Optimization and validation method of watersoluble vitamins in dermocosmetics by HPLC-UV

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Vitamins are biologically active compounds which act as controlling agents for an organism's normal health and growth. Laboratories are required to certify the concentration of all components although matrix, polarity and other factors make their analysis difficult. This is the reason why determining and validating a method to simultaneously quantify vitamins in pharmaceutical products by HPLC-UV is necessary.

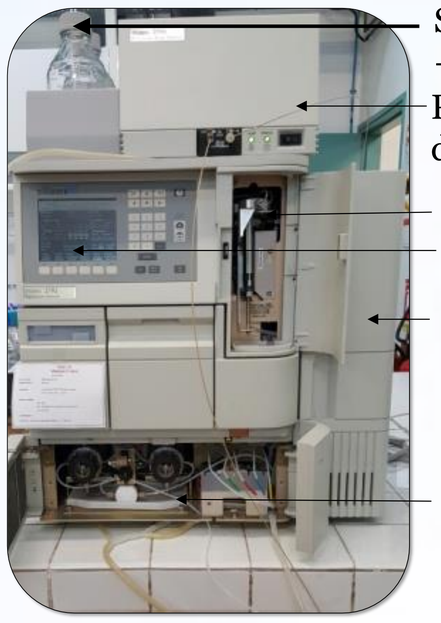
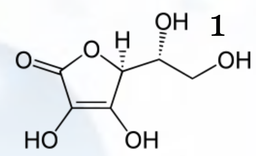
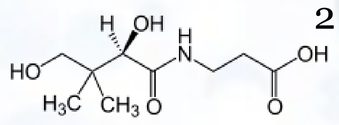


*Figure 1:*Waters® HPLC-UV 2795 (≈ 40 000€)

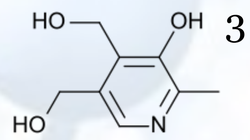
Goal: Simultaneous quantification of vitamins contained in a hair care product



Ascorbic acid (pKa 4.1; 11.8)

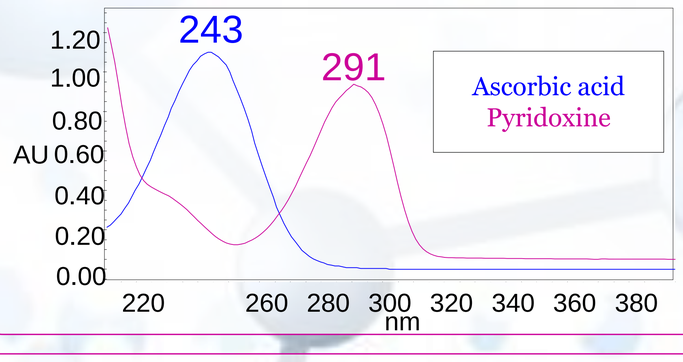


Pantothenic acid (pKa 4.35)



Pyridoxine pKa(5.0; 9.0)

Figure 2: Stuctures of all molecules analyzed



Pantothenic acid absorbance in UV (λ= 268nm) is too low to give a proper spectrum. However, it gives a quantifiable peak on the chromatogram.

Figure 3: UV spectrum of ascorbic acid (240 mg/L) and pyridoxine (180 mg/L in 55% phosphate buffer and 45% ACN)

Chromatographic conditions

- Standard sampling (in 100mL of phosphate buffer 25mM pH=2.5) :

	Compound	Introduced mass (mg)	Concentration (mg/L)	Tr (min)
1	Ascorbic acid	24	240	4.33
2	Pantothenic acid	60	600	7.94
3	Pyridoxine	18	180	7.42

Optimized chromatographic conditions

Time	%A	%B
0	99	1
2	99	1
6	55	45
9	55	45
11	99	1
14	99	1

Column: ODS Nucleosil RP 18, Phenomenex (4x150 mm, 5 µm)
Detection at 220 nm
Temperature : 25 °C
Flow : 0.6 mL/min
Analysis time : 14 min
A= Phosphate buffer (25 mM, pH 2.5)
B= ACN
Injection volume : 10 µL

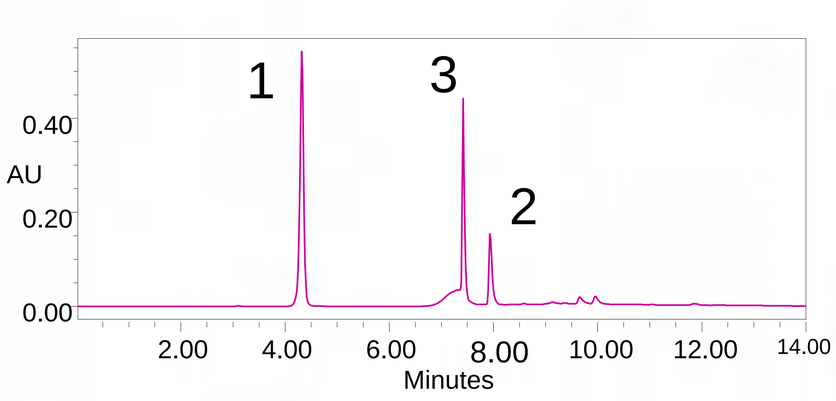


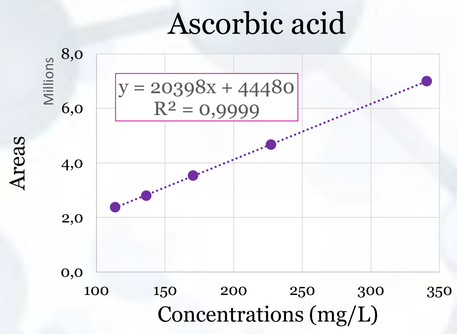
Figure 4: Mix of 3 vitamins: [Ascorbic acid]= 159 mg/L, [Pantothenic acid]= 322 mg/L, [Pyridoxine]= 121 mg/L

