

Nitrogen determination in diphtheria and tetanus toxoids

KjelMaster System K-375 / K-376, KjelDigester K-449, Scrubber K-415.

Nitrogen and total protein determination in pharmaceutical products are described in the European Pharmacopoeia (Ph.Eur.) section 2.5.9 and 2.5.33 method 7 [1, 2]. In the Pharmacopoeia of the United States (USP), nitrogen determination is described in section 461 [3]. Therein, different titration techniques are specified.

The Ph.Eur. describes back titration in the presence of the indicator methyl red mixed solution and the USP potentiometric and colorimetric boric acid titration.

1. Introduction

Aim of the following study was to determine the reproducibility and to compare the three different titration techniques, i.e. boric acid titration (potentiometric detection), boric acid (colorimetric detection) and back titration (potentiometric detection).

Therefore, the nitrogen content of two vaccine samples, a diphtheria and tetanus toxoid, was determined by sulfuric acid digestion followed by distillation and titration applying the BUCHI KjelMaster System K-375 / K-376.

The two samples were kindly provided by the Staten Serum Institute, Denmark.

2. Experimental

The toxoid samples were pipetted (1 mL) into a sample tube and 15 mL conc. sulfuric acid and two Kjeldahl Tablets Titanium were added. The samples were digested using the KjelDigester K-449.

Subsequently, distillation and three titration techniques; boric acid titration (potentiometric), boric acid titration (colorimetric) and back titration (potentiometric) were applied using the KjelMaster System K-375 / K-376. The varying distillation and titration parameters of the three titration techniques are listed in Table 1 – 3.

Table 1: Distillation parameters for boric acid titration (potentiometric).

Stirrer speed distillation	5	Sensor type	Potentiometric
Titration type	Boric acid	Endpoint pH	4.65
Receiving solution vol.	50 mL (boric acid)	Stirrer speed titration	7
Titration solution	HCl 0.01 mol/L		

Table 2: Distillation parameters for boric acid titration (colorimetric).

Stirrer speed distillation	3	Titration solution	HCl 0.01 mol/L
Titration type	Boric acid	Sensor type	Colorimetric
Receiving solution vol.	60 mL (boric acid)	Stirrer speed titration	10

Table 3: Distillation parameters for back titration (potentiometric).

Stirrer speed distillation	3	Sensor type	Potentiometric
Titration type	Back titration	Titration solution	NaOH 0.01 mol/L
Receiving solution	HCl 0.01 mol/L	Endpoint pH	5.6
Receiving solution vol.	20 mL (HCl)	Stirrer speed titration	5

3. Results

The results of the three titration techniques, boric acid titration (potentiometric), boric acid titration (colorimetric) and back titration (potentiometric) are shown in Table 4. Samples were measured four-fold (n=4).

Table 4: Results obtained by the three titration techniques.

	Diphtheria toxoid [mg N/mL]	Tetanus toxoid [mg N/mL]
<i>Expected value</i>	0.80 – 1.02	0.30 – 0.38
<i>Boric acid titration (potentiometric)</i>	0.95 ± 0.01 (RSD 0.68)	0.31 ± 0.00 (RSD 0.45)
<i>Boric acid titration (colorimetric)</i>	0.93 ± 0.01 (RSD 1.35)	0.31 ± 0.00 (RSD 1.19)
<i>Back titration (potentiometric)</i>	0.94 ± 0.01 (RSD 1.29)	0.30 ± 0.00 (RSD 0.54)

All three titration techniques lead to similar and reproducible results and were within the sample specification (0.91 ± 0.11 mg N/mL for diphtheria toxoid, 0.34 ± 0.04 mg N/mL for tetanus toxoid).

4. Conclusion

The nitrogen content of the toxoid samples were successfully determined using the KjelDigester K-449, Scrubber K-415, and the KjelMaster System K-375 / K-376. All experiments were performed smoothly and reproducibly, applying automated distillation and titration procedures in accordance to Ph.Eur. and USP.

Depending on the standard followed, boric acid or back titration are required for nitrogen determination. Boric acid titration (potentiometric and colorimetric) and back titration led to similar results using the KjelMaster System K-375 / K-376.

A point-wise discussion of the Ph.Eur. 2.5.9. and USP 461 requirements and the compliance of the BUCHI instrumentation is detailed in the appendix in application note No. 279/2016 [4]. Furthermore, advantages and disadvantages of each titration technique are discussed in the Result and Discussion section of the application note [4].

5. References

- [1] European Pharmacopoeia (01/2008:20509, 9th edition) 2.5.9., Determination of nitrogen by sulfuric acid digestion.
- [2] European Pharmacopoeia (01/2008:20533, 9th edition) 2.5.33. Method 7, Total protein.
- [3] Pharmacopoeia of the United States 461, Nitrogen determination (2016).
- [4] Application note: 279/2016_Nitrogen determination in diphtheria and tetanus toxoid samples. www.buchi.com