

Development and validation of a stability-indicating assay method for a hospital-compounded oral suspension of ferrous fumarate for preterm neonates

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Introduction

Iron deficiency affects 25 to 85 % of preterm neonates during the first six months of life, with the risk increasing as gestational age and birth weight decrease. *FERROSTRANE® NOURRISSONS*, the only liquid formulation with marketing authorisation in France for this indication, is unsuitable (excipients with known effects, osmolality > 4000 mOsm/L). A hospital-compounded oral suspension of ferrous fumarate containing 5 mg/mL of elemental iron has been developed to meet these specific needs.[1]

Objective: To develop and validate a stability-indicating assay method and to establish the degradation profiles of ferrous fumarate, alone and within the matrix

Materials and methods

Developed formulation

Ferrous fumarate
Microcrystalline cellulose and sodium carmellose
Sucralose
Potassium sorbate
Citric acid
Raspberry Flavour
Sterile water

Equipment [2]

Reverse-phase HPLC in isocratic mode
Mobile phase: water : acetonitrile 40:60 (v:v) Water containing 0.06 M methanesulfonic acid
Column: TSKgel Super ODS 50 × 4.6 mm, 2 µm
Temperature : 35°C
Flow rate : 1 mL/min
λ: 282 nm
Analysis time : 6 min

Assay method validation (ICH Q2R2)

