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Research Article

A VALIDATED SIMULTANEOUS HPLC METHOD FOR DETERMINATION OF ASSAY OF TEMAZEPAM AND FORCED DEGRADATION STUDIES

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ABSTRACT

A new HPLC method was developed for selective and simultaneous determination of of Teamazepam. The developed method is also applicable for the related substances determination in bulk drugs. The chromatographic separation was achieved on a Zorbax SB C-18, 4.6 x 250mm, and 5µ column. The mobile phase consisted of Acetonitrile and methanol (60:40, *v/v*) delivered at a flow rate of 2.0 mL min^{-1}. Buffers consisted of dissolve 5.22 g of dipotassium hydrogen orthophosphate in 1000 mL of water and add 2 mL of triethylamine, adjust pH to 3.0 with ortho phosphoric acid. The mobile phase was pumped at a flow rate 2.0 mL per minute and detector of UV at 245 nm. In the developed HPLC method, the resolution between Teamazepam and its potential impurities, namely Imp-A, Imp-B, Imp-C, and Imp-G was found. Accuracy found by % recovery from 99.2-100.5 at 80.0% to 120.0% level and the linearity results for Teamazepam and its related compounds in the specified concentration calibration curves linear with coefficient of variation (r) not less than 0.99 The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur acid hydrolysis conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 95% - 105%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness. The validation was performed according to the current requirements as laid down in the ICH guidelines.

Keywords: HPLC, ICH guidelines, chromatography

INTRODUCTION

Temazepam (marketed under brand names Normison, Temtabs, Euhypnos, Restoril, Remestan, Tenox, Temaze and Norkotral) is an intermediate-acting 3-hydroxy benzodiazepine. It is generally prescribed for the short-term treatment of sleeplessness in patients who have difficulty maintaining sleep. In addition, temazepam has anxiolytic (anti-anxiety), anticonvulsant, and skeletal muscle relaxant properties[1-3]. Temazepam was first synthesized in 1964, but it first came into use in 1969 when its ability to counter insomnia was realized[4]. By the late 1980s, temazepam was one of the most popular and widely prescribed hypnotics on the market and it became one of the most widely prescribed drugs. Temazepam is a hypnotic agent. In sleep laboratory studies, temazepam significantly decreased the number of nightly awakenings[5] but has the drawback of distorting the normal sleep pattern[6].

Temazepam is officially indicated for severe insomnia and other severe or disabling sleep disorders. The prescribing guidelines in the UK limit the prescribing of hypnotics to two-to-four weeks due to concerns of tolerance and dependence[7].

Temazepam should not be used in pregnancy, as it may cause harm to the fetus. The safety and effectiveness of temazepam has not been established in children; therefore temazepam should generally not be given to individuals under 18 years of age, and should not be used at all in children under 6 months old. Benzodiazepines also require special caution if used in the elderly, alcohol or drug-dependent individuals and individuals with comorbid psychiatric disorders[8].

The smallest possible effective dose should be used in elderly or very ill patients, as there is a risk of apnea and/or cardiac arrest. This risk is increased when temazepam is given concomitantly with other drugs that depress the central nervous system[9].

Chronic or excessive use of temazepam may cause drug tolerance, which can develop rapidly[10], so this drug is therefore not recommended for long-term use. In 1979 the Institute of Medicine (USA) and the National Institute on Drug Abuse stated that most hypnotics lose their sleep-inducing properties after about 3 to 14 days[11]. In use longer than 1–2 weeks, tolerance will frequently develop towards the ability of temazepam to maintain sleep, so that the drug loses effectiveness[12]. Some studies have observed tolerance to temazepam after as little as one week's use[13]. Another study examined the short-term effects of the accumulation of temazepam over 7 days in elderly inpatients, and found that little tolerance developed during the accumulation of the drug[14]. Other studies examined the use of temazepam over six days and saw no evidence of tolerance[15,16].

MATERIALS AND METHODS

Reagents and Chemicals

HPLC grade Acetonitrile and methanol, triethylamine, anhydrous potassium dihydrogen orthophosphate and Ortho Phosphoric acid (85 %) from Merck Research Laboratories, India.

