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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF PENTAZOCINE IN PARENTRAL DOSAGE FORM

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ABSTRACT

A reverse phase isocratic HPLC was developed and validated for the determination of Pentazocine in bulk and parental dosage forms. Method development was carried out on ODS C_{18} column 250x4.6mm, particle size 5 µm, maintained at ambient temperature, Shimadzu UFLC-LC 20. The mobile phase was a mixture of Methanol: Phosphate Buffer (68:32 v/v), with apparent pH of 3 and the flow rate was set at 1.0 ml/min and UV detection at 285 nm. The chromatographic retention time of proposed method was 3.96 min and the mean assay of content was found to be 99.30%. After completion of chromatographic separation of pentazocine going to do validation of the drug according to USP. The proposed method was successfully applied for the quantitative determination of Pentazocine in bulk form and could be used for routine analysis with phenomenal accuracy and precisions.

KEYWORDS: Pentazocine, Mobile phase, Validation, Accuracy, precision, Retention time.

INTRODUCTION

Pentazocine is a synthetically-prepared prototypical mixed agonist–antagonist narcotic (opioid analgesic) drug of the benzomorphan class of opioids used to treat moderate to moderately severe pain. This compound may exist as one of two enantiomers, named (+)-pentazocine and (-)-pentazocine. (-)-pentazocine is a κ-opioid receptor agonist, while (+)-pentazocine is not, instead displaying a ten-fold greater affinity for the σ receptor. Usually, in its oral formulations, it is combined with naloxone so as to prevent people from crushing the

tablets, dissolving them in a solvent (like water) and injecting them for a high (as naloxone is not orally bioavailable it produces no effect when the formulation is used orally, but it blocks the opioid effects of pentazocine if injected intravenously for a high). Its chemical name is 2-dimethyl allyl-5,9-dimethyl-2'-hydroxy benzmorphan and molecular formula is $C_{19}H_{27}NO$. It belongs to a category of opioid analgesic. Its MOA includes κ-opioid receptor agonist.

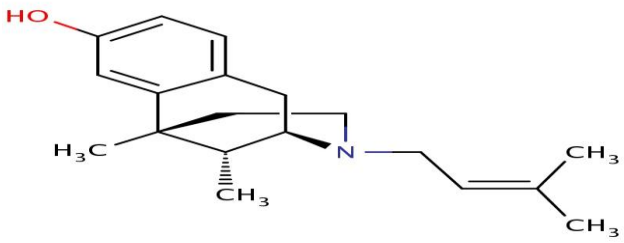

Fig.1: Chemical structure of Pentazocin.

MATERIALS AND METHODS

All reagents and chemicals used are of HPLC grade Methanol purchased from Merck, India and HPLC grade water is used to prepare the mobile phase. Stock solutions of pentazocine and sample solutions are prepared using HPLC grade water. All solutions are filtered through 0.45 µm membrane filter and degassed using a sonicator. The formulations are purchased locally from market.

METHOD DEVELOPMENT

Preparation of Mobile Phase

Methanol HPLC Grade mixed with Phosphate buffer in the ratio of 68:32 v/v, which was then filtered through membrane filter of 0.45µ Millipore membrane filter before use and degassed in an ultrasonic bath.

Buffer Used

1.36gms of potassium dihydrogen ortho phosphate and 2ml of 3 % (v/v) solution of triethylamine are dissolved in 800ml of water. pH is adjusted to 3 with ortho phosphoric acid and the volume is made up to 1000ml with water.

Preparation of Standard Solutions

An accurately weighed quantity of Pentazocine were transferred to 100 ml volumetric flask, and made up to volume with water and individual chromatograms were recorded.

Working Standard Solution (150µg/Ml)

Working standard solution is prepared daily by accurately measuring 1ml of the stock solution into 10ml volumetric flask and made up to the mark with water and standard chromatograms of pentazocine were depicted.

Optimized Chromatographic Conditions

Stationary Phase : C_{18} ODS with particle size of 5µm.
Mobile Phase : Buffer: Methanol
Solvent Ratio : 32:68v/v
pH : 3
Detection λ : 285 nm.
Flow Rate : 1.0 ml/min.
Injection Volume : 20µl.
Temperature : Ambient.
Mode : Isocratic.
Run Time : 3.96 minutes.

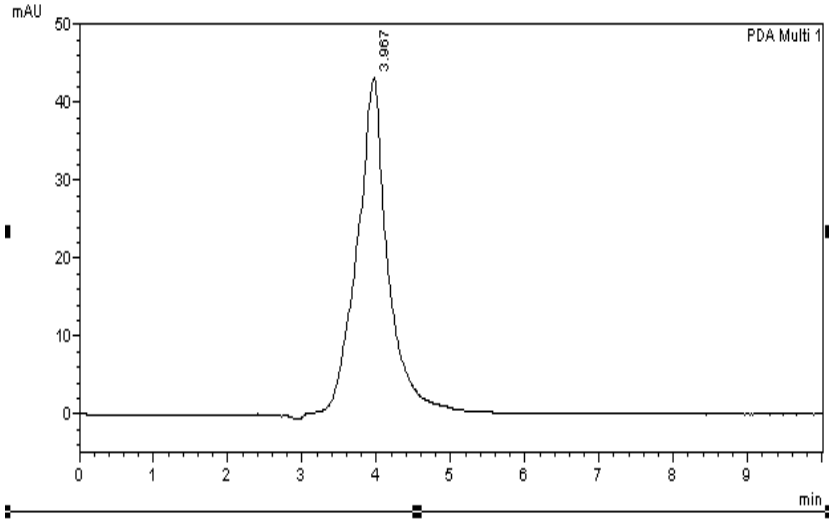

Fig.2: Chromatogram of Pentazocine in injection formulation by HPLC method.