Ferrous fumarate complexed with bathophenanthroline (Bphen) → Fe(Bphen)²⁺₃

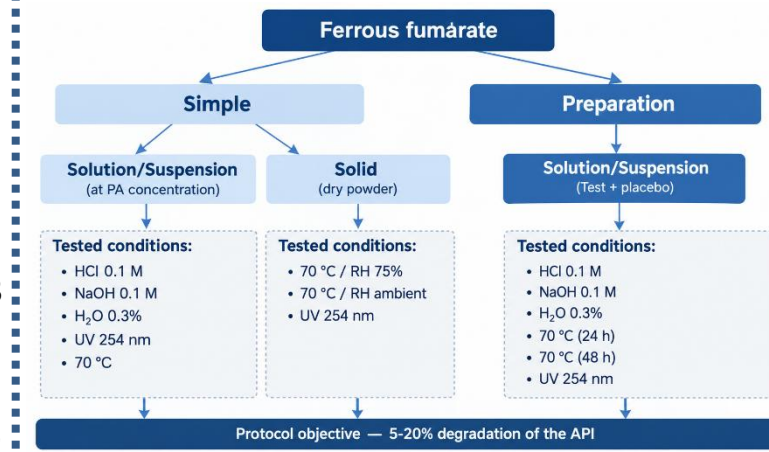
On ferrous fumarate

- 5 calibration standards
- 1 validation standard (100%) repeated x6

On the formulation

- 5 calibration standards x3

Forced degradation

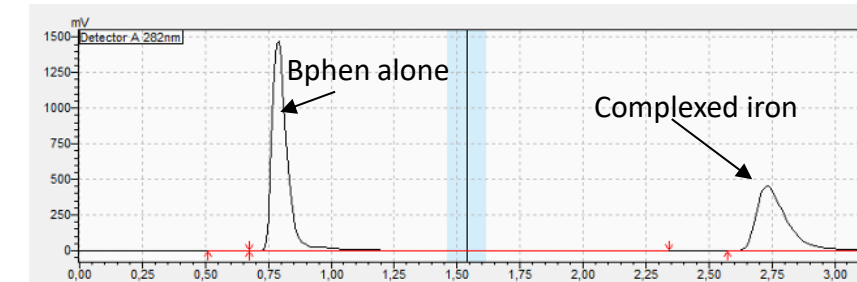


Results & Discussion

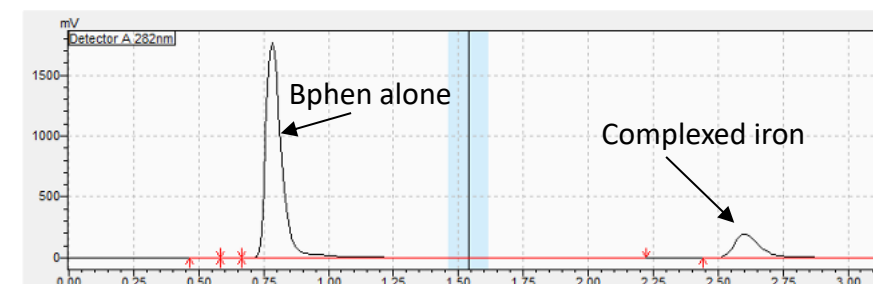
Assay method validation

Parameters		Results
Specificity		Absence of interference
Response function	Dose-response relationship	Linear response D1 (API only) : $Y = 1,26 \cdot 10^6 \cdot X + 93\,494$; $R^2 = 0,996$ D2 (API + matrix) : $Y = 1,20 \cdot 10^6 \cdot X - 107\,944$; $R^2 = 0,991$ No lack-of-fit detected : D1 $p = 0,477$; D2 $p = 0,783$.
	Validation interval	60 – 140 % of the nominal content (2,83 µg/mL = 100 %).
	Matrix effect	No significant matrix effect Comparison of slopes : $p = 0,104$ Comparison of intercepts : $p = 0,086$
Precision	Repeatability (within a single day)	CV = 0,65 % — 95 % CI [0,48 ; 1,00] %
	Intermediate precision (between days)	CV = 0,75 % — 95 % CI [0,53 ; 1,31] %
Accuracy		Recovery : 101.19 % — 95 % CI = [100.23 ; 102.15]
LOD / LOQ		LOD = 0,10 µg/mL LOQ = 0,29 µg/mL

- The method is linear, accurate, and precise
- No matrix effect



Chromatogram of complexed iron



Chromatogram of degradation in the simple matrix (basic hydrolysis, 3h)

Forced degradation

Forced degradation study — summary of results						
Matrix	Status of the API	Degrading	Time	Degradation (%)	C1*	C2**
Simple	Solu/suspension	HCl 0,1 M	24 h	10,4	✓	✗
	Solu/suspension	NaOH 0,1 M	30 min	21,1	✗	✓
	Solu/suspension	H ₂ O ₂ 0,3 %	1 min	89,6	✗	✓
	Solu/suspension	UV 254 nm***	24 h	16,0	✓	✗
	Solu/suspension	70 °C	24 h	61,8	✗	✓
	Solid	70 °C / HR 75 %	24 h	17,2	✓	✗
	Solid	70 °C / HR amb.	24 h	19,6	✓	✗
Preparation	Solu/suspension	UV 254 nm***	24 h	13,4	✓	✗
	Solu/suspension	HCl 0,1 M	24 h	7,2	✓	✗
	Solu/suspension	NaOH 0,1 M	2 h	53,5	✗	✓
	Solu/suspension	H ₂ O ₂ 0,3 %	5 min	64,8	✗	✓
	Solu/suspension	70 °C	24 h	12,9	✓	✗
	Solu/suspension	70 °C	48 h	18,6	✓	✗
	Solu/suspension	UV 254 nm***	24 h	29,4	✗	✓

- **No inert conditions** : loss ≥ 5% under all conditions.
 - **H₂O₂/NaOH** : loss > 20% in less than 2 h (Fenton [3]; hydrolysis of Fe²⁺ accelerates its oxidation).
 - **Heat** (suspension) : API alone > preparation; protection attributed to acidic pH (citric acid).
 - **UV-C**: preparation > API alone; photoreactivity of Fe(III)-carboxylate complexes [4]; not covered by ICH Q1B.
 - **Solid state** : loss of 13–20%, with no aggravating effect from moisture.
- **Common pathway: oxidation of Fe²⁺ to Fe³⁺, modulated by oxygen and pH.**[3][4]

*C1 : degradation 5-20 % (objective)

**C2 : excluding 5-20 % (>20 % for early-stage or <5 % for advanced-stage)

***UV 254 nm : UV-C exposure

Conclusion

The method meets the criteria of ICH Q2(R2) with matrix calibration. Ferrous fumarate degrades primarily through the oxidation of Fe²⁺, which is accelerated by oxidizing agents, basic pH, heat, and UV light. These data will guide the packaging and storage of the preparation, which are being evaluated in the ongoing stability study.