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A COMPREHENSIVE REVIEW ON STABILITY-INDICATING UPLC METHOD FOR SIMULTANEOUS ANALYSIS OF *XANOMELINE* AND *TROSPIUM CHLORIDE* IN FORMULATION

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ABSTRACT This article is focused on developing a fast, sensitive and dependable analytical technique which is essential for the simultaneous measurement of Trospium chloride & Xanomeline medications meant to treat neuropsychiatric conditions, especially Schizophrenia and Alzheimer's disease in biological matrices and pharmaceutical formulations. The advantage of Ultra-Performance Liquid Chromatography (UPLC) over traditional HPLC method is emphasized in this article and includes higher resolution, increased sensitivity and shorter processing times. The investigation includes important elements like the composition of the mobile phase, the choice of column, the wavelength of detection, and the validation of the method (accuracy, precision, linearity, limit of detection, and limit of quantification) and also with stability indicating studies.

KEYWORDS : Xanomeline, Trospium chloride, Method Development and Validation, Stability studies.

INTRODUCTION

Two pharmacologically different substances that have drawn a lot of interest in the fields of neuropsychiatric and urological treatments are xanomeline and trospium chloride. Originally created to treat Alzheimer's disease. Xanomeline-3-hexoxy-4-(1-methyl-3,6-dihydro-2H pyridin-5yl)- 1,2,5-thiadiazole is a muscarinic receptor agonist that selectively targets M1 and M4 receptors. It has demonstrated encouraging results in treating schizophrenia by enhancing cognitive function and lowering psychotic symptoms. However there are peripheral cholinergic side effects such as nausea, vomiting. Its quaternary ammonium structure of Trospium chloride (1,3,5)-3-[Hydroxy diphenyl acetyl) oxy] spiro [8 azoniabicyclo octane-8,1-pyrolidinium) chloride, a muscarinic antagonist mostly used to treat overactive bladder, gives it a distinct pharmacokinetic profile by preventing considerable penetration of the central nervous system. Its combination has generated interest as a potential substitute for conventional antipsychotic therapies in the treatment of neuropsychiatric diseases including schizophrenia, as a unique therapeutic approach. The development of a UPLC method that can simultaneously detect and quantify trospium chloride and Xanomeline is a unique and most required analytical challenge which is not developed upto now.

MATERIALS AND METHODS

Chemicals

The methanol, acetonitrile and water are employed for sample dilution and mobile phase preparation. To create aqueous buffer phases, either potassium dihydrogen phosphate or ammonium acetate are utilised. Orthophosphoric acid or Trifluoroacetic acid (TFA) are employed as an acidic modifier in the mobile phase.

Instrumentation

A Water-acquired UPLC or Agilent 1290 Infinity II UPLC with PDA detector and empower 2.0 software, analytical balance, an ultrasonic cleaner and a pH meter is required.

CHROMATOGRAPHIC CONDITION

The muscarinic receptor agonist Xanomeline has moderate lipophilicity of 3.77 makes it easier for it to interact with hydrophobic stationary phases and affect retention time, and molecular weight is around 281.42 g/mol. Basic amine groups have a pKa value of 6.70, which affects chromatographic separation and peak characteristics by influencing their ionization state based on the pH of the mobile phase (3.0 to 5.0). When using the right solvent in RP-UPLC analysis its polarity and hydrophobic interactions control how it partitions between the mobile and stationary phases. Trospium chloride, a quaternary ammonium muscarinic receptor antagonist, has a molecular weight of about 427.97g/mol and a pKa value of 11.05, which indicates that it is highly hydrophilic and provides high aqueous solubility with minimal lipophilicity of 0.50. Its strong ionic nature has a significant impact on its retention on hydrophobic stationary phases, frequently leading to shorter retention times unless specific mobile phase modifications or ion-pairing agents (such as alkyl sulfonates and per fluorinated carboxylic acids) are used. For the Trospium chloride, in contrast to lipophilic substances. The pH (5.0–7.5) and makeup of the mobile phase conditions must be carefully chosen. Buffer selection is important for peak form and resolution because of its strong ionic nature; acidic pH conditions usually improve retention by modifying ion-exchange interactions.

Column Selection

Xanomeline and trospium chloride are polar medications. A C8 (octylsilane) or C18 (octadecylsilane) column works well on a non-polar stationary phase. The retention duration is moderate and is mostly dictated by hydrophobic interactions between the non-polar parts of the medication and the C18 stationary phase.

Mobile Phase

Trospium chloride as well as xanomeline using an isocratic elution process can help to allow controlled separation. One possible solution is to use a mixture of acetonitrile and water with a small amount of organic solvent. Due to the high ionic strength of trospium chloride, a heptane sulfonic acid or tetra butyl ammonium ion-pairing agent may be required to help in enhancing retention.

pH range

The pH of the sample rises when the analyte is more hydrophobic. A pH of ±1.5 pH units above or below pka and an equal concentration of ionic and non-ionic species are required for proper 100% uninization.The pH range for Xanomeline mobile phase should be 3.0-5.5 based on the pKa of 6.70 and Trospium chloride has a pH range of 5.0 to 7.5 based on the pka 11.05 by switching up a buffer, like acetate or phosphate buffers is used.

Flow Rate

For both compounds In RP-UPLC, the normal flow rate is between 0.2 and 0.5 mL/min; however, this can be changed according to the size of the column and the required analysis duration. The effectiveness of separation can be increased with lower flow rates.

