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

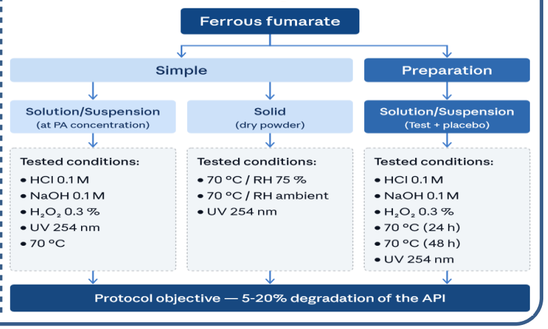
} x3

On the formulation

- 5 calibration standards

} x3

Forced degradation

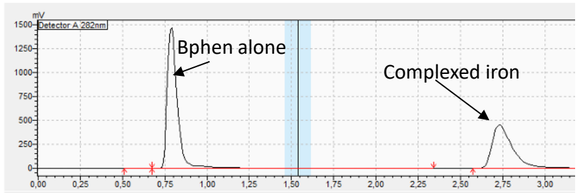


Results & Discussion

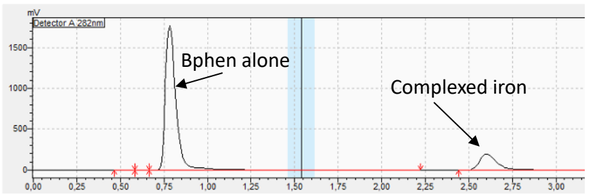
Assay method validation

Parameters		Results
Specificity		Absence of interference
Response function	Dose-response relationship	Linear response D1 (API only) : Y = 1,26·10⁶·X + 93 494 ; R² = 0,996 D2 (API + matrix) : Y = 1,20·10⁶·X − 107 944 ; R² = 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	✓	X
	Solu/suspension	NaOH 0,1 M	30 min	21,1	X	✓
	Solu/suspension	H_2O_2 0,3 %	1 min	89,6	X	✓
	Solu/suspension	UV 254 nm***	24 h	16,0	✓	X
	Solu/suspension	70 °C	24 h	61,8	X	✓
	Solid	70 °C / HR 75 %	24 h	17,2	✓	X
	Solid	70 °C / HR amb.	24 h	19,6	✓	X
	Solid	UV 254 nm***	24 h	13,4	✓	X
Preparation	Solu/suspension	HCl 0,1 M	24 h	7,2	✓	X
	Solu/suspension	NaOH 0,1 M	2 h	53,5	X	✓
	Solu/suspension	H_2O_2 0,3 %	5 min	64,8	X	✓
	Solu/suspension	70 °C	24 h	12,9	✓	X
	Solu/suspension	70 °C	48 h	18,6	✓	X
	Solu/suspension	UV 254 nm***	24 h	29,4	X	✓

*C1 : degradation 5-20 % (objective)

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

***UV 254 nm : UV-C exposure

- **No inert conditions** : loss ≥ 5% under all conditions.
- **H_2O_2/NaOH** : loss > 20% in less than 2 h (Fenton [3]; hydrolysis of Fe^{2+} 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 Fe^{2+} to Fe^{3+}, modulated by oxygen and pH.[3][4]

Conclusion

The method meets the criteria of ICH Q2(R2) with matrix calibration. Ferrous fumarate degrades primarily through the oxidation of Fe^{2+}, 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.

1. Rao R et al. Clin Perinatol. 2009. — 2. Dias NC et al. J AOAC Int. 2011. — 3. Neyens E et al. J Hazard Mater. 2003. — 4. Zepp RG et al. Environ Sci Technol. 1992.