DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING CHROMATOGRAPHIC ASSAY METHODS FOR DRUGS AND ITS COMBINED PHARMACEUTICAL FORMULATIONS ACTING ON GI TRACT

Synopsis of the PhD thesis submitted to

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Title of the thesis and abstract

Title of the thesis: "DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING CHROMATOGRAPHIC ASSAY METHODS FOR DRUGS AND ITS COMBINED PHARMACEUTICAL FORMULATIONS ACTING ON GI TRACT"

Abstract:

The gastrointestinal (GI) tract includes the tongue, esophagus, stomach (glandular and nonglandular portions), small intestine (duodenum, jejunum, and ileum), and large intestine (cecum, colon, and rectum). Medications that exert an effect on the gastrointestinal tract are among the most frequently used drugs worldwide. Gastrointestinal agents are one of the most commonly cited class of drugs recorded on the medication histories. International Conference on Harmonization (ICH) guidelines demand validated stability-indicating liquid chromatography (LC) methods established through forced degradation studies for stability testing of drug product and drug substances.International Conference on Harmonization (ICH) guidelines demand validated stability-indicating liquid chromatography (LC) methods established through forced degradation studies for stability testing of drug substances. The current studies were to develop a simple, accurate, precise and rapid stability indicating reversed-phase HPLC method and subsequent validation using ICH suggested approach for the determination of Clidinium bromide (CLBr), Chlordiazepoxide(CDZ), and Pantoprazole sodium(PNT) in their combined capsule dosage forms. Sofalcone (SFL) was subjected to the ICH-prescribed acidic, alkaline, oxidative, photolytic, and thermal stress conditions, and it undergoes degradation with well- resolved degradation products. These degradation products were further analyzed by mass spectrometry to elucidate the structure of degradation products and proposed the prediction of the degradation pathway and Mass Balance.

Brief description on the state of the art of the research topic

- In recent years, mechanical systems have been developed that more closely mimic the full dynamic, physical and biochemical complexity of the GI Tract. The development of these complex systems raises the possibility that they could be used to support formulation development of poorly soluble compounds and importantly may be able to replace clinical BE studies in certain circumstances (1).
- Sofalcone, 2 -carboxymethoxy-4,4 -bis(3-methyl-2butenyloxy)chalcone, is an anti-ulcer agent with mucosa protective effect and directly inhibits growth of Heliobacter pylori. It is therefore useful for the treatment of gastric ulcer. Sofalcone is a type of flavonoid and a synthetic derivative of sophoradine which is isolated from the root of the Chinese medicinal plant Sophora subprostrata3 (2,3). Clidinium bromide (3-[(2-hydroxy-2, 2diphenylacetyl)-oxy]-1-methyl-1-azoniabicylo- [2.2.2] octan-1-ium bromide) is used for anticholinergic and

antisecretory agent which exerts its action by inhibiting the action of parasympathetic innervations thus reducing the secretions of stomach acid and is also a mild antispasmodic (4,5).

