

FORCED DEGRADATION STUDIES ON AGENTS OF THERAPEUTIC INTEREST

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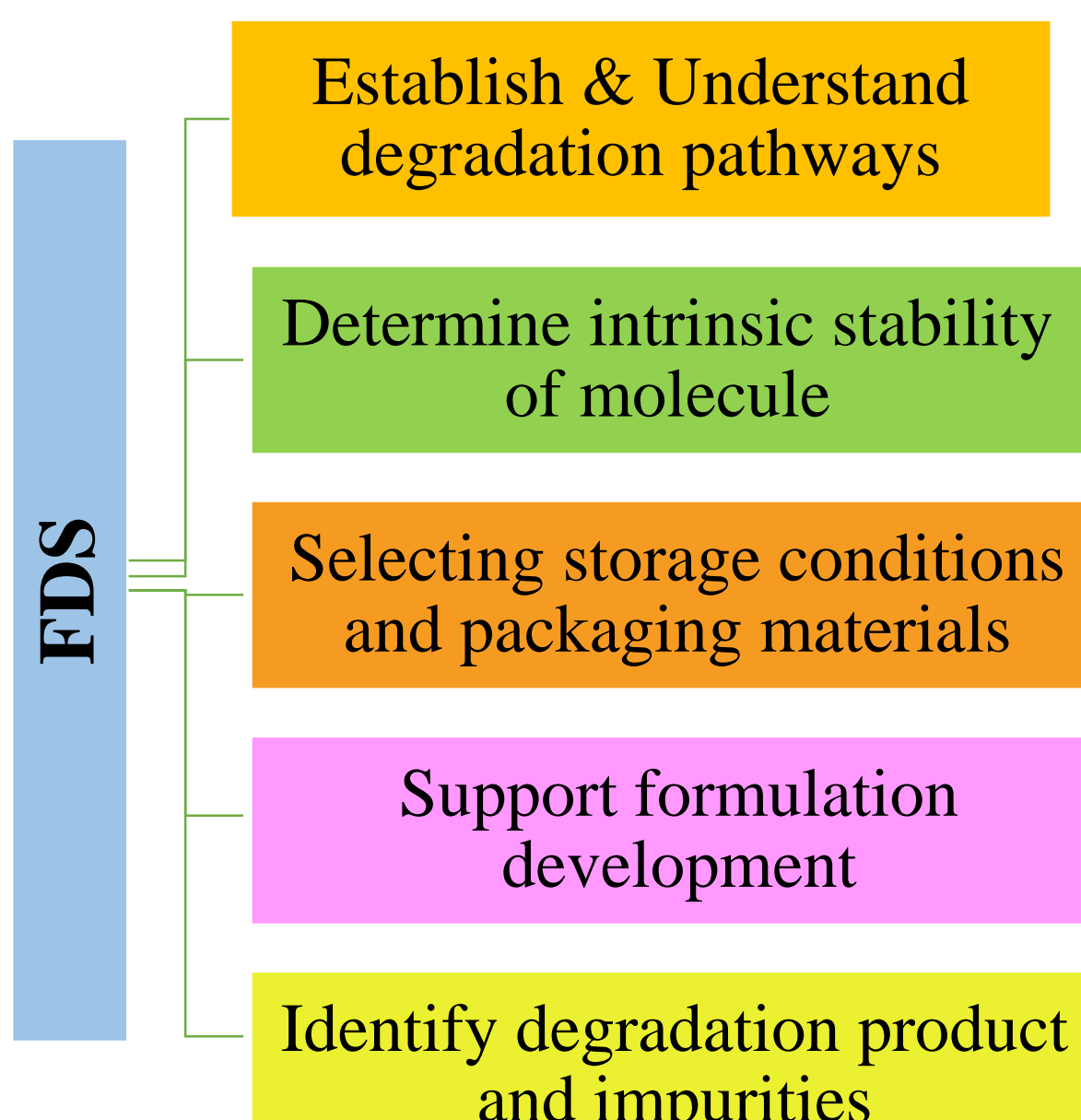
ABSTRACT

Chalcones possessing potential anti Alzheimer's activity were synthesised in our lab using the Claisen Schmidt reaction. FDS protocols in accordance with ICH guidelines were applied to three thiophene chalcones TC1, TC2, TC3. HPLC method was developed and validated for the individual untreated molecules and was found to be specific, selective, precise, reproducible, robust and linear in the range of about 5-15 ppm of the working standard concentration. The chalcones were stable under thermal and thermal-humidity stress, but degraded to different extents under acid-and base-catalysed hydrolysis, oxidative stress and photolytic conditions, as seen by HPLC analysis.

