Development and validation of an assay method for the determination of pyrimethamine in an oral suspension by HPLC-UV

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COM21-98359

Introduction : Pyrimethamine (PYR), combined with sulfadiazine and folinic acid, is the first line treatment of congenital toxoplasmosis in neonates and infants. No pharmaceutical form is available to this age group in France: the development of an oral suspension of pyrimethamine is therefore essential

Objective : Development and validation of a stability-indicating method by HPLC-UV..

Materials and methods

Dosage method

Stationary phase	Kinetex Coreshell C18 column (250 x4,6 mm)			
Mobile phase : Gradient	**Time**	**KH_2PO_4 pH=3, 10 mM**	**MetOH**	**ACN**
	0 → 5 min	60%	30%	10%
	5 → 25 min	50%	40%	10%
	27→ 30 min	60%	30%	10%
Flow rate / temperature	1,0 mL/min at 40°C			
Wavelenght	230 nm, 280 nm and scan (190-400 nm)			

Analytical method validated according to **ICH Q2 :**
linearity, accuracy, precision, and specificity
Calibration range (with and without excipients) from 40 mg/L to 120 mg/L
Precision (repetability and intermediate precision) evaluated by a control at 80 mg/L

Forced degradation study (FD)

Hydrolysis	pH = 2 (HCl 0,01N), pH = 6 (KH_2PO_4 10 mM buffer) and pH = 12 (NaOH 0,01N)	D0, D3, D16
	60°C, 20°C and 4°C	
Light-UV	Natural light and 365 nm	
Oxydation	H_2O_2 3%	
	Magnesium MonoPeroxyPhthalate (MMPP) 0,01 mM	D0 and 6 h

Results

Dosage method

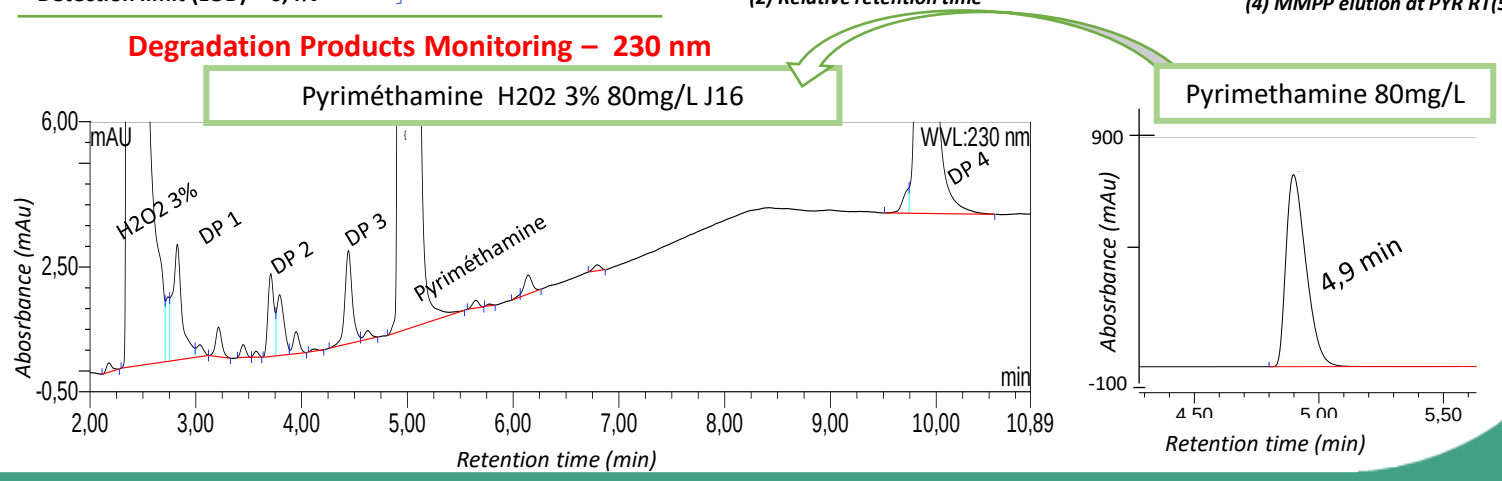
Parameter	Results
Linearity	From 40 to 120 mg/L, r^2 = 0,997 and 0,990 (with and without excipients)
Accuracy	R = [99,41-100,54]
Repetability/ Intermediate precision	CV(r) = 1,77 % CV(if)= 2,11 %
Specificity α=5%	No interference of excipients (sucralose, citric acid, sodium citrate, xanthane gum, potassium sorbate)
Retention time	4,98 +/- 0,02 min
Quantification limit (LOQ) < 0,08% **Détection limit (LOD) < 0,4%**	Compliant with ICH Q3B

Forced degradation study (FD)

	condition	DP(1)(3): 230 nm RRT(2) [Area (% AN)]	%degradation (m/m)	Dura-tion
hydrolysis	pH = 2 pH=6 pH=12	None	-4,0% -0,2% -0,2%	16 d
	60°C	None	-1,6%	16 d
Oxydation	H_2O_2 3%	0,57; 0,75; 0,89; 2,0 **[0,15; 0,18; 0,25; 5,7]**	**11,3%**	16 d
	MMPP 0,01 mM	1,25; 1,95 **[8,43; 13,2]**	ininterprétable (4)	**6 h**
	UV	0,53; 0,54; 0,71; 0,77 **[0,07; 0,35; 0,1; 0,23]**	**1,4%**	16 d
	light	non	0,7%	16 d

(1) Degradation products
(2) Relative retention time
(3)Area normalisation if area > 0,1% PYR area
(4) MMPP elution at PYR RT(5,0 min)

Degradation Products Monitoring – 230 nm



Conclusion : The analytical assay method is validated and the DF tests have demonstrated the stability indicator character: PYR is very sensitive to oxidation, not very sensitive to light and not sensitive to hydrolysis. This will allow a stability study to be carried out on the formulation under development in order to determine the duration and conditions of conservation