0.531	99.89
Mean	0.531	99.89	0.533	100.27
Std Dev	0.001291	0.2428	0.003	0.5643778
% RSD	0.24	0.24	0.56	0.56

Table no:2 Precision results of Methohexital

S. No	Validation parameter	Results
1	Limit of detection (LOD)	0.080 µg/ml
2	Limit of Quantitation (LOQ)	0.282 µg/ml

Table no:3 LOD & LOQ results of Methohexital.

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