Oral suspension of spironolactone : developement and validation of an analytical method by HPLC-UV

A. MUSICKI[a], C. MERIENNE[b,c], C. MARCHAND[b], M. BRENIAUX[a], F. PIROT[b,c], T. QUESSADA[a],

[a] Pharmacy, Groupement Hospitalier Est (Louis Pradel) – Hospices Civils de Lyon
[b] Pharmacy, Groupement Hospitalier Centre (Edouard Herriot) - Hospices Civils de Lyon
[c] Unit of Preparation and Control of Medicine[a], FRIPHARM, pharmacy, Groupement Hospitalier Centre (Edouard Herriot) - Hospices Civils de Lyon

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Introduction :

The Louis Pradel Hospital currently treats children with heart failure with an oral suspension of spironolactone (5 mg/mL).
The European Pharmacopoeia requires quality controls to release batches of this hospital pharmaceutical preparation, including the determination of the active ingredient content.

Purpose: To develop and validate an analytical method of quantification by High Performance Liquid Chromatography coupled to an ultraviolet detector in order to evaluate the content of spironolactone within a complex matrix (Ora-Blend®).

Material and method :

→ In collaboration with the Hospital Edouard Herriot equipped with a control laboratory and FRIPHARM.
→ According to the methodological guide for stability studies of preparations (GERPAC)

Use of an Agilent Technologies 1260 Infinity HPLC + UV detector

Chromatographic conditions	Defined conditions
Stationnary phase	Column Luna ® : 3 µm, 150 mm x 4.6 mm Octadecyl silica
Mobile phase	Water for injectable preparation + acetonitrile (30:70, v:v)
Flow rate	1.0 mL / min
Injection volume	20 µL
Detection wavelength	254 nm

Calibration standards : preparation = pure spironolactone powder " Chemical Reference Substance " (CRS) + mobile phase.
- 3 concentration levels (low, medium, high): 15, 50 and 75 µg/mL.
Repeated twice, i.e. 6 calibration points per day.

Validation standards : preparation = spironolactone powder " Active Pharmaceutical Ingredient " (API) + Ora-Blend®.
- 3 concentration levels (low, medium, high): 35, 50 and 65 µg/mL.
Repeated 3 times i.e. 9 validation points per day.

Parameters	Validation protocol	Specifications
Specificity	Comparison between CRS and API chromatograms : retention times (RT), areas under the curve (AUC)	No matrix effect
Linearity	2 calibration ranges per day for 3 days with a different operator each day	The average coefficient of determination of the ranges R^2 must be greater than 0.95
Precision	3 validation ranges per day for 3 days	The mean coefficients of variation (CV) within day (repeatability) and between days (reproducibility) must be less than 5% and 8% respectively.
Accuracy	3 validation ranges per day for 3 days	The average relative bias of the concentrations must be < ± 10% and the recovery within the range [100 % ± 10 %]
Accuracy profile	Interval of acceptability and tolerance, values and average of validation standards	Values and average of the validation standards within the tolerance and acceptability interval

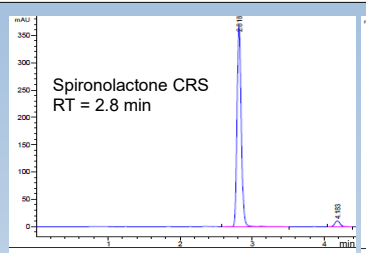
Results :

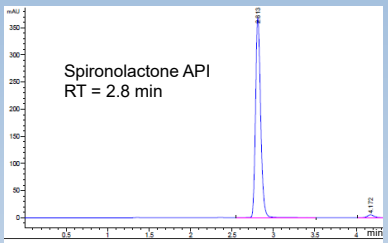
RT and AUC of these chromatograms are similar
No matrix effect
→ **Specificity**

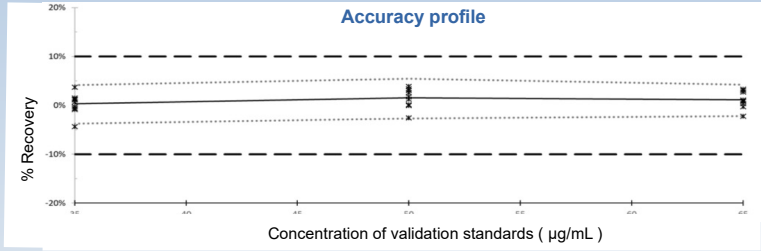
$R^2 = 0.99 > 0,95$ → **Linearity**

Repetability : CV = 1.5 % < 5 %
Reproductibility : CV = 1.8 % < 8 %
→ **Precision**

Relative bias = 1.0 % ± 0,52 ϵ] - 10 % ; + 10 % [
Recovery = 100.99 % ± 0,50 ϵ [90 % ; 110 %]
→ **Accuracy**







Conclusion :

Statistical parameters are within specifications. This reinforces the quality and security of the release of a hospital pharmaceutical preparations batch.
A comparison of the results of the spironolactone quantification in Syrspend® or Inorpha® as vehicles would be interesting (they differ from Ora-Blend® in particular by their osmolarity, their texture and the potentially harmful excipients).