Temperature

The temperature used for both compounds is between 30 and 40°C.Temperature influences the viscosity of the mobile phase and the interaction between the analyte and the stationary phase, so a moderate temperature enhances resolution.

Detection

UV detection at roughly 200-250 nm may be used for both xanomeline and Trospium chloride,

METHOD VALIDATION

The method used to do the analysis is called the analytical technique. A thorough explanation of every step required to carry out each analytical test should be included. The reference standard, the sample, the equipment, the reagent preparations, the calibration curve, the application of the calculation formula, etc. The approach needs to be

validated in terms of its specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness.

System Suitability
The chromatographic system's analytical appropriateness and repeatability are assessed by its system applicability. Prior to sample analysis, five replicate injections of the reference solutions are carried out to assess the system's suitability. Acceptance criteria include resolution (Rs) >2%, USP plate count (N) >5000, USP tailing factor (T) <2.0%, and USP plate count (N) <2% RSD for peak areas.

Accuracy
It describes the degree of agreement between the measured and real values. Accuracy is the proportion of recovery that happens when the assay is used to calculate the known amount of analyte that was given to the sample. At least three concentration levels (50, 100, and 150 %) within the designated range should be taken, according to the ICH recommendations, and three replicates of each concentration should be analysed, yielding nine results in total. Recovery concentration RSDs up to 2.0% and FDA recovery percentages ranging from 98 to 102% are acceptable.

Precision
It describes how closely several measurements taken from the same homogeneous material under particular circumstances agree. One typically utilizes the standard deviation or relative standard deviation of a set of measurements to characterize the precision of a test procedure. Measured RSDs should be upto 2%.

Repeatability
Using six determinations made on the same day for 100% level of the test concentrations like medium quality control (MQC) repeatability is evaluated. Requirement for acceptance is % RSD<2.0%.

Intermediate Precision
Using six determinations made at 100% of the test concentration on several days and with various instruments were used to gauge intermediate precision. Acceptance criteria is %RSD≤2.0%.

Reproducibility
Reproducibility was assessed using 6 determinations at 100% of the test concentration in two different laboratories. Acceptance criteria is %RSD≤2.0%.

Linearity
In a mixed solution, a linearity study is conducted using two analytes at a minimum of five concentrations, which covered the whole expected drug concentration range for the assay and dissolution research. Acceptance criteria the regression line must have a correlation coefficient of NLT 0.999 in order to be accepted.

Specificity
Specificity of the method is performed by injecting the placebo solution and comparing with the blank solution. (5 to 6 determinations are performed).

Limit Of Detection (LOD)
The amount of the analyte that reliably distinguishes it from noise. Five to six injections are administered using this procedure in order to determine LOD.
LOD=3.3 σ/slope
(σ standard deviation)

Limit Of Quantification (LOQ)
It indicates the lowest analyte concentration that can be accurately measured; a signal to noise ratio of 10:1is usually needed. To measure LOQ, five to six injections are made in this procedure**.**
LOQ=10.1σ/slope

Ruggedness
The extent to which test results can be replicated when the same material is analysed using a different instruments, different reagents, various days, and a different analyst. The recommendations stated below are implemented. Criteria for acceptance: All system stability parameters must be met.

Robustness
The following minor but intentional changes in the analytical condition were used to demonstrate robustness: Variations represents light increases or decreases.
Flow Rate: ± 0.2 ml per minute

Forced Degradation Studies
Forced degradation studies are conducted by subjecting the medications to different stress settings in order to establish the analytical approach. For a stability indicating method. According to ICH QIA, stress tests were conducted in the following conditions: hydrolysis, oxidation, and reduction of acids and bases (R2). It is planned to carry out a number of experiments with varying degrees of stress in order to attain a deterioration of between 10 and 30 percent.

Acid degradation In a 10-milliliter volumetric flask, one milliliter of the sample stock solution and one milliliter of I N HCl are combined, and the mixture is let to settle for fifteen minutes. Use diluents after adding 1 milliliter of 1N NaoH after 15 minutes to make up the difference. Measure the absorbance to measure degradation.

Alkali Degradation One milliliter of the sample stock solution is placed in a10ml volumetric flask, followed by one milliliters of 1N NaOH,and allowed to sit for 15 minutes. Add1 ml of 1N HCl after15 minutes, and use diluents to make up the difference. Measure the absorbance to measure degradation.

Peroxide Degradation1 ml of the sample stock solution is transfer to a10ml volumetric flask, then add 0.3ml of 30% hydrogen peroxide and to pitup with diluents. Measure the absorbance to measure degradation.

Reduction Degradation A 10 ml volumetric flask is filled with 1 ml of the sample stock solution, 1 ml of a 30% sodium bisulphate solution, and diluents to make up the difference. Measure the absorbance to measure degradation.

Thermal Degradation For 6 hours, the sample stock solution is placed in an oven set at 105°. In UPLC, the resulting solution was injected. Measure the absorbance to measure degradation.

Hydrolysis Degradation In a 10 ml volumetric flask with 1 ml of the sample stock solution, 1 ml of UPLC water was added. After that, the residual volume is altered using diluents. Measure the absorbance to measure degradation.

CONCLUSION
The current study offers thorough insight into the development and validation of a stability indicating method for the UPLC-based quantification of Trospium chloride and Xanomeline in a controlled dosege form.

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