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Stability indicating HPLC method for methocarbamol and diclofenac sodium in combined dosage form

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Abstract
High performance liquid chromatography is the most accurate method widely used for the quantitative as well as qualitative analysis of drug product and is used for determining drug product stability. Stability indicating HPLC method is used to separate various drug related impurities that are formed during the synthesis or manufacture of drug product. In this article, we will discuss the strategies and issues regarding the development of stability indicating HPLC system for drug substance**.** Many key chromatographic factors were evaluated in order to optimize the detection of all potentially relevant degradants. The method should be carefully examined for its ability to distinguish the primary drug components from its impurities. Newer chemical entities and drug products rely to undergo forced degradation studies which may be helpful in developing and demonstrating the specificity of such stability indicating methods. At every stage of drug development, practical recommendations are provided which will help to avoid failuresms.

Keywords: HPLC, Stability indicating HPLC method, Injection Dosage form

Introduction

What is forced degradation study?

Study designed to intentionally degrade a drug substance or drug product. Forced degradation or accelerated degradation is a process whereby the natural degradation rate of a product or material is increased by the application of an additional stress. Forced degradation studies are used to identify reactions which may occur to degrade processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities.

Aim to perform Forced Degradation Study

To check- any Impurity or Degraded product generated at different stress condition or not. If any Impurity &/or Degraded product is generated then, whether generated any Impurity &/or Degraded product interfere with the main Analyte peak or not.
Whether proposed method able to separate & identify them or not in the presence of main Analyte peak.

Testing of stress samples are required to demonstrate the following abilities.

- To evaluate the stability of drug substance and drug products in solution.
- To determine structural transformations of drug substance and drug product.
- To detect low concentration of potential degradation products.
- To detect unrelated impurities in the presence of the desired product or product related degradants.
- To separate the product related degradants from those derived from excipients and intact placebo.
- To elucidate possible degradation pathways.

Parameters in forced degradation

The typical forced degradation studies on drug substance include:
Temperature and/or with humidity, Acid-Base stress testing, Oxidation, Photo degradation and pH variations (high and low).

Experimental procedure

Chromatographic condition

Mobile phase: buffer (ph5)-Methanol. 30:70

Buffer prepn: (0.05M potassium dihydrogen ortho phosphate, ph-5)

Take about 6.8gm potassium dihydrogen ortho phosphate reagent into a 1000ml beaker. Add 800ml Methanol. and dissolve. Adjust ph 3.5 of this solution with 0.1N NaOH. Make up volume upto 1000ml with Water. (use this solution as buffer)

Flow rate: 1ml/mint

Injection volume: 20Diclofenacrolit.

Column: 250*4.6mm C18, Hypersil BDS

Wavelength-278nm

Std stock soln of Methocarbamol: 300mg→100ml with Methanol. (300mcg/ml)

Std stock soln of Diclofenac: 50mg→100ml with Methanol. (500mcg/ml) →10ml→100(50 mcg/ml)

Working std prepn (Combine std prepn): Take 1ml from Methocarbamol stock and 1ml from Diclofenac std stock soln →10ml with Mobile phase (Methocarbamol-30mcg/ml, Diclofenac-5mcg/ml)

Assay preparation (Marketed formulation)

Label claim: Methocarbamol-500mg, Diclofenac-100mg

Sample stock solution

Weigh and powdered 20 tablets. Take tablet powder equivalent to 30mg Methocarbamol/5mg Diclofenac in to a 100ml volumetric flask. Add 60 ml Methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with Methanol. Filter this solution with Whatman filter paper no-1. (Methocarbamol-300mcg/ml, Diclofenac-50mcg/ml)

Working sample preparation

Take 1ml from sample stock solution into a 10ml and make up with mobile phase. (Methocarbamol-30mcg/ml, Diclofenac-5mcg/ml)

Results and Discussion

Stress Study (Stability study)

Stress study was carried out using acid, base, oxidation, photo degradation and thermal stability. Results of which are as given below