INTRODUCTION

Forced degradation which is also called as stress testing is a process in which the drug compounds are subjected to severe chemical and environmental conditions to generate the possible degradation products and their degradation pathway and also to detect the probable impurities present.

The degradation of drug product and drug substances are carried out under conditions which are severe and more stringent than those used in accelerated conditions.



OBJECTIVE



- 1) To develop a validated HPLC method for analysis of the chalcones TC1, TC2 and TC3 and their degradation products
- 2) To perform Forced Degradation Studies of TC1/TC2/TC3 as per ICH guidelines.
- 3) To propose degradation pathways based on data obtained by LC-MS for TC1

RATIONALE

Chalcones are privileged scaffolds with potential to address many of the causative mechanisms leading to Alzheimer's disease. However, the inherent high reactivity of this motif makes it prone to several degradation pathways which impact its stability. The nature of the substituents on the terminal rings is known to affect the reactivity of the α , β -unsaturated carbonyl moiety of the chalcones.

This project aims to study the degradation potential of three promising thiophene-phenyl chalcones in accordance to protocols given as guidelines under ICH

Method Development

Chromatographic conditions:	Methoxy Chalcone (TC1)	Benzyl Chalcone (TC2)	Hydroxy Chalcone (TC3)
HPLC System	Agilent 1260 infinity	Agilent 1260 infinity	Agilent 1260 infinity
Column	Thermo Scientific C18 (250 x 4.6, 5 μ)	Thermo Scientific C18 (250 x 4.6, 5 μ)	Cyno column (150 x 4.5, 5 μ)
Mobile Phase	ACN : Sodium acetate buffer pH 3 (60:40)	ACN : Sodium acetate buffer pH 3 (60:40)	ACN : Sodium acetate buffer pH 3 (25:75)
Flow rate	1.0 ml/min	1.0 ml/min	1.0 ml/min
Injection vol.	20 μ l	20 μ l	20 μ l
Diluent	Methanol	Methanol	Methanol: Water (80:20)
Column Temperature	25 $^{\circ}$ C	25 $^{\circ}$ C	25 $^{\circ}$ C
Retention Time	10.5 minute	27.4 minute	10.2 minute
Wavelength	280nm	280nm	280nm
Detector	PDA	PDA	PDA