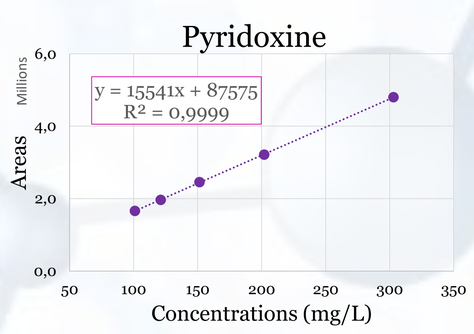
Calibration

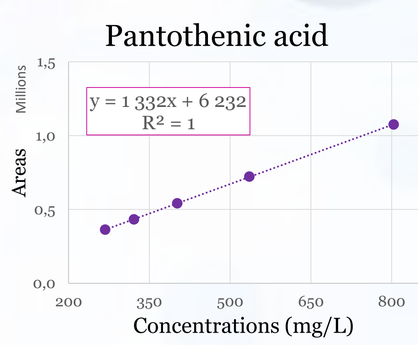
- Sampling for the calibration

So : Stock solution

So	Mass (mg)	Volume of So	Concentration (mg/L)
Ascorbic acid	39.8	100mL phosphate buffer 25mM pH2,5	398
Pantothenic acid	80.4		804
Pyridoxine	30.3		303







Method validation

	Linearity (R^2)	LOD/LOQ (mg/L)	Repeatability (CVr)	Accuracy <10%
Ascorbic acid	0.9999	5.54/13.38	1.92	17.1
Pantothenic acid	1	9.31/20.12	3.52	3.3
Pyridoxine	0.9999	3.95/8.53	5.06	1.8

Linearity: R^2> 0.99..... ✔
Repeatability: CVr<20% ..... ✔
Accuracy: Deviation< 10%..... ✔

Analysis of hair care product

Sampling preparation : 500µL diluted in 25mL of phosphate buffer.

	Compound	tr (min)	Peak area	Concentration in hair care product (g/L)
1	Ascorbic acid	4.34	57252	< LOD
2	Pantothenic acid	7.92	566987	21.05
3	Pyridoxine	7.42	1745054	5.33

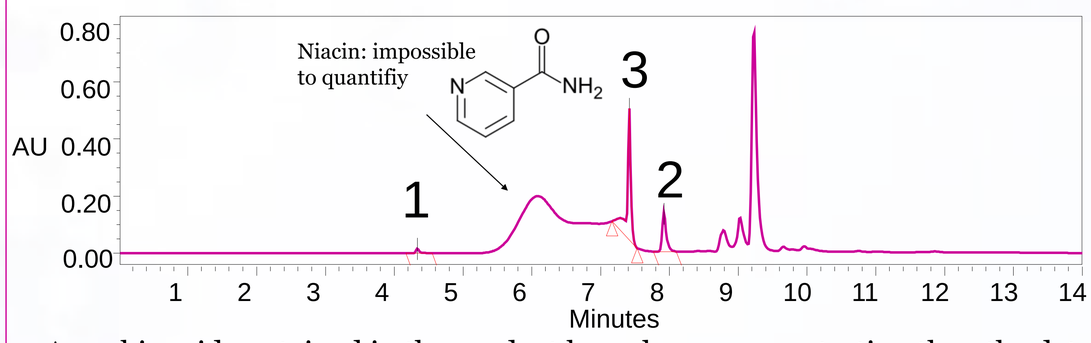


Figure 5 : Chromatogram of the analysis of a hair care product

Ascorbic acid contained in the product has a lower concentration than the detection limit that is why it was not measured. We could explain this observation by means of the spontaneous degradation of the ascorbic acid in aqueous solution.

Thanks to the different analyses, experimental conditions have been optimized and allow the simultaneous quantification of three hydrosoluble vitamins in aqueous solution. The study of different parameters like repeatability, linearity and accuracy have permitted to validate this method for hair care product. In conclusion, this method is easy, reliable and it could be used to measure hydrosoluble vitamins in different complex matrices like in effervescent tablets.

Aknowledgments : We would like to thank our professors and the laboratory for their help and their advices. A special thank to Mr Jammes from Pierre Fabre for his collaboration.

References : Reversed Phse HPLC of Water soluble Vitamins on Agilent ZORBAX Eclipse Plus Columns, A.Glincko, MJ Bozym, M.L Usher, **2008**, Agilent Technologies
Rapid analysis of water-soluble vitamins, Dr.S.Marten,**2014**, Knauer