Acid Stress Study (Stability study)

Table 1: Acid Stability study data for Methocarbamol and Diclofenac by HPLC

Area of standard Methocarbamol (30 µg/ml)			1583.404	
Stressed Coditions	Methocarbamol		Diclofenac	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1603.079		1306.106	0
2 Hr	1477.401	7.839788	1233.824	5.53416
6 Hr	482.492	69.90217	492.061	62.32611

Base Stress Study (Stability study)

Table 2: Base Stability study data for Methocarbamol and Diclofenac by HPLC

Area of standard Methocarbamol (30 µg/ml)			1583.404	
Stressed Conditions	Methocarbamol		Diclofenac	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1551.568		1291.085	
2 Hr	1504.239	3.050398	1204.366	6.716754
6 Hr	432.024	72.15565	352.462	72.70033

Oxidative Stress Study (Stability study)

Table 3: Oxidative Stability study data for Methocarbamol and Diclofenac by HPLC

Stressed Conditions	Methocarbamol		Diclofenac	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1551.58		1298.668	
2 Hr	1436.496	7.417213	1211.932	6.678843
6 Hr	465.298	70.01134	425.057	67.26977

Photo Stability study

Table 4: Photo Stability study data for Methocarbamol and Diclofenac by HPLC

Stressed Conditions	Methocarbamol		Diclofenac	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1552.58		1299.468	
12 Hr	1516.159	2.345837	1213.779	6.59416
72 Hr	694.581	55.26279	505.609	61.09108

Thermal Stress Study (Stability study)

Table 5: Thermal Stability study data for Methocarbamol and Diclofenac by HPLC

Stressed Conditions	Methocarbamol		Diclofenac	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1553.478		1297.523	
2 Hr	1446.398	6.89292	1194.376	7.949532
6 Hr	358.757	76.90621	294.425	77.30869

Table 6: Stability study data for Methocarbamol standard by HPLC

Area of standard Methocarbamol (30 µg/ml)			1583.404	
Stressed Conditions	Standard		Sample	
	Area Found	% Degradation	Area Found	% Degradation
Acid	1105.73	30.16754	1065.679	32.69696
Base	1378.409	12.94647	1350.243	14.7253
Thermal	1213.339	23.37148	1217.497	23.10888
Oxidation	1240.017	21.68663	1244.217	21.42138
Photo	1114.432	29.61796	1161.942	26.61746

Upon study of Methocarbamol in different stress condition, it was found that Methocarbamol having highest stability in basic condition for standard as well as in sample while least stable in acid and on exposure of UV light. The result indicates that drug having basic nature due to presence of amine group in its structure so it is more stable in basic condition and more degraded in acidic condition and on UV exposure.

Table 7: Stability study data for Diclofenac by HPLC

Area of standard Diclofenac (5 µg/ml)			1583.404	
Stressed Conditions	Standard		Sample	
	Area Found	% Degradation	Area Found	% Degradation
Acid	1031.536	19.95	1030.448	20.03
Base	1046.381	18.80	1067.493	17.16
Thermal	875.864	32.03	927.591	28.02
Oxidation	954.014	25.96	982.501	23.75
Photo	869.288	32.54	900.758	30.10

Upon study of Diclofenac in different stress condition, it was found that Diclofenac having highest stability in basic and acid condition for standard as well as in sample while least stable in thermal exposure and on exposure of UV light i.e. photo stability.