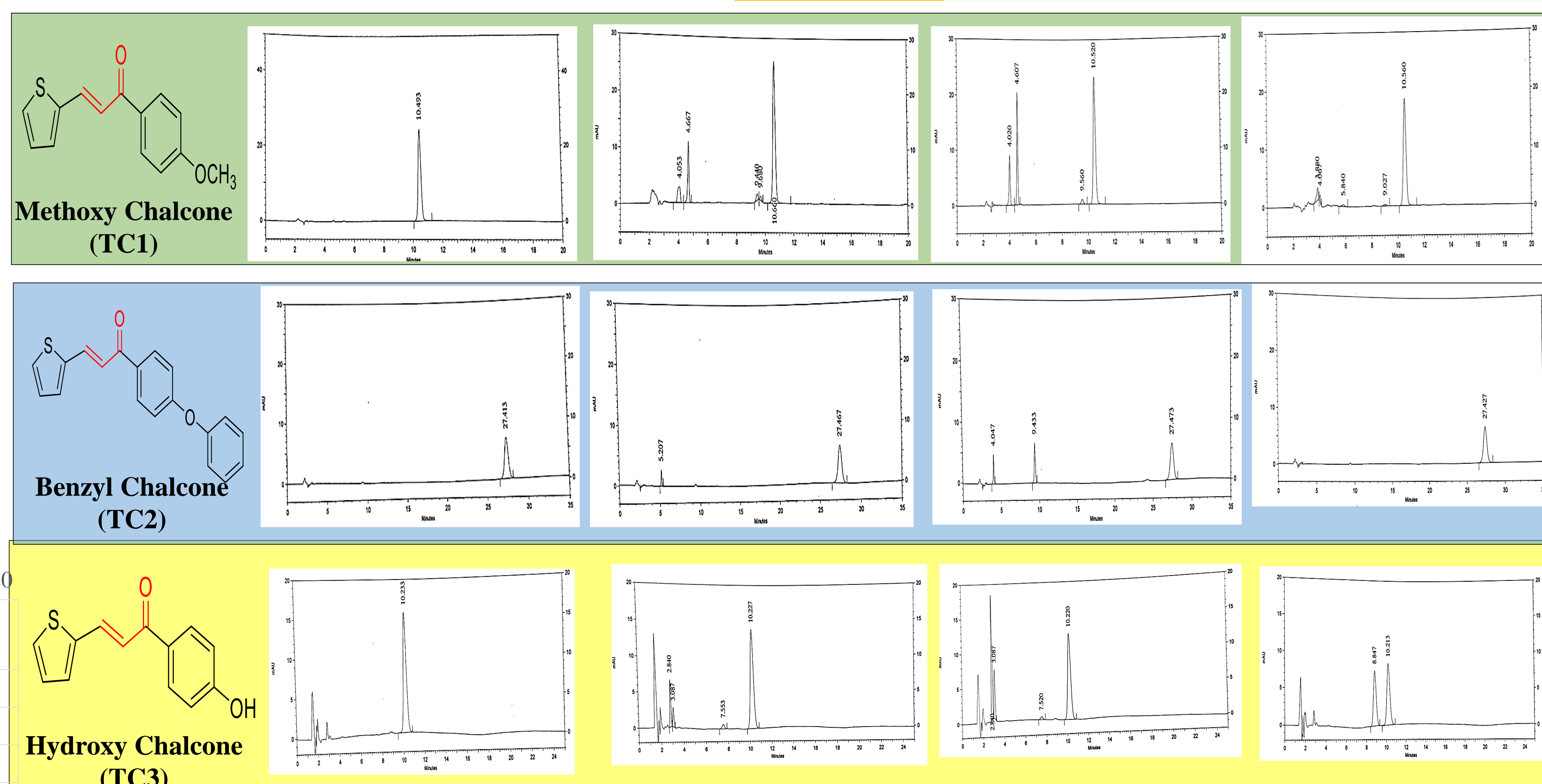
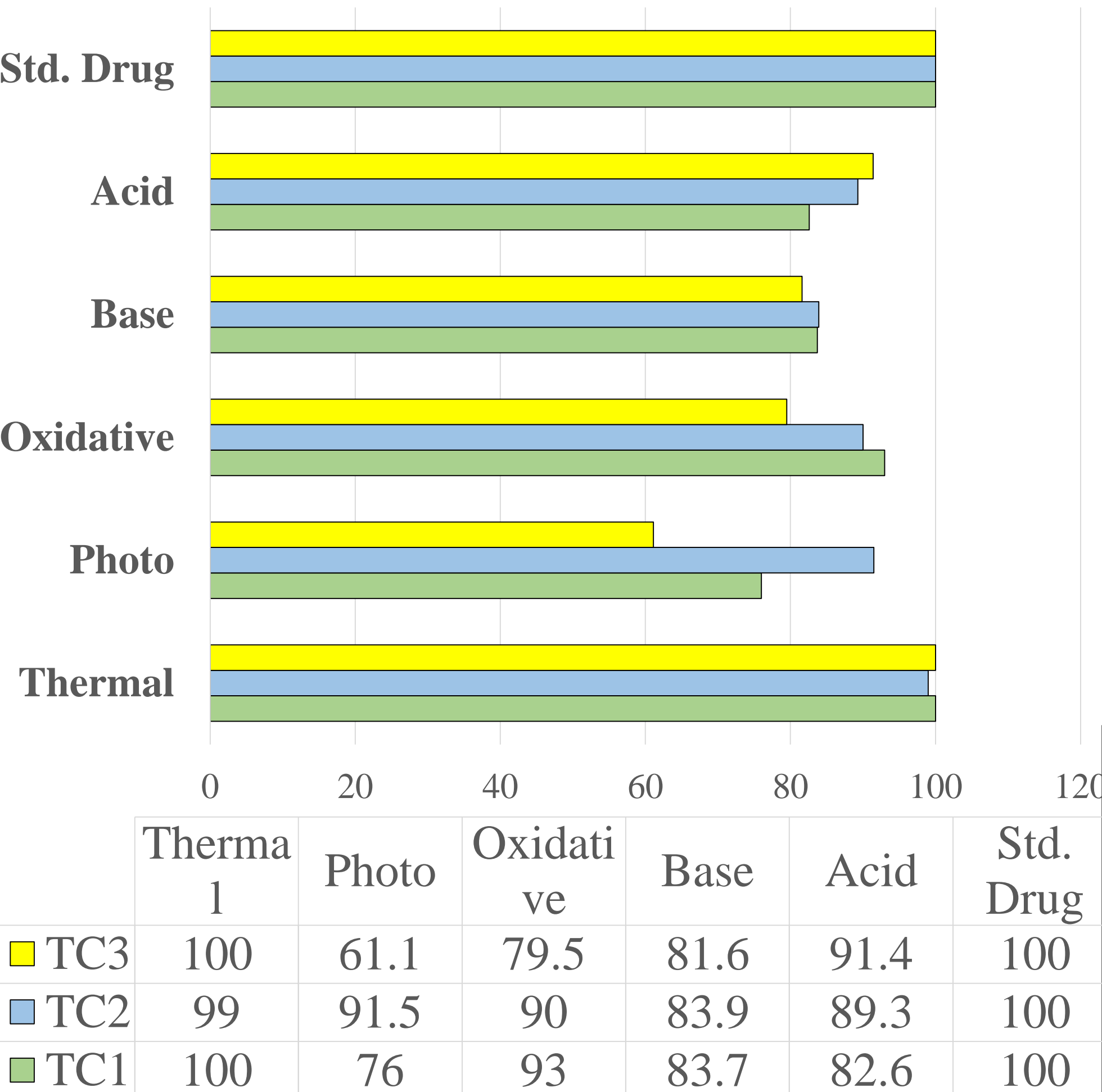
Method Validation