Method Validation

Validation of analytical methods is important in the analysis of pharmaceutical formulations. The ability to control the quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria. Analytical method validation is now required by regulatory authorities for marketing authorizations and guidelines have been

published. It is important to isolate analytical method validation from the selection and development of the method. The most widely applied validation characteristics are given below.

System Precision

Working standard solution (90µg/ml) of pentazocine is injected six times into the HPLC system separately and the chromatograms are recorded. The system precision is evaluated based on the area of the peaks and found to be within the limits. The results are incorporated in Fig.3. The % RSD for peak areas of six replicate injections of pentazocine is determined and found to be 0.68.

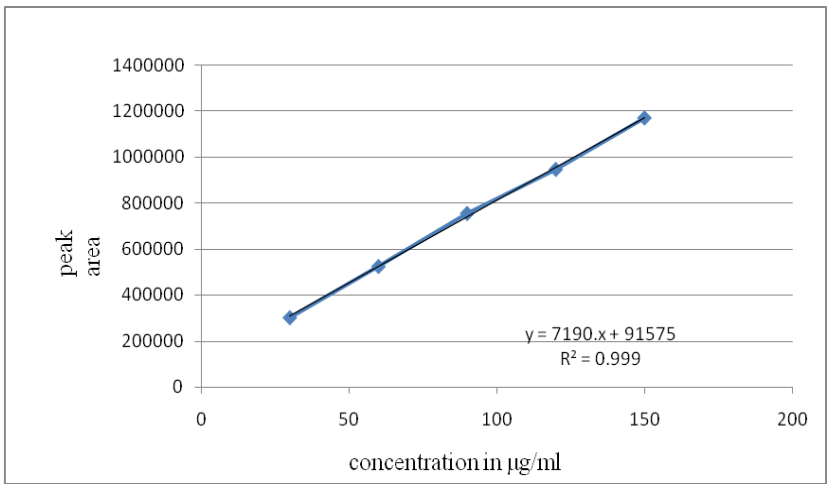

Fig.3: Calibration plot of Pentazocine by HPLC method.

Linearity of detector response

Linearity was assessed by injecting 20µl of five different standard concentrations obtained by diluting standard stock solution with water under optimized chromatographic conditions, which provides 30, 60, 90, 120,150 µg/ml of pentazocine. A graph is plotted to concentration in µg/ml on X-axis versus response on Y-axis. The detector response is found to be linear with a squared correlation coefficient of 0.999.

Tab.1: Linearity of Pentazocine

Concentration of Pentazocine (µg/ml)	Peak area of Pentazocine
30	301261.9
60	523722.5
90	754165.4
120	945627.4
150	1168915.8

Accuracy and Recovery

Recovery assessment was performed by adding known quantities of pure standards at three different levels in 50%, 100% and 200% to the preanalyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate and chromatograms were presented.

Tab.2: Accuracy Study Results.

Drug	Amount Added (mg)	Amount Recovered* (mg)	% Recovery*	Average Recovery (%)	% RSD
PENTAZOCINE 30mg	15 30 60	121.73 243.82 486.35	97.39 97.53 97.27	97.39	0.13

*Mean of three determinations at each level

Precision of test method

Commercial formulation of pentazocine injection is successfully analyzed. The precision of the test method is evaluated by taking six replicate measurements. The average percent of assay of pentazocine is found to be 99.30% and %RSD is found to be 0.286 and 0.564 respectively.

Robustness

Robustness is a measure of the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters such as per cent organic content in the mobile phase, pH of the mobile phase, buffer concentration, temperature, injection volume and column temperature. These parameters may be evaluated one factor at a time or simultaneously as part of a factorial experiment. It also provides some indication of the reliability of an analytical method during normal usage Six separately prepared sample solute.

CONCLUSION

Although different methods by using different techniques have been reported for the estimation of pentazocine, till now no RP-HPLC method development and validation has been published for this drug. The proposed HPLC method employed is found to sensitive, accurate, precise, non-destructive and economically reliable for the routine determination of pentazocine. The methods also provide efficient reproducible results obtained were within

limits. The determination of pentazocine by RP-HPLC analysis yielded well resolved peaks within short analysis time of < 3.96 min. The values of standard deviation were satisfactory low and recovery was close to 100% for both the methods indicates its accuracy and good precision (<2%) and also shows that the method is free from interferences of the excipients used in the formulation. The method no where involves the use of complicated sample preparations and its speedy nature appears to be equally suitable for the routine quantitative analysis in Pharmaceutical formulation.

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