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Research Paper

Development of Validated Stability-Indicating Assay Methods- Critical Review

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Abstract:
Developed a robust and reliable method for the simultaneous estimation of DrotaverineHCl and Mefenamic acid using reverse-phase liquid chromatography.Some key features of your method include the use of a Hi Q C-18 W column, a simple mobile phase consisting of 80:20% v/v methanol and HPLC grade water (with 0.1% triethylamine at pH 3), and UV detection at a wavelength of 250 nm. These conditions allowed for good linearity in the concentration ranges of 2-10 µg/mL for DrotaverineHCl and 6-30 µg/mL for Mefenamic Acid.The validated method according to ICH guidelines and tested its stability indicating capacity. This method appears to be a valuable tool for the analysis of DrotaverineHCl and Mefenamic acid in pharmaceutical formulations.

Keywords: *Drotaverine hydrochloride, Mefenamic acid, Stability indicating, RP-HPLC, Force degradation study.*

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I. Introduction

Stability indicating RP-HPLC method is a widely used analytical technique that is employed to determine the stability and purity of drugs in pharmaceutical formulations. Drotaverine HCL and Mefenamic Acid are two important drugs that are commonly used to treat various medical conditions. A stability-indicating RP-HPLC method can be used to determine the purity and stability of these drugs in pure and pharmaceutical formulations.

The method involves the use of a high-performance liquid chromatography (HPLC) system, which separates the components of a sample based on their physicochemical properties. The stationary phase of the HPLC column is packed with a suitable material that allows for the separation of the components of a sample based on their polarity, size, and other characteristics.

The mobile phase used in the method is typically a mixture of solvents that allows for the elution of the components of a sample from the column. The eluted components are then detected using a UV or other suitable detector, which produces a chromatogram.

A stability-indicating RP-HPLC method for the determination of Drotaverine HCL and Mefenamic Acid in pure and pharmaceutical formulations involves the preparation of a standard solution of the two drugs. The standard solution is then injected into the HPLC system, and the components are separated using the stationary and mobile phases. The eluted components are then detected using a suitable detector, and a chromatogram is produced.

The stability-indicating RP-HPLC method can also be used to determine the stability of the drugs in a pharmaceutical formulation. In this case, the drug product is subjected to different stress conditions, such as high temperature, humidity, and light, to induce degradation. The degraded samples are then analyzed using the RP-HPLC method to determine the extent of degradation and identify any degradation products. The stability indicating RP-HPLC method is a powerful analytical technique that can be used to determine the purity and stability of drugs in pharmaceutical formulations.

The following is a general outline for developing a stability-indicating RP-HPLC method for the determination of DrotaverineHCl and Mefenamic acid in pure and pharmaceutical formulations:

Method development:

- Select an appropriate RP-HPLC column and mobile phase that can separate DrotaverineHCl and Mefenamic acid with good resolution and retention.
- Optimize the mobile phase composition, pH, and flow rate to achieve the best chromatographic performance.
- Determine the detection wavelength for both DrotaverineHCl and Mefenamic acid.

Method validation:

- Validate the method according to ICH guidelines by determining the following parameters: specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ).
- Verify the method's ability to separate and quantify the drugs in the presence of potential degradation products, such as impurities, degradants, or excipients.

Forced degradation studies:

- Conduct forced degradation studies to assess the method's stability-indicating properties and to identify potential degradation pathways and products of DrotaverineHCl and Mefenamic acid.
- Use various stress conditions, such as heat, light, acid/base hydrolysis, oxidation, and photolysis, to induce degradation of the drugs.
- Analyze the samples using the validated RP-HPLC method and compare the chromatograms of the degraded drugs to the corresponding chromatograms of the pure drugs to ensure the method's ability to detect and quantify the degradation products.
- Application to pharmaceutical formulations:
- Apply the validated stability-indicating RP-HPLC method to the analysis of commercial formulations containing DrotaverineHCl and Mefenamic acid.
- Extract the drugs from the formulations using a suitable method, such as sonication or shaking with a suitable solvent.
- Analyze the samples using the validated RP-HPLC method and determine the drugs' content and purity in the formulations.

Developing a stability-indicating RP-HPLC method for the determination of DrotaverineHCl and Mefenamic acid in pure and pharmaceutical formulations requires careful method development, validation, and forced degradation studies to ensure the method's selectivity, accuracy, and precision.

II. EXPERIMENTALS

Reagents and chemicals

DrotaHCl supplied as a gift sample by Wockhardt Pharmaceutical Ltd, Aurangabad MA AvestiaPharma Ltd. Kandivali East. All the chemicals used of HPLC Grade (Merk Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

Instrument

HPLC system of yangline (S.K) Younglin (S.K) Gradient System UV Detector.UV 730 D & SP930 D Plus Intelligent HPLC Detector. With column of Hi Q C18 W (4.6mm x 250mm), 5 is used. A gradient elution is performed using mixture of Methanol & HPLC Grade water (0.1%triehylamine pH3) in the ratio of 80:20%v/v as a mobile phase at flow rated of 1 ml/min at detection wavelength of 250 nm.