- Selective
- Linear (Range of 5-15 ppm)
- Precise (System precision & Method precision)
- Robust (wavelength of ± 2 nm, the mobile phase composition of $\pm 2\%$ and flow rate of ± 0.1 mL/min)
- Solution Stability (24hrs)

Optimized FDS Conditions

Conditions	Methoxy Chalcone(TC1)	Benzyl Chalcone(TC2)	Hydroxy Chalcone(TC3)
Base Degradation	0.1N NaOH at 100 $^{\circ}$ C for 10min	0.1N NaOH at 100 $^{\circ}$ C for 10min	1N NaOH at 100 $^{\circ}$ C for 30min
Acid Degradation	10N HCl at 100 $^{\circ}$ C for 2 hrs	1N HCl at 100 $^{\circ}$ C for 1hr	10N HCl at 100 $^{\circ}$ C for 1hr
Oxidative Degradation	30% H ₂ O ₂ at 100 $^{\circ}$ C for 15min	30% H ₂ O ₂ at 100 $^{\circ}$ C for 15min	3% H ₂ O ₂ at 100 $^{\circ}$ C for 15min
Thermal Degradation	85 $^{\circ}$ C for 24hrs	85 $^{\circ}$ C for 24hrs	85 $^{\circ}$ C for 24hrs
Photo Degradation	1.2 million Lux hours for 1 hr	1.2 million Lux hours for 1 hr	Expose to sunlight for 5 minutes
Thermal & Humidity	40 $^{\circ}$ C / 75% RH for 24hrs	40 $^{\circ}$ C / 75% RH for 24hrs	40 $^{\circ}$ C / 75% RH for 24hrs

% DRUG RECOVERED



CONCLUSION

FDS protocols in accordance with ICH guidelines were applied to three thiophene chalcones under five forced degradation conditions. The chalcones were stable under thermal and thermal-humidity stress, but degraded to different extents under acid-and base-catalysed hydrolysis, oxidative stress and photolytic conditions. and results obtained for the analytical method validation for the determination of the three chalcones were found to be specific, selective, precise, reproducible, robust and linear in the range of about 5-15 ppm of the working standard concentration.

REFERENCES

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