Degradation profiling of cefixime and azithromycin (antibiotics)

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Introduction

Stability is the capacity of a drug product to remain within specification for a given time. It is a prime requirement to ensure its identity, strength, quality and purity. Forced degradation studies are an integral part of drug development programs. For detecting the number and types of degradation products that are formed under various conditions, different chromatographic techniques in conjunction with UV and MS are used.

HPTLC is especially beneficial for stability testing due to its disposable stationary phase, as well as in the case of forced degradation, when appreciable amounts of acidic, alkaline and peroxide reagents are used. Furthermore, HPTLC permits the analysis of a large number of samples in a short time. There are also multiple derivatization and detection options that can help to characterize degradation products. Apart from this, simplicity and low cost of analysis add to the benefits.

Standard solutions

10 mg of cefixime trihydrate (CEFI) or azithromycin dihydrate (AZI) dissolved in 10 mL methanol

Sample preparation

1) Hydrolytic degradation: 1 mL of drug standard solution was mixed with 1 mL water or 0.5 N HCl or 0.5 N NaOH, kept for 1 h at RT and neutralized with acid or base
2) Oxidative degradation: 1 mL of drug standard solution was mixed with 1 mL of H_2O_2 (3 and 30 %) and kept for 1 h at RT
3) Thermal degradation: The drug powder placed in a sealed glass ampoule was heated at 100 °C and 200 °C for 1 h and 2 h in a hot oven
4) Photolytic degradation (according to ICH guidance Q1B, option 2): A thin layer of solid drug powder was exposed with fluorescent cold white light (1.25 million lux h) and UV light (200 Whm^{-2}) in a photo stability chamber

All degradation samples were dissolved (solid samples) or diluted with methanol to a final concentration of 100 µg/mL.

Chromatogram layer

HPTLC plates silica gel 60 F_{254}, aluminium backed (Merck), 20 x 10 cm, prewashed by developing first with methanol and then with the mobile phase followed by drying for 15 min with cold air

Sample application

Bandwise application with Linomat 5 or Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volumes 10.0 µL for sample solutions and 2.0 and 5.0 µL for standard solutions (CEFI resp. AZI); for preparative isolation: band length 180 mm and application volume 200.0 µL

Chromatography

In the Twin Trough Chamber 20 x 10 cm with chamber saturation (with filter paper) for 20 min, development with ethyl acetate – methanol – acetone – toluene – ammonia 2:10:14:1:1 to the migration distance of 80 mm (from the lower edge), drying for 15 min with cold air

Postchromatographic derivatization

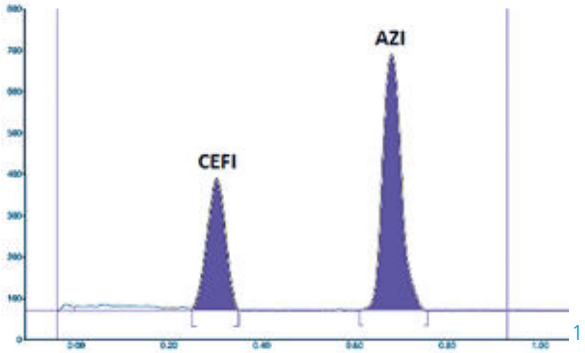
For detection of AZI, the plate was immersed with the Chromatogram Immersion Device into sulfuric acid reagent (1:4 in ethanol; immersion speed 3 cm/s, immersion time 6 s), dried for 30 s with cold air, and heated at 100 °C for 5 min using the TLC Plate Heater.

Documentation

TLC Visualizer under UV 254 nm, UV 366 nm, and white light

Densitometry

TLC Scanner 3 and winCATS, absorption measurement at 235 nm for CEFI and 530 nm for AZI, slit dimension 6.00 × 0.45 mm, scanning speed 20 mm/s, spectra recording from 190 to 550 nm



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Densitogram (absorption measurement) at 254 nm of the standard mixture; reprinted from [1]

Mass spectrometry

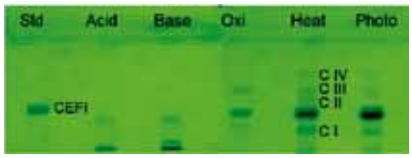
The target zones were marked with a pencil, and plates were cut carefully to separate different bands. Individual bands were cut and sonicated with methanol for extraction of degradation products. Methanol fractions were concentrated and evaporated to obtain solid residues which were analysed by Q-TOF and Ion Trap mass spectrometry in the positive ionization mode.

Results and discussion

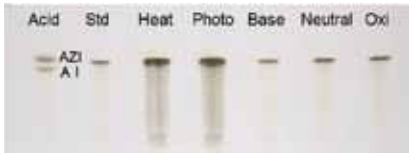
The developed method was validated according to the International Conference on Harmonization guideline Q2 (R1). All parameters were within the acceptance criteria, indicating that the method is suitable for the analysis of AZI and CEFI.

Parameter		Result	
		CEFI	AZI
Linearity	Linear calibration	y = 2.1614x+1002	y = 27.89x-313
	Determination coefficient r^2	0.9924	0.9952
	Range	500–2500 (ng/band)	50–250 (ng/band)
Sensitivity	LOD (ng/band)	58	3
	LOQ (ng/band)	175	10
Trueness and precision	Level spiked: mean amount (ng/band) ± intra-day precision (*%RSD*)	800: 799.3 ± 0.3 1000:1000.6 ± 0.4 1200:1201.4 ± 0.4	100: 99.5 ± 0.3 150:149.7 ± 0.4 200:200.6 ± 0.5
	Level spiked: mean amount (ng/band) ± inter-day precision (*%RSD*)	800: 798.6 ± 0.9 1000: 999.0 ± 0.9 1200:1199.1 ± 0.7	100: 98.9 ± 0.7 150:148.8 ± 0.9 200:199.9 ± 0.8
Accuracy	Level spiked: mean recovery (%) ± precision (*%RSD*)	800: 98.9 ± 1.3 1000: 99.9 ± 0.2 1200:101.1 ± 0.8	100: 99.5 ± 0.3 150:100.7 ± 0.5 200:100.1 ± 0.3
Robustness	Precision (*%RSD*)	< 2	< 2

In the forced degradation studies, CEFI was found to degrade to 4 major products (CI – IV) at different stress conditions. AZI showed only one additional peak (AI) upon acid and neutral hydrolysis. The specificity of the method was verified by comparing the hR_F values and UV spectra of the standard solutions with the degraded samples. Peak purities were investigated by spectrum scans at three positions within each zone and calculating their correlations (peak purities between 0.9993 and 0.9997).



2 *Degradation study of CEFI ; reprinted from [1]*



3 *Degradation study of AZI; reprinted from [1]*

Four degradation products for CEFI and one degradation product for AZI were isolated by preparative TLC and subjected to MS/MS for characterization. The β-lactam ring of CEFI did open under all conditions (chemical structures at [1]). AZI degraded to azithralosamine (loss of cladinose ring).

[1] V. Gawande *et al.* Acta Chromatographica, 2017, DOI: 10.1556/1326.2017.00199

Further information is available on request from the author.

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