Optimization and validation method in HPLC of the dosage method of an IPA (Ingredient Pharmaceutical Active)

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Introduction

The structure of HPLC columns allows for a considerable decrease in the time it takes to analyse dosage methods, without any loss of efficiency. This translates to a reduction in time-cost as well as utilized solvent amount. The method set up previously for the dosage of an IPA, produced by RHODIA in HPLC, utilizing a long column, required a total time of approximately 50 minutes. Replacing it with a shorter column caused a considerable reduction to the analysis time. The aim of this study was to decrease analysis time while maintaining good separation between the measured peaks: X1, X2 and X3. After this, a validation of the new method was done, following a validation protocol.

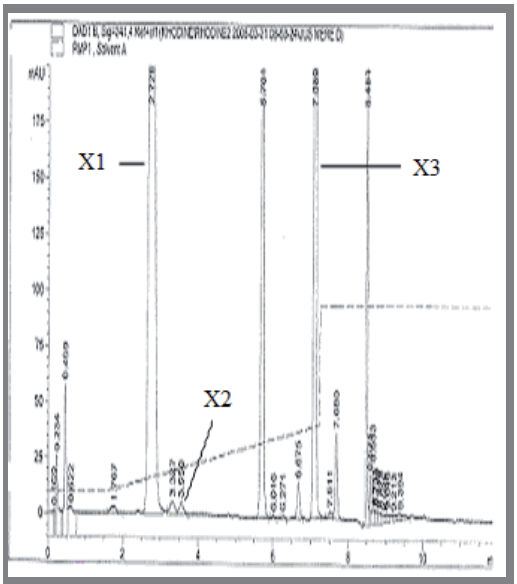

Figure 1. Typical chromatogram after optimization

Experimental conditions

The column used on the old method was an INERTSIL-ODS 250*4.6 mm, 5 µm. The temperature, the wavelength and the flow were 40°C, 241nm and 1mL/min respectively for this method. This column was replaced by a 3 µm, 55*2 mm PUROSPHER Lichrocart column. The temperature and the wavelength were kept the same for the new method. However, the gradient elution and the flow were changed in order to reduce the time analysis. Here is the comparative statement for both methods:

Results and discussion

First, the samples had to be solubilised in acetonitrile and water (1/1), for the new method, to keep symmetrical peaks. The new parameters showed good optimization.
After that, the new method had to be validated. Specificity, repeatability, reproductibility, linearity, detection and quantification limits, precision and comparison between the two methods were all validated.

	Old method			New method		
Diluent	Acetonitrile			Acetonitrile/water (1/1)		
Mobile phase flow (mL/min)	1			0.8		
Column temperature (°C)	40			40		
Elution gradient	Time (min)	%ACN	%H_2O+H_3PO_4 (2‰)	Time (min)	%ACN	%H_2O+H_3PO_4 (2‰)
	0	40	60	0	10	90
	15	45	55	1.5	10	90
	20	60	40	7.2	41	59
	25	80	20	7.21	95	5
	30	80	20	15	95	5
	35	40	60			
	40	40	60			

Conclusion

The new method had a time analysis of 20 minutes, which was almost 3 times faster than the old method. Moreover, the validation gave satisfactory results, as the measured peaks maintained good separation.

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