- Chlordiazepoxide (7-chloro-2-methylamino-5- phenyl-3H-1, 4-benzodiazepene-4-oxide) is a benzodiazepine. It has GABA facilitator action. It is used as anxiolytic, sedatives, hypnotics, skeletal muscle relaxants. The drug may inhibit monosynaptic and polysynaptic reflexes by acting as inhibitory neuronal transmitters or by blocking excitatory synaptic transmission (6,7). It shares the actions of other benzodiazepines and is used for the management of anxiety disorders or for short-term relief of symptoms of anxiety and for the management of agitation associated with acute alcohol withdrawal. Combination of Clidinium Bromide and Chlordiazepoxide in the ratio 1:2, respectively, produce antispasmodic effects, antianxiety action, and also help in treatment of peptic ulcer disease and irritable bowel syndrome (8,9).
- Pantoprazole is chemically, 6-(difluoromethoxy)-2- {[(3,4-dimethoxypyridin-2-yl)methane]sulfinyl}-1H1,3-benzodiazole. It is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease. The chemical formula is $C_{16}H_{15}F_2N_3O_4S$. The molecular formula is 383.37 g/mol. Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H+,K+)- ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect is dose related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus (10,11).
- There are several Bioanalytical method has been developed for the estimation of Sofalcone However, to the best of our knowledge, no reports on stability-indicating HPLC/UHPLC Methods with subjected to the ICH-prescribed acidic, alkaline, oxidative, photolytic, and thermal stress conditions and it undergoes degradation with well- resolved degradation products. These degradation products were further analyzed by mass spectrometry to elucidate the structure of degradation products and proposed the prediction of the degradation pathway and Mass Balance with LC-MS/MS.
- There are many methods reported in the literature for the analysis methods involving spectrophotometry, HPLC, HPTLC have been reported for Clidinium Bromide, Pantoprazole and Chlordiazepoxide in single form and in combination with other drugs while few analytical methods have been reported for Clidinium Bromide and Chlordiazepoxide in combination with each other including HPLC, and HPTLC.

- To the best of our knowledge, there is no published chromatographic method for these three combinations of drugs in the presence of their degradants using the ICH approach of stress testing.

Definition of the problem

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidances state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used (12-13).

In general, pharmaceutical items intended for the global pharmaceutical market are currently tested for stability under normal storage conditions for as long as 36 months, though, typically, regulatory agencies would initially assign only a 24-month conformance period, thereby providing an extra stability reserve. This is achieved through exhaustive research and thorough understanding of the stability characteristics of the drug substances (DSs) and DPs, and a long-term testing program. Meaningful product-expiration dates are obtained only after meticulous, scientifically-designed studies using specific stability indicating assays, rigorous computer-assisted analysis of the resulting data, and appropriate statistics. Thus, a satisfactory 3-month accelerated data submission may also permit granting a 24-month tentative expiry date, providing that the room temperature data also meet specifications. Hence, stability-indicating methods (SIMs) play a key role in current pharmaceutical regulation.

The development of a SIM likely to meet regulatory requirements is a seven-step process that entails

(a) Critical study of the drug structure to assess the likely decomposition route(s);

(b) Collection of information on physicochemical properties;

(c) Conducting stress (forced decomposition) studies;

(d) Preliminary separation studies on stressed samples;

(e) Final method development and optimization;

(f) Identification and characterization of degradation products, and preparation of standards; and,
(g) Validation of SIMs (14-15).

Objectives and scope of work

- Validated stability-indicating UHPLC method for the estimation of Sofalcone in drugs and the LC–MS identification of its degradation products.
- Force Degradation with Mass Balance Study for Sofalcone in drugs.
- RP-HPLC method development and validation for the estimation of Sofalcone in bulk drug and formulations with forced degradation studies.
- Development and validation of a rapid RP-HPLC method for the determination of clidinium bromide, chlordiazepoxide and pantoprazole sodium in their combined capsule dosage form.
- RP-HPLC method development and validation for the estimation of clidinium bromide, chlordiazepoxide and pantoprazole sodium in bulk drug and formulations with forced degradation studies.

Scope of work

Drug analysis is a comprehensive area that encompasses a diverse variety of academic fields and expertise. Specifically, the goal of this research is to collect data that will help in the creation of high-quality pharmaceuticals with maximum efficacy and safety while also allowing for the production of drugs with the best possible efficiency in the manufacturing process. The current research has a very promising future in terms of the development of a GI formulation for a difficult indication such as Gastro intestinal disease. As a result, researchers are investigating new formulation challenges and good manufacturing practices (GMPs), as well as future analytical method for Sofalcone and in combined clidinium bromide, chlordiazepoxide and pantoprazole sodium, as well as the assessment and validation of a well-developed dosage form. For routine determination of the drug under investigation in bulk and pharmaceutical dosage forms, the procedures recommended below, which are alternatives to the ones listed above, may be used in place of the ones specified.