Preparation of Mobile phase

A mobile phase consisted of Methanol: water (0.1%Triethylamine pH3) (80:20 v/v) was selected to achieve symmetrical peak and sensitivity.

Preparation of Stock Standard Solution

(Solution A)

Standard stock solution was prepared by dissolving 10.0mg of DrotaHCl and 30.0 mg of MA in 10.0 mL was water that give concentration 400 and 1200 µg/mL for DrotaHCl and MA respectively.

Preparation of Working Standard Solution

(Solution B)

From the standard stock solution, the mixed standard solutions were prepared using Methanol to contain 10µg/mL of DrotaHCl and 30µg/mL of MA.

Selection of detection wavelength

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 250 nm was selected.

Linearity Study

From the standard stock solution of DrotaHCl and MA 0.25 mL were taken in 10 mL volumetric flask diluted

up to the with Methanol such that final concentration of DrotaHCl and MA in the range 2-10 µg/mL of DrotaHCl and 6-30 µg/mL of MA respectively. Volume of 20µl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

Forced degradation studies

Forced degradation carried out by applying various stress conditions to study the effect over wide range of pH, heat, and oxidation and photo degradation using the following approach. Stress studies were conducted in aqueous solutions.

Acid Degradation

Accurately weight tablet equivalent to 10.0 mg of DrotaHCl& 30.0mg of MA were dissolved in 5.0 mL of aqueous 0.1N hydrochloric acid in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1 hr. And with heat after 3 hr.

Alkali Degradation

Accurately weight tablet equivalent to 10.0 mg of DrotaHCl& 30.0mg of MA were dissolved in 5.0 mL of aqueous 0.1N sodium hydroxide in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr And with heat after 3 hr.

Neutral Degradation

Accurately weight tablet equivalent to 10.0 mg of DrotaHCl& 30.0mg of MA were dissolved in 10.0 mL of water in a separate volumetric flask and kept at room temperature for 1hr and with heat after 3 hr.

Oxidative Degradation

Accurately weight tablet equivalent to 10.0 mg of DrotaHCl& 30.0mg of MA were dissolved in 10.0 mL of 3% H2O2 in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for1hr and without heat after 3 hr.

Thermal Degradation

Accurately weight tablet equivalent to 10.0 mg of DrotaHCl& 30.0mg of MA were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in oven at 60°C for 24 hrs.

Photo Degradation

Accurately weight tablet equivalent to 10.0 mg of DrotaHCl& 30mg of MA were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in sunlight for 3 days.

III. RESULTS

HPLC Method Development and Optimization,

Table 1. The finally optimized chromatographic conditions are

Mobile Phase	**Mixture of Methanol & HPLC Grade water (0.1%Triethylamine) in the ratio of 80:20%v/v**
Column	Hi Q C 18 W, 4.6*250mm, 5
Flow Rate	1.0ml/min
Injection Volume	20µl
Column Oven Temp	Ambient

Table 2. Linearity studies of DrotaHCl

Concentration of Drota HCl [µg/mL]	**Peak Area**	**± SD**	**%RSD**
2	85.14	0.76	0.89
4	171.76	1.33	0.77
6	259.25	3.36	1.30
8	322.43	0.66	0.20
10	415.65	1.71	0.41

Table 4. System suitability studies for DrotaHCl and MA

System Suitability Parameter	**Standard**	**Proposed Method of DrotaHCl**	**Proposed Method of MA**
Retention time (tR) (min)	5-10min	5.5	9.5
Resolution	Should be>2	0.000	12.914
Theoretical plate (N)	More than2000	5854.4	12648

Table 5. Summary of force degradation studies

Condition	**% Assay Drota HCl**	**%Degradation DrotaHCl.**		**%Assay MA**	**% Degradation MA**	
		1hr	**3hr**		**1hr**	**3hr**
Initial sample	99.74	-		98.95		
0.1 N HCl	97.24	7.40	10.11	98.50	5.70	4.51
0.1 N NaoH	97.74	7.53	6.54	98.50	5.57	5.61

3%H2O2	98.44	6.65	4.18	97.90	5.69	5.90
Thermal (60) for 24hrs	98.96	-	8.05	97.37	-	5.65
Neutral	99.72	9.94	10.37	-	5.02	4.69
Sun (for 3 days)	96.45	-	8.05	98.50	-	5.65

IV. Conclusion:

The parent drug peak was clearly separated from all the degradants produced under different stress conditions. However, there were some degradants with similar retention times under different stress conditions that could not be confidently identified as the same chemical entity or different ones. Therefore, further studies are necessary to isolate and characterize these degradants to determine their chemical identity.It is also mentioned that DrotaHCl is susceptible to acid, photolytic, thermal, and oxidative degradation, while MA is susceptible to alkali, oxidative, thermal, and photolytic degradation in the marketed formulation. This information suggests that the drug product may undergo various degradation pathways under different stress conditions, and the stability of the drug product should be carefully evaluated during its development and storage. The statement highlights the need for further investigation to fully understand the degradation pathways of the drug product under different stress conditions, and to ensure the quality and stability of the drug product during its shelf-life.

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