Chromatographic conditions

Chromatographic system consisted of a Waters Model Alliance 2489 separation module equipped with auto sampler Photodiode array ultraviolet (UV) detector. The data recorded using empower software. A gradient HPLC analytical column; Zorbax SB C-18, 4.6 x 250mm, particle size 5 µm; and detector of UV at 225 nm.

Preparation of standard solution

About 100 mg of Teamazepam r standard was transferred into a 50 mL volumetric flask. Dissolved in and diluted to volume with diluent of methanol. Diluted 5.0 mL of this solution to 10 mL with diluent of methanol. Prepared in duplicate.

Validation of HPLC method

In order to confirm method suitability during routine quality control use, the proposed method was checked critically for the following validation characteristics as per ICH guidelines.

Linearity

Linearity for Teamazepam was determined in the concentration range 80 to 120 % of working concentration of standard. The peak area responses were plotted against the

corresponding concentrations and the r^2 values were calculated.

Precision

System precision: The system precision was performed by analysing system suitability standard solution six times. Results of Peak area of the impurities. The peak area variation observed for Teamazepam and impurities was less than 5.0

Method precision: Six samples of Teamazepam were analyzed as per the method. Each named impurity and total impurities were calculated on these replicates.

Intermediate precision or inter-day precision

The intermediate or inter-day precision of the method was determined by six replicate analysis of Teamazepam from sample, as per the proposed method by different instruments and (Waters Alliance 2489 and Shimadzu), by same analyst on different days. The average drug content and the % RSD were calculated in each case.

Accuracy (recovery studies)

Recovery studies were performed by standard addition method at three levels i.e. 80%, 100% and 120%. Known amounts of standard Teamazepam were added to pre-analyzed samples and they were subjected to proposed HPLC method. Results of recovery studies are shown in Table 1.

Stability of analytical solution

A sample solution of Teamazepam was prepared as per the proposed method. To this sample all known impurities were quantitatively spiked at specification limit concentration and stored at 10°C. The sample was injected into the system initially and at various time intervals. Sample solution spiked with impurities was found to be stable up to 1600 minutes at 10°C.

Robustness

The robustness study was done by making small changes in the optimized method parameters as indicated in Table 2. Results are also shown there.

Ruggedness

The ruggedness study was done by the two analysts by using the proposed method by same instrument on same day. The % RSD for each analyst for each drug was calculated.

Evaluation of system suitability

Inject blank (diluent), followed by reference solution in six times into the chromatograph and record the chromatograms. The system is suitable for analysis, if and only if, the resolution between Teamazepam and intermediate peaks not less than 2.0. The tailing factor for Teamazepam peak not more than 2.0. The number of theoretical plates for Teamazepam peak not less than 3000. Inject sample solutions into the chromatograph and record the chromatograms. Note sown the area response of peaks eluting. Disregard the peaks due to blank. The retention time of Teamazepam peak is about 14 minutes

Table 1: Assay results of intermediate precision

Sample	Analyst 1 - Day1	Analyst 2 - Day2	Bias
Sample-1	99.4	99.9	-0.5
Sample-2	99.2	99.9	-0.7
Sample-3	99.6	99.4	+0.2
Sample-4	99.2	99.7	-0.5
Sample-5	99.3	99.5	-0.2
Sample-6	99.5	99.9	-0.4
% RSD	0.16	0.24	

Table 2: Result of recovery studies

Drugs	Recovery levels	Mean % of recovery	% RSD
Teamazepam	80 %	100.1	0.25
	100 %	100.5	0.15
	120 %	99.2	0.05

Table 3: Robustness for Teamazepam

Parameters	Actual	Low level	High level
Flow variation	2.0 ml/min	1.8 ml/min	2.2 ml/min
Column oven temperature	30^0C	28^0C	32^0C
Buffer pH variation	3.0	2.8	3.2
Buffer composition variation	60:40	62:38	58:42

RESULTS AND DISCUSSION

In linearity study, the graphical representation of data proves that Teamazepam and linearity in the range of 0.16 mg/ml to 0.24 mg/ml with r^2 value 0.9997.