Original contribution by the thesis

The current study provides validated stability-indicating HPLC and UHPLC methods for the estimation of Sofalcone and it undergoes degradation with well- resolved degradation products. These degradation products were further analyzed by mass spectrometry LC-MS/MS to elucidate the structure of degradation products and proposed the prediction of the degradation pathway and Mass Balance, While validated stability-indicating RP-HPLC method for the estimation of clidinium bromide, chlordiazepoxide and pantoprazole sodium in bulk drug and formulations. This

investigation is useful for further evaluation of Sofalcone and clidinium bromide, chlordiazepoxide and pantoprazole sodium in Quality control department.

The entire work in this synopsis, is the original work.

Methodology of Research and Results:

Materials and Reagents:

All reagents and solvents were analytical and HPLC grades, except Formic acid (Rankem, India) and Ammonium Acetate (Rankem, India). The water used was distilled and deionised by using Millipore (ELIX) system. Sofalcone, clidinium bromide, chlordiazepoxide and pantoprazole sodium of the highest grade (purity>98.0%) were used as the external standards. In which Sofalcone was procured from Zeta Scientific LLP. – Mumbai. Clidinium bromide and chlordiazepoxide were procured as gift sample from Ontop pharmaceuticals Pvt. Ltd., Bangalore whereas pantoprazole sodium was procured as gift sample Aum research Laboratories, Ahmedabad.

Instrumentation and Chromatographic conditions:

Instruments used were mentioned in table-1.

Table 1: Instrumentation:

Instruments	**Model No.**	**Manufacturer**
HPLC	1260 Infinity II	Agilent
UHPLC-MS QQQ Mass Spectrometer	-	Agilent
UHPLC Column	Eclipse Plus C18 RRHD (100mm x 2.1mm, 1.8µm)	Agilent
HPLC Column	Eclipse Plus C18 (150mm x 4.6mm, 5µm)	Agilent
Detector	Photo Diode Array	-
FT-IR	IR Spirit	Shimadzu
UV-Visible Spectrophotometer	UV- 1900	Shimadzu
PH meter	EQ-610	Lab Line
Ultra Sonicator	LMUC 6	-
Water purification system	-	Mili- Q
Analytical Weighing Balance	ME204/A04	Shimadzu

For Sofalcone

Methos-A: *Validated Stability-indicating RP-UHPLC method for the estimation of Sofalcone in drugs, Reconciling Mass Balance in Force Degradation studies and LC-MS identification of its degradation products*:

Method-B: *RP-HPLC method development and validation for the estimation of Sofalcone in bulk drug and formulations with forced degradation studies:*

Chromatographic conditions are mentioned in table-2a and table-2b for Method-A and Method-B respectively:

Table 2a: Optimized Chromatographic condition for Method-A:

Parameters	**Optimized condition**
Elution mode:	Gradient
Mobile phase composition	Mobile Phase A: Water (0.1% Formic Acid) Mobile Phase B: Ammonium Acetate in Methanol Mobile Phase A : Mobile Phase B (Initial 30:70)
Column	Agilent Eclipse Plus C18 RRHD (100mm x 2.1mm, 1.8µm)
Flow rate	0.300 mL/min
Detection wavelength	350 nm
Injection volume	2.00 µL
Run time	20 minutes
Retention time	5.3 minutes
Sample concentration	100 µg/mL

Table 2b: Optimized Chromatographic condition for Method-B:

Parameters	**Optimized condition**
Elution mode:	Isocratic
Mobile phase composition	Mobile Phase A: Ammonium Acetate buffer + Tri-ethylamine (pH 5.6 Adjusted with Glacial Acetic Acid) Mobile Phase B: Acetonitrile Mobile Phase A : Mobile Phase B (50:50)
Column	Eclipse Plus C18 (150mm x 4.6mm, 5µm)
Flow rate	1 mL/min

