N/Protein Determination in Vaccines according to the Dumas combustion method

Reference: **VELP Internal Procedure**

Tested with **VELP Scientifica NDA 701 Dumas Nitrogen Analyzer** (Code F30800070)

Introduction

A vaccine contains a part of a germ (bacteria or virus) that is called an antigen. The antigen has already been killed or disabled before it's used to make the vaccine, so it can't make you sick. Antigens are substances, often a protein, that stimulate the body to produce an immune response to protect itself against attacks from future actual disease exposure. In addition, vaccines contain other ingredients that make them safer and more effective, including preservatives, adjuvants, additives and residuals of the vaccine production process.

Vaccines have reduced and, in some cases, eliminated many diseases that killed or severely disabled people just a few generations ago.

Vaccines, like any medication, are continually monitored for safety.

Protein Determination in two different vaccine solutions

The Dumas method starts with a combustion furnace (CF) to burn the sample, obtaining elemental compounds.

Water is removed by a first physical trap (WT1 - **DriStep™**), placed after the combustion, and a second chemical one (WT2). Between the two, the elemental substances passed through a reduction furnace (RF).

The auto-regenerative CO_2 absorbers (CO_2) let pass only the elemental nitrogen that is detected by the **LoGas™** innovative Thermal Conductivity Detector (TCD) with no requirement for a reference gas.

The NDA 701 is controlled via PC through the intuitive **DUMASoft™**.
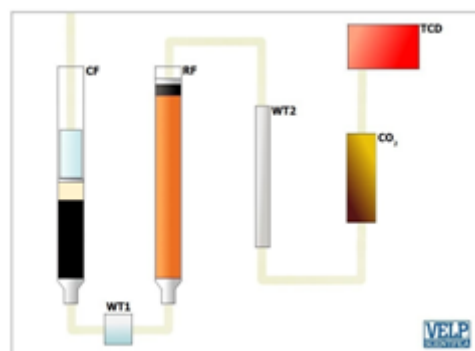
NDA 701 Preliminary Operations (daily)

Follow the operating manual to start the NDA 701 and check that the following parameters are set:

Temperature Combustion reactor (Code A00000158): 1030 °C
Temperature Reduction reactor (Code A00000226): 650 °C
Flow rate MFC1 He: 190 ml/min
Flow rate MFC2 He: 220 ml/min

Condition the system by testing 2 EDTA standard (Code A00000149) and 3 to 5 empty tin foils (Code A00000153) as Check up. Verify the calibration curve with one or more tests as Standard by testing the same standard used for the curve creation.

Sample Preparation

Two liquid vaccine samples have been analyzed: Dipthteria and Tetanus. Samples have been kept in vials at refrigerated temperature until the test.

In order to homogenize the sample, shake the vial through VELP Vortex TX 4 (Code F202A0270).

Put into the tin foil 50-70 mg of Super-Absorbent Powder (Code A00000317).

Pour into the tin foil around 200 μl (200-220 mg) of vaccine solution using a micropipette.

Carefully close the tin foil, obtaining a capsule.

Load the capsule into the autosampler.

Analysis Procedure

Fill the following fields in the database: **Sample name, Weight, Method, Sample type, Calibration number**

Create a new customizable method with the following parameters:

Protein factor: 6.25
O_2 flow rate: 300 ml/min
O_2 factor: 0.7 ml/mg

Press 🟢 to start the analysis.

Analysis time: from 3 minutes for one run.

Typical Results on Vaccine samples

Results have been obtained with the following calibration curve: in a range of 0 – 1.7 mg N with 5 measurements of Rice flour standard (N% = 1.38) (Code A00000235). The data obtained are included in the tolerance admitted by the Rice flour certificate.

The table below shows the nitrogen/protein results, obtained by the Dumasoft™.

Sample	Sample quantity (mg)	Nitrogen %	Protein %
Diphteria	220.90	0.100	0.628
	217.00	0.099	0.619
	216.90	0.091	0.570
	219.00	0.100	0.624
	215.70	0.098	0.612
	Average ± SD%	**0.098 ± 0.004**	**0.611 ± 0.023**
Tetanus	199.50	0.127	0.791
	201.80	0.131	0.816
	206.80	0.131	0.821
	204.30	0.130	0.811
	210.90	0.130	0.814
	Average ± SD%	**0.130 ± 0.002**	**0.811 ± 0.012**

Protein Factor: 6.25

Conclusion

Nowadays the attention on safety of vaccines has increased due to their potential adverse reactions after injection. Nitrogen/protein analysis according to the Dumas principle with the combustion apparatus is a credible alternative to the acid digestion method according to Kjeldahl.

NDA 701 ensure reliable results in a easy and fast way. Despite low sample weights, excellent reproducibility are possible and the data confirm the complete combustion of the sample with no memory effect observed.

With high productivity and non-stop performances, NDA 701 Dumas combustion apparatus is ideal for high throughput, being fully automated and requiring just 3-4 minutes per analysis.