

Validation of a stability-indicating HPLC-UV method: a prerequisite for conducting a stability study of an oral solution of isavuconazonium

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Introduction

Isavuconazole (ISA) is a triazole antifungal indicated for invasive aspergillosis and mucormycosis in adults and children over 1 year of age. The active ingredient is present as a prodrug (isavuconazonium, ISN). To date, ISA is available in two forms: 100 mg capsules and powder for solution for injection. The raw material (ISN) is not available in France. Regella et al. (1) characterized the forced degradation products of ISN, but stability data under real-world conditions remain absent from the literature.

(1) Identification and structural characterization of four novel degradation products and a process impurity of isavuconazonium sulfate for injection formulation bulk by LC-ESI-QTOF-MS/MS, 2019, Regella et al.

Dosage forms not suited for:

low body weight swallowing difficulties limited venous access

Oral solution

Objectives

1 Validate a stability-indicating HPLC-UV method

2 Study the preliminary stability of the extemporaneous preparation

Materials & Methods

Chromatographic conditions

HPLC	Shimadzu Nexera CI series, UV diode array detector		
Column	Nucleodur C18 inverse phase (150x4.6mm, 5µm)		
Flow rate	1mL/min		
Wavelength	220nm : qualitative detection of ISN and of mannitol (in powder for IV solution) 280nm : qualitative and quantitative detection of ISN and its degradation products		
Temperature	Column : 40°C, Vial : 6°C		
Injection volume	20µL		
Mobile phase	A = methanol, B = phosphate buffer 16mM pH4.2		
Elution method	Isocratic (forced degradation)	Isocratic until elution of ISN, then gradient (analytical method validation and stability study)	
Analysis time	50min	16min	
Elution conditions	A/B 58/42% V/V	Time (min)	Mobile phase A (%)
		0	58
		5.5	58
		6	70
		11	70
		11.01	58
		16	58
			Mobile phase B (%)
			42
			42
			30
			30
			42
			42

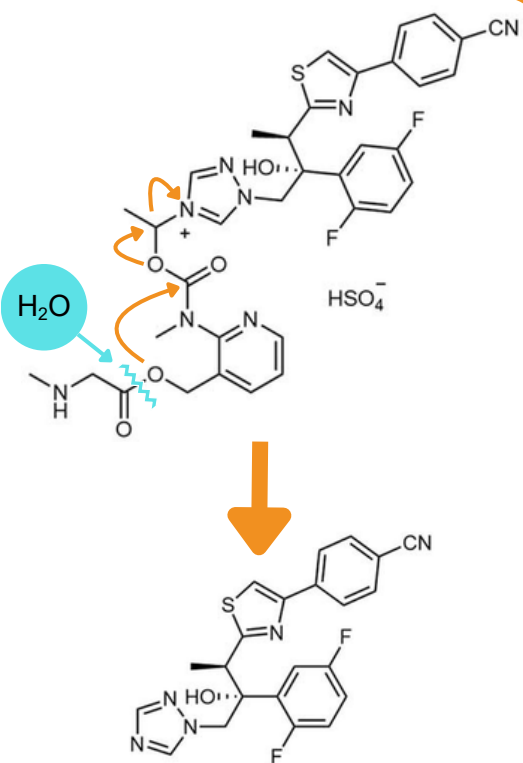


Fig. 1 : Degradation of isavuconazonium (ISN) into isavuconazole (ISA)

Methods' validation (normes ICH Q2)

Linearity	5 range points - 3 replicates - 3 days
Repeatability	5 range points - 6 replicates - 1 day
Intermediate precision	5 range points - 3 replicates - 5 days
Accuracy	3 levels of quality control (QC) - 3 replicates - 5 days
Specificity (forced degradation until 20-30% of ISN is degraded)	HCl 0.01M - Room temp. NaOH 0.05M - Room temp. H ₂ O ₂ 3% - 40°C UV - Room temp. 40°C / Room temp.
ISN retention time	4.5-4.6 min

Preliminary stability study

Source of API	capsules 100mg ISA	IV 200mg ISA
Concentration	10 mg/mL in ISA equivalent	
Excipient	Inorpha®	H ₂ O (reconstitution), Inorpha®
Storage	2-8°C	
Sampling time	0 - 7 - 21 days	
Parameters monitoring	Visual examination Content (concentration), pH Degradation products	