Detection wavelength	348 nm
Injection volume	5 µL
Run time	10 minutes
Retention time	4.7 minutes
Sample concentration	100 µg/mL

For Clidinium bromide, Chlordiazepoxide and Pantoprazole sodium:

Method-C: *Development and Validation of a Rapid Rp-Hplc Method for the Determination of Clidinium Bromide, Chlordiazepoxide and Pantoprazole Sodium in their Combined Capsule Dosage Form*

Method-D: *Rp-Hplc method development and validation for the estimation of Clidinium bromide, Chlordiazepoxide and Pantoprazole sodium in bulk drug and formulations with forced degradation studies.*

Chromatographic conditions are mentioned in table-2c and table-2d for Method-C and Method-D respectively:

Table 2c: Optimized Chromatographic condition for Method-C:

Parameters	**Optimized condition**
Mobile Phase	0.4% TEA: Methanol:Acetonitrile (pH-6 adujsted by OPA) (50:30:20 v/v/v)
Pump mode	Isocratic
Stationary phase	Sunshell_Coreshell Column C18, (100 x 4.6 mm i.d), Particle size 2.6 µm
Flow rate (ml/min)	1.6
Run time (min)	10min
Volume of Injection (µl)	10.0
Detection wavelength (nm)	220nm
Retention time (min)	CLBr : 1.318 PNT : 2.434 CDZ:4.691
Diluent	Mobile phase

Table 2d: Optimized Chromatographic condition for Method-D:

Parameters	**Optimized condition**
Sample Concentration	160+20+40 ppm
Column	Zorbax SB Phenyl (150*4.6mm, 3.5µm)
	Make: Agilent
	Stationary Phase: Phenyl
Flow rate :	1.5mL/min
Column temprature :	30°C
Wavelength :	230 nm
Injection volume :	20µL
Run time :	10 minutes
Diluent :	Water:Acetonitrile_65:35% v/v
Mode :	Isocratic
Mobile phase :	MP-A: Sodium perchlorate buffer
	MP-B: Acetonitrile

Results and Discussion

For Sofalcone

Methos-A: *Validated Stability-indicating RP-UHPLC method for the estimation of Sofalcone in drugs, Reconciling Mass Balance in Force Degradation studies and LC-MS identification of its degradation products*:

Method-B: *RP-HPLC method development and validation for the estimation of Sofalcone in bulk drug and formulations with forced degradation studies:*

Mass Balance Study in Table 3, Degradation conditions are mentioned in table 4, Analysis of degradation products in Figure-1, Summary of developed analytical method are mentioned in table-5 for both methods.

Table-3 Mass Balanec Study for Method-A:

Mass Balance							
Conditions	**Unspecified impurity at RRT 0.58**	**Unspecified impurity at RRT 4.7**	**Unspecified impurity at RRT 1.39**	**%Total impurities**	**Assay (%)**	**Mass Balance (% Total impurities + % Assay) (%)**	**Mass Balance wrt to as such sample**
As such (Unstressed Sample)	ND	ND	ND	0	102.5	102.5	NA
Acid degradation (1N HCl, RT, 3 Hours)	8.9	ND	ND	24.33	77.67	102.0	99.5
Base degradation (1N NaOH, RT, 3 Hours)	2.14	ND	ND	3.27	95.71	98.98	96.6
Oxidation degradation (5ml 30 % H2O2, RT , 1 Hours)	ND	1.55	5.21	8.57	92.47	101.04	98.6
Thermal degradation (80°C, 3 hours)	ND	ND	2.12	3.49	97.01	100.5	98
PhotoDegradation (UV light for 3hours)	ND	ND	19.73	32.48	66.92	99.4	97

Table 4: Forced Degradation study for Method-B:

Parameter	**Condition**	**% Degradation**	**Peak purity**	**Peak purity pass/ fail**
		Sofalcone	**Sofalcone**	
Acid degradation	At 1hrs.	8.9306	999.9	True

Parameter	Condition	% Degradation	Peak purity	Peak purity pass/ fail
		Sofalcone	Sofalcone	
(1N HCl, RT, 3 Hours)	At 2 hrs.	14.6016	999.9	True
	At 3 hrs.	44.1016	999.9	True
Base degradation (1N NaOH, RT, 3 Hours)	At 3hrs.	4.5116	999.9	True
Oxidation degradation (5ml 30 % H2O2, RT , 1 Hours)	At 3hrs.	4.5116	999.9	True
Thermal degradation (80°C, 3 hours)	30min 80° C	0.3162	1000.0	True
PhotoDegradation (UV light for 3hours)	UV Light 24 Hours	56.8268	999.9	True

Figure-1 Analysis of degradation products from Method-A:

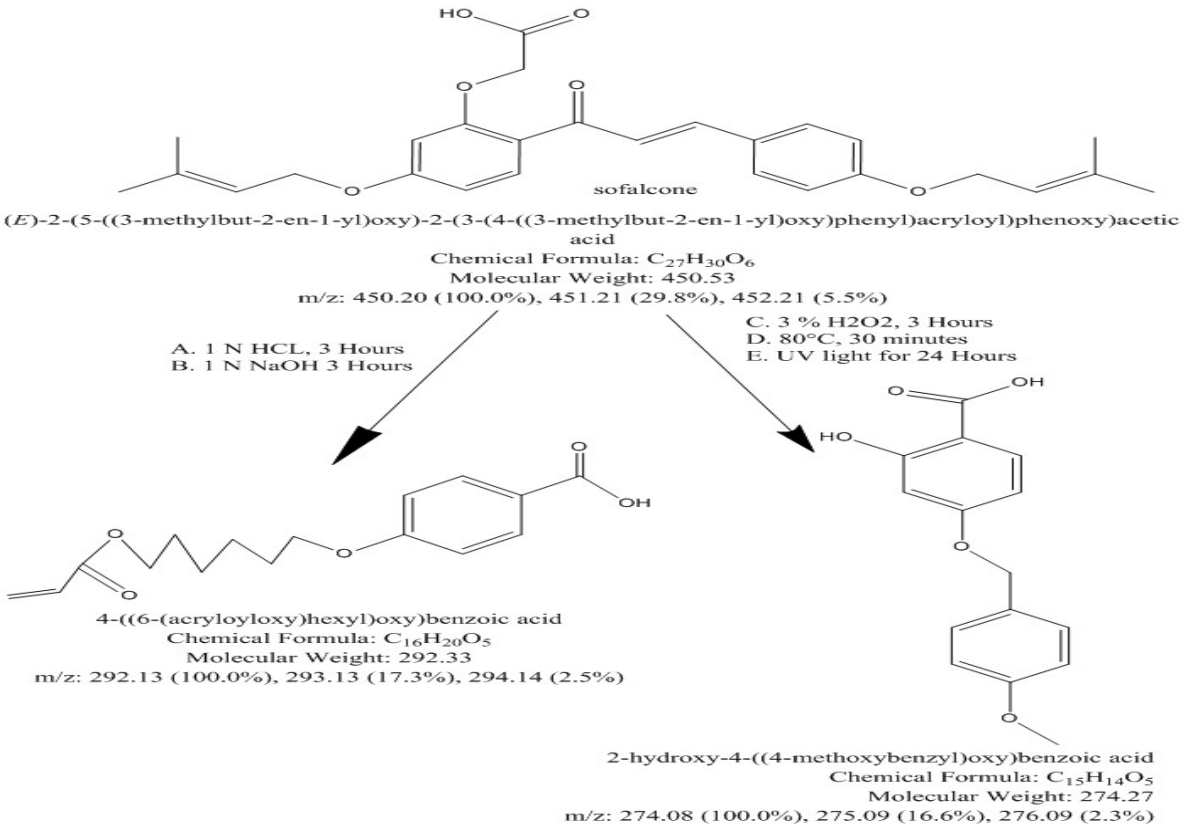

Table 5: Summary of Developed Stability Indicating Method

Parameter	**Result**
	Sofalcone
Linearity Regression equation (R2)	50-150µg/ml 0.9954
Accuracy	100.02%- 100.55%.
Precision Repeatability Intraday Inter-day	RSD was found to be less than 2
Robustness Change in Wavelength Change in flow rate Change in Column Temp.	RSD was found to be less than 2
LOD(µg/ml)	10.419
LOQ(µg/ml)	31.575

For Clidinium bromide, Chlordiazepoxide and Pantoprazole sodium:

Method-C: *Development and Validation of a Rapid Rp-Hplc Method for the Determination of Clidinium Bromide, Chlordiazepoxide and Pantoprazole Sodium in their Combined Capsule Dosage Form*

Method-D: *Rp-Hplc method development and validation for the estimation of Clidinium bromide, Chlordiazepoxide and Pantoprazole sodium in bulk drugs and formulations with forced degradation studies.*

Force Degradation Study of Formulation in Table-6 and Individual API in Table-7 and Summary of developed analytical method are mentioned in table-8.