In system precision study, the % RSD for Teamazepam was found to be 0.12 respectively. The % RSD observed on the replicate indicates the precision of the system.

In method precision study, the mean % drug content for Teamazepam was found to be 99.2 % and 99.6 % respectively. The % RSD for Teamazepam was found to be 0.16. The results indicate that the method is validated for method precision. No interference forms other components or excipient was found during determination.

In intermediate or inter-day precision study, the mean % drug content for Teamazepam found to be 99.2 and 99.9 respectively. The % RSD for Teamazepam found to be 0.16 and 0.24 respectively. There is no significant difference by same analyst by different instruments on different day. Therefore the intermediate or inter-day precision of the method can be considered to be acceptable as shown in table 1.The bias in assay determination for each parameter less than ± 1.5.

In accuracy or recovery studies, the results are shown in Table 2. The overall % of recovery and % RSD for Teamazepam in marketed formulation indicated that there is no significant difference in percentage of recovery. Therefore, accuracy of the method considered acceptable as it was well within 99.2 to 100.5 %.

A sample solution of Teamazepam was prepared as per the proposed method. To this sample all known impurities were quantitatively spiked at specification limit concentration and stored at 10°C. The sample was injected into the system initially and at various time intervals. Sample solution spiked with impurities was found to be stable up to 1600 minutes at 10°C.

In robustness or system suitability study, there was no significant impact on the % RSD and tailing factor. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions.

Degradation study

Analyze the impurities and Teamazepam individually as per above method to verify the retention time. In order to assess the stability indicating nature of the HPLC method,

Teamazepam samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples are analyzed using a photodiode-array detector.

Sample Preparation

Accurately weigh and transfer about 50.0 mg of substance into a 100 mL volumetric flask, dissolve in and dilute of methanol. Dilute 5.0 mL of this solution to 10 mL with diluents.

Acid hydrolysis, Base hydrolysis and Oxidation

At room temperature accurately weigh and transfer about 100 mg of substance into a 100 mL volumetric flask, dissolved in and diluted to volume with diluents methanol. From this take 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N hydrochloric acid solution. The solutions were kept at room temperature for 3 hours, then neutralized with 0.2 mL of 1N sodium hydroxide solution and diluted to 10 mL with diluents methanol. At 600C one solution was prepared by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N hydrochloric acid solution. The solutions were kept at 600C for 3 hours, then neutralized with 0.2 mL of 1N sodium hydroxide solution and diluted to 10 mL with diluent of methanol.

At room temperature four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N sodium hydroxide solution. The solutions were kept at room temperature for 3 hours, 6 hours, 12 hours and 24 hours, then neutralized with 0.2 mL of 1N hydrochloric acid solution and diluted to 10 mL with diluent of methanol. At 600C four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N sodium hydroxide solution. The solutions were kept at 600C for 3 hours, 6 hours, 12 hours and 24 hours, then neutralized with 0.2 mL of 1N hydrochloric acid solution and diluted to 10 mL with diluent of methanol.

At room temperature four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 5% of hydrogen peroxide solution. The solutions were kept at room temperature for 3 hours, 6 hours, 12 hours and 24 hours and diluted to 10 mL with diluents of methanol.At 600C four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 5% of hydrogen peroxide solution. The solutions were kept at 600C for 3 hours, 6 hours, 12 hours and 24 hours and diluted to 10 mL with diluents of methanol.

Heat degradation, UV degradation

Four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask. The solutions were kept at 600C for 3 hours, 6 hours, 12 hours and 24 hours and diluted to 10 mL with diluents of methanol.

UV degradation was carried out by accurately weighed and transferred about 50.0 mg of substance into a 100 mL volumetric flask, dissolve in and diluted to volume with diluents of methanol. Taken 5.0 mL of above solution into a 10 mL volumetric flask and exposed to an integrated near Ultra violet energy (UV light) of not less than 200 watt/square meter and then diluted to 10 mL with the diluents.

CONCLUSION

Hence, it can be concluded that the newly developed RP-HPLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for Teamazepam without any interference from other components or additives. This method can be employed conveniently, reliably and successfully for the estimation of Teamazepam for routine quality control and stability studies.

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