Results

Methods' validation

Range	30 - 40 - 50 - 60 - 70 mg/L equivalent ISA		
Linearity	Y = 64298x - 57565		R ² = 0.9990 > 0.998
Equation of the line			
Repeatability (RSD)	RSD < 0.47% < 5%		
Intermediate precision (RSD)	RSD < 2.04% < 5%		
Accuracy (% relative error)	QC1 = 32 mg/L +/- [0.99-4.52]% < 5%	QC2 = 48 mg/L +/- [0.60-2.60]% < 5%	QC3 = 64 mg/L +/- [0.27-3.88]% < 5%
Limit of quantification	203 ng - 10.15 ng/µL equivalent ISA		
Limit of detection	61 ng - 3.05 ng/µL equivalent ISA		

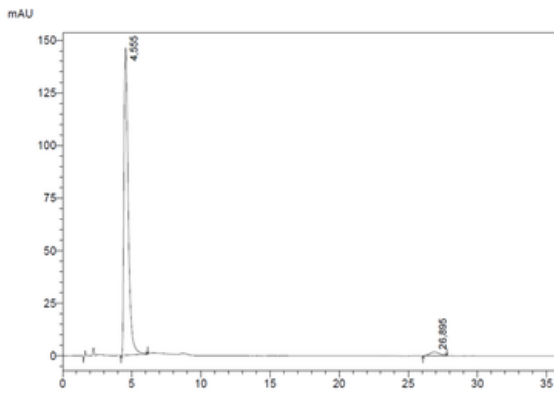


Fig. 2 : Chromatogram of non-degraded ISN (280 nm)

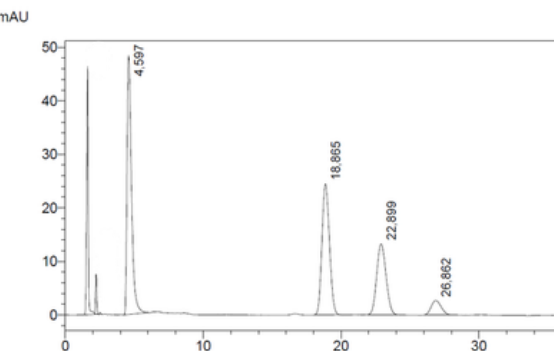
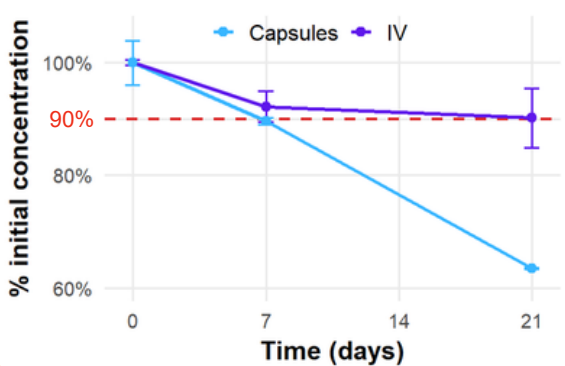


Fig. 3 : Chromatogram of the ISN degraded by 65% in NaOH (280nm)

Forced degradation

	HCl 0.01M	NaOH 0.05M	H ₂ O ₂ 3% + 40°C	UV	40°C	Room temp.
Degradation (%)	34%	65%	22%	20%	34%	29%
Time to degradation	5 min	20 sec	24h	18h	4h	24h
Retention time (Rt) of degradation products (min)	18.9 22.9 26.9	18.9 22.9 26.9	26.9	2.2 26.8	26.9	26.9

Evolution of ISN content according to the source of API



No visual changes from Day 0 to Day 21

Evolution of pH according to the source of API

	D-0	D-7	D-21
IV	2.53	2.63	2.49
Capsules	4.77	4.71	4.66

Percentage of relative area for each degradation product (DP)

	Identification	D-0	D-7	D-21
IV	DP1	0.4%	0.6%	1.1%
	DP2	2.7%	3.5%	4.3%
	DP3	2.4%	3.0%	4.5%
Capsules	DP1	0.8%	3.0%	7.1%
	DP2	4.3%	7.4%	7.9%
	DP3	4.1%	10.9%	24.4%

Discussion & Conclusion

1 The stability-indicating HPLC-UV method has been validated

2 The preliminary stability study shows poor stability in the oral solution, although stability is better for the formulation derived from the injectable form due to the more acidic medium⁽²⁾

(2) Biowaiver Monograph for Immediate-Release Solid Oral Dosage Forms: Isavuconazonium Sulfate, 2024, Plano et al.

Perspectives :

- Optimize the formulation by adjusting the pH to extend its stability
- Conduct a physicochemical and microbiological stability study in accordance with ICH guidelines and the European Pharmacopoeia
- Identify degradation products by mass spectrometry

To date, this is the first robust study investigating the stability of isavuconazonium in oral solution