Table 6: Forced Degradation study in Formulation for Method-D:

Parameters	**Ulrax Capsule**	**Retention time (min)**	**Peak area**	**Resolution**	**% Assay**	**% Degradation**
Alkali Degradation (at 1hr)	PNT	1.827	4468	-	101.6	0.3
		2.040	976730	1.6		
		2.807	16098	3.2		
	CLBr	3.867	226002	3.9	101.7	-0.3
	CDZ	6.773	288616	11.2	101.4	-0.2
		7.760	2947	2.4		
Alkali Degradation (at 2hr)	PNT	1.827	5243	0.0	101.3	0.6
		2.040	973850	1.6		
		2.876	18246	3.2		
	CLBr	3.863	224550	3.9	101.1	0.4
	CDZ	6.760	286675	11.1	100.7	0.5
		7.780	3247	2.4		
Alkali Degradation (at 4hr)	PNT	1.827	9463	0.0	100.7	1.1
		2.040	968320	1.6		
		2.807	33249	3.2		
	CLBr	3.827	211864	3.9	95.4	6.1
	CDZ	6.767	272564	11.2	95.8	5.4
		7.770	6978	2.4		

Table 7: Forced Degradation study in individual API for Method-D:

Condition applied	**Area**	**% Assay**	**% Degradation**	**Remark**
Pantoprazole Sodium				
Untreated Sample	976730	102.1	---	---
HCl Treated	813941	85.49	14.51	Degradation products
NaOH Treated	880972	92.1	7.9	

H_2O_2 Treated	766062	80.03	19.97	Degradation products
Thermal Treated (80oC)	881272	88.07	8.4	
UV Light Treated	765062	82.2	19.6%	Degradation products
Clidinium Bromide				
Untreated Sample	225745	101.6	---	---
HCl Treated	219252	98.7	2.8	---
NaOH Treated	211864	95.4	6.1	---
H2O2 Treated	204561	92.1	9.4	---
Thermal Treated (80^0 C)	221565	99.7	1.7	---
UV Light Treated	199352	89.7	11.7%	Degradation products
Chlordiazepoxide				
Untreated Sample	288478	101.4	---	---
HCl Treated	278548	97.9	3.3	---
NaOH Treated	272564	95.8	5.4	---
H2O2 Treated	275568	96.8	4.4	---
Thermal Treated (80o C)	281259	98.8	2.4	---
UV Light Treated	282158	99.1	2.1%	---

Table 8: Summary of Developed Method for Method-C:

Parameter	**CLBr**	**PNT**	**CDZ**
Linear range (µg/ml)	10-30	80-240	20-60
Slope	385.1	713.1	1016
Intercept	758.6	11033	296.4
SD of Slope	0.8687	0.1471	0.4082
SD of Intercept	18.2012	20.0574	21.5566
Limit of Detection (µg/ml)	0.1559	0.09281	0.07001
Limit of Quantitation (µg/ml)	0.4725	0.2812	0.2121

Conclusion

FOR SOFALCONE:

Method-A: *Validated Stability-indicating RP-UHPLC method for the estimation of Sofalcone in drugs, Reconciling Mass Balance in Force Degradation studies and LC-MS identification of its degradation products*:

Mass balance correlates the measured loss of a parent drug to the measured increase in the amount of degradation products. It is a good quality control check on analytical methods to show that all degradation products are adequately detected and do not interfere with quantitation of the parent drug (i.e., stability-indicating methods). Regulatory agencies use mass balance to assess the appropriateness of the analytical method as a stability-indicating method and determine whether all degradants have been accounted.

Stability-indicating property:

The stability-indicating properties were tested by applying various stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic degradation on standard sofalcone samples. The degradation studies were optimized to achieve the degradation different reagents, concentrations, and time intervals were set to allow appropriate degradation of sofalcone at room temperature. For acidic, the sofalcone undergoes 24.33% degradation by 1 N HCl and 3.27% degradation by 1 N HCl within 3 hours. The acidic degradation product was formed at RRT value of 0.58 and well resolved from standard sofalcone peak at RRT value of 0.58. It indicates that the sofalcone drug is more prone to degrade and sensitive towards acidic condition. For alkaline, it undergoes 3.27% degradation by 1 N NaOH within 3 h. The alkaline degradation product was formed at RRT value of 0.52. For oxidative, sofalcone experiences 8.57%degradation by 3% H2O2 with Two degradation product at RRT value of 4.78 and 1.39 within 3 h. The degradation products were observed and well resolved from the standard peak. For Photolytic, sofalcone experiences 32.48 % degradation by 3% H2O2 with one degradation product at RRT value of 1.39 within 24 h. The sofalcone drug was stable over thermal conditions because no degradation was observed at 80^{o} C for 30 minutes.

Analysis of Degradation Products:

To identify the degradation products by LC MS/MS, the standard and processed test solutions were direct-injected to MS in full scan mode (Q1 with positive and negative ionization modes) with water (0.1% FA)–Ammonium acetate in methanol (30:70, v/v) as the carrier mobile phase at 0.3 mL flow rate. The applied collision energy was 25 eV, and the total run time was 20 min. The ESI positive ionization mode showed the mass spectra of major peaks, and the obtained values of mass to charge ratio (m/z) were used to identify degradation products.

The mass spectra of standard and degradation products are shown in Fig. The standard mass spectra of sofalcone have observed values of m/z 293.50, 201, 327, 543, 274.8 and 465 as major fragments out of which m/z 451.7 is molecular ion peak of sofalcone. The mass spectra of degradation product of acidic, alkaline, and oxidation samples showed common m/z at 293.50 apart from the major observed values of m/z in standard sofalcone mass spectra. Likewise, the mass spectra of another oxidative & Photolytic degradation product showed 293.50 and 274.8 m/z values. From these, it could be proposed that the values m/z 293.50 (impurity 1)i.e M-C11H10O and m/z 274.8 (impurity 2) i.e M-C12H16O are matched with the corresponding molecular formula [C16H20O5]+ (calc. 292.33) and the molecular formula [C15H14O5]+ (calc. 274.27) respectively.

The small and constant difference in the observed and calculated values indicates that the efficient protonation of sofalcone in positive mode could be expected due to the mobile phase used for LC MS/MS. Therefore, the m/z 293.50 was found to be a major degraded product in acidic, alkaline, and oxidative conditions, and m/z 274.8 was found as a second degradation product in oxidative and photolytic condition. The proposed structure and molecular formulas of all degraded products are shown in Fig. and the outcome of this study was counterpart in terms of degradation seen under acid hydrolysis, Photolytic, and oxidative degradation conditions and stable over thermal and Base conditions.

