Trolamine quantification in Biafine®

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Introduction

Biafine is a pharmaceutical product which is sold all over the world. The laboratory which produces it is Janssen, in Val de Reuil, Normandy. Many analyses are made on Biafine, such as, sulphuric ash, water content, pH and viscosity measurements, dry extract and other tests which are necessary for marketing. This article discusses quantification of trolamine by High-Performance Liquid Chromatography (HPLC), as this molecule is the active principle in Biafine.

Experimental

For the quantification of trolamine by HPLC, we used the following analytical conditions:
Nucleosil 100NH2 RP column
Flow at 1.0mL/min
Solvent and mobile phase: Acetonitrile/water 85/15 (v/v)
UV detection, wavelength: 205 nm
Injection volume: 20µL

For trolamine quantification, it is necessary to prepare standards which will be used to quantify the amount in an unknown sample. For sample preparation, 1000mg of sample was weighed and put in a 100 mL low actinic volumetric flask and diluted with Acetonitrile/water 85/15 (v/v).
After one hour of agitation, we put the solution in a vial.
The standard solution, S1, was injected five times which permitted us to check the stability of the column and standard before the sample injection. We determined the relative standard deviation (RSD) and confirmed that it did not exceed a value fixed by the procedure. Then the sample was injected between two standards (S1 and S2). This enabled us to verify the stability of the chromatographic system and standards. This last verification allowed us to determine the final RSD for the analyses completed. In fact, if this deviation had been out of specification, the result for the sample would be incorrect and the analysis would have to be done again with a new standard and samples.
We then wrote the analysis sequence on the software and after the analysis, checked the result (Fig. 1).

The detector is set at 205nm to obtain the trolamine UV spectrum. To realize HPLC analysis it is necessary to verify this value that is why we weighed 30mg of Trolamine and diluted it in a 50mL volumetric flask with Ethanol. Then, 1mL of this solution was put in a 100mL volumetric flask and 10mL of this solution was transferred to a 50mL flask. A sample of this last solution was introduced in the UV cell and analyzed. From the spectrum, we obtain a maximum peak value at a wavelength of 202.17nm (Fig. 2). This is in correlation with the value imposed by the procedure. So, we can begin the HPLC quantification.

Figure 1 : Chromatogram of Trolamine
Figure 2 : UV spectrum of Trolamine

Fig.1

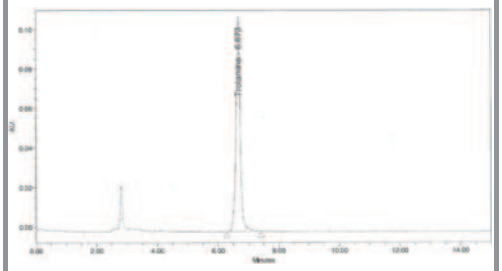
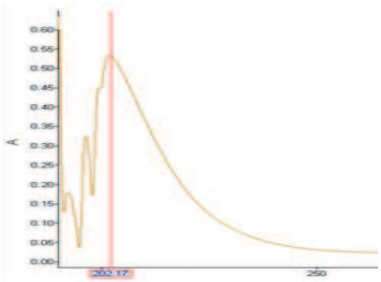


Fig. 2



Results and discussion

Results : property of Janssen

Biafine is considered as a drug which is why specifications are made by the Research & Development according to European Pharmacopeia.
The results we obtained for the quantification of trolamine by HPLC must be between the upper and lower limits defined in the specifications where the target value is 0.67%, which is the average value. Our analyses were between the limits.