Table 8: Analysis of Marketed Formulation by HPLC

Brand	Serial No	Label Claim (mg) (w/w)	Result (mg) (w/w)	% Assay	Avg % Assay	SD	% RSD
Diclofenac	1	50	46.51	93.01	94.26	1.087	1.154
	2	50	47.37	94.74			
	3	50	47.51	95.02			
Methocarbamol	1	300	316.63	105.54	105.16	1.154	1.097
	2	300	311.59	103.86			
	3	300	318.22	106.07			

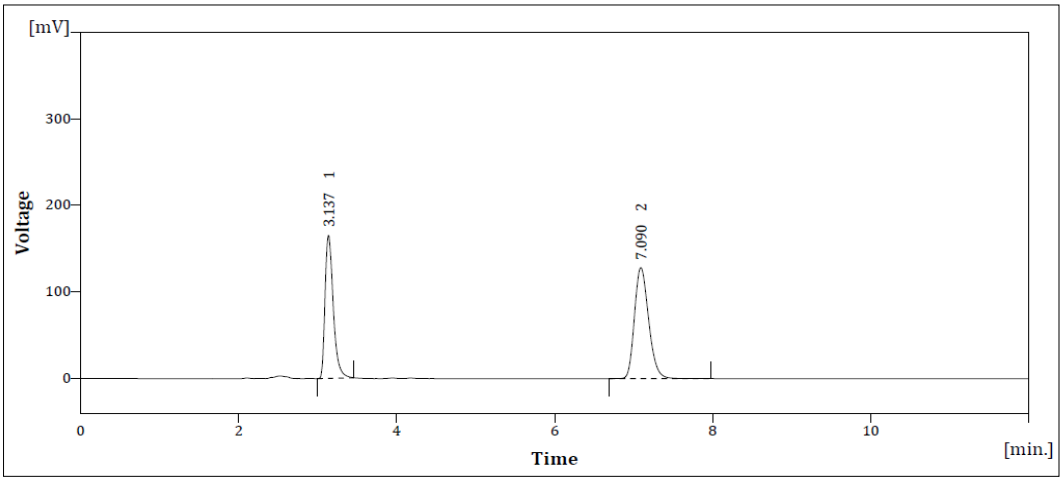

Fig 1: Chromatogram of Assay study for Sample MET (30 µg/ml) and DIC (5µg/ml) DATA 1

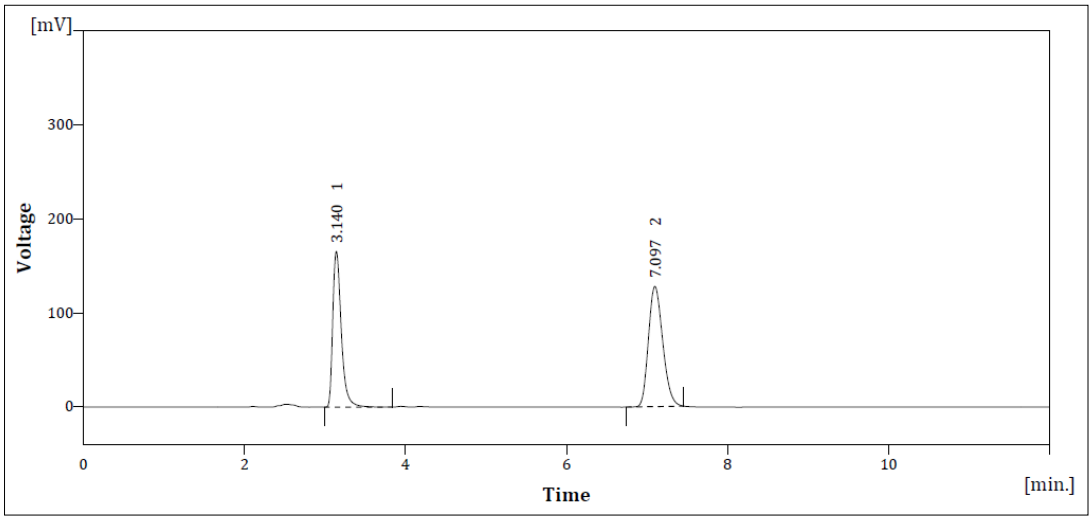

Fig 2: Chromatogram of Assay study for Sample MET (30 µg/ml) and DIC (5 µg/ml) DATA 2

Application of Proposed Method for analysis of Marketed formulation

The proposed method was applied successfully for analysis of marketed formulation and results obtained are shown in following table.

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