The first UHPLC–MS/MS method for the identification and quantitative determination of sofalcone and its degradation products has been developed.

Method-B: *RP-HPLC method development and validation for the estimation of Sofalcone in bulk drug and formulations with forced degradation studies:*

Developed HPLC method can resolve all degradants peaks of drugs, so this method can give analysis of drugs in presence of its degradants products. Hence, this HPLC method is stability indicating in nature. The developed RP-HPLC method was found to be simple, precise, specific and accurate. Range of % degradation in forced degradation was 5-30% which follows here. Therefore, this method can be applied for routine analysis of drugs in marketed formulation and in bulk drug. Major degradation impurity peaks are separated from the analyte peak. The purity was found above 990. Hence, the method can be termed as specific

FOR CLIDINIUM BROMIDE, CHLORDIAZEPOXIDE AND PANTOPRAZOLE SODIUM:

Method-C: *Development and Validation of a Rapid Rp-Hplc Method for the Determination of Clidinium Bromide, Chlordiazepoxide and Pantoprazole Sodium in their Combined Capsule Dosage Form*:

Using this single procedure, it is possible to perform quantitative analysis of three drugs in pharmaceutical dosage forms within a short analysis time less than 5 min, so less consumption of solvent which is very beneficial for routine analysis. The developed method reported herein was validated by evaluation of the validation parameters as described in ICH- Q2 (R1) guideline. System suitability, specificity, linearity, LOD, LOQ values, within- and between-day precision and accuracy of the proposed technique were obtained during the validation studies.

Method-D: *Rp-Hplc method development and validation for the estimation of Clidinium bromide, Chlordiazepoxide and Pantoprazole sodium in bulk drugs and formulations with forced degradation studies.*

Developed RP-HPLC method can resolve all degradants peaks of three drugs, so this method can give analysis of three drugs in presence of its degradants products. Hence, this HPLC method is stability indicating in nature. The developed RP-HPLC method was found to be simple, precise, specific and accurate. Range of % degradation in forced degradation was 5-30% which follows here. Therefore, this method can be applied for routine analysis of drugs in marketed formulation and in bulk drug.

Copies of papers published and a list of publications arising from the thesis

Sr. No.	Details
Papers published	
1	**Dharati Rami**, Nehal J. Shah, "Development and validation of a rapid RP-HPLC method for the determination of clidinium bromide, chlordiazepoxide and pantoprazole sodium in their combined capsule dosage form", World journal of pharmacy and pharmaceutical sciences, 2021, Vol: 10, Issue: 12, ISSN: 2278 – 4357 (Accepted)
2	**Dharati Rami,** Nehal J. Shah, "RP-HPLC method development and validation for the estimation of Sofalcone in bulk drug and formulations with forced degradation studies", Asian Journal of Pharmacy and Pharmacology, 2022, Vol: 8, Issue: 1, ISSN: 2455-2674 (Accepted)
Paper Communicated	
1	**Dharati Rami**, Dr.Nehal J. Shah, "Validated Stability-indicating RP-UHPLC method for the estimation of Sofalcone in drugs, Reconciling Mass Balance in Force Degradation studies and LC-MS identification of its degradation products" Communicated to *International Journal of Pharmaceutical Sciences and Research* **Current Status**- review is in process
Probable Paper	
1	**Dharati Rami**, Dr.Nehal J. Shah, "Rp-Hplc method development and validation for the estimation of Clidinium bromide, Chlordiazepoxide and Pantoprazole sodium in bulk drugs and formulations with forced degradation studies." Probable Journal- Indo global journal of pharmaceutical sciences

Patents (if any) ---------- NA----------

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