Nitrogen determination in pharmaceutical active peptides

KjelMaster System K-375 / K-376, SpeedDigester K-439, 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 boric acid titration.

1. Introduction

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

Therefore, the nitrogen content of a biotechnologically prepared peptide sample for intravenous use was determined by sulfuric acid digestion followed by distillation and titration applying the BUCHI KjelMaster System K-375 / K-376.

2. Experimental

The peptide sample was pipetted (0.35 mL) into a micro sample tube and 5 mL conc. sulfuric acid and one Kjeldahl Tablet Titanium Micro were added. The samples were digested using the SpeedDigester K-439.

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*	*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 in triplicate (n=3).

Table 4: Results obtained by the three titration techniques.

	Nitrogen content [mg N/mL]
Boric acid titration (potentiometric)	***6.24 ± 0.01 (RSD 0.16)***
Boric acid titration (colorimetric)	***6.25 ± 0.01 (RSD 0.23)***
Back titration (potentiometric)	***6.23 ± 0.04 (RSD 0.58)***

A nitrogen content of 6.24 ± 0.01 mg N/mL, 6.25 ± 0.01 mg N/mL and 6.23 mg N/mL ± 0.04 was determined in the peptide sample applying the three different titration techniques. In general, all three titration techniques lead to similar and reproducible results.

4. Conclusion

The nitrogen content of the peptide sample was successfully determined using the SpeedDigester K-439 with Micro Kjeldahl sample tubes, 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 (potentiometric and colorimetric) and back titration lead 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. 264/2016 [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: 264/2016_Nitrogen determination in pharmaceutical active peptides. www.buchi.com