

Bovine serum albumin is a protein that is applied in many biochemical and medical applications. A simple and fast procedure for nitrogen determination in bovine serum albumin, according to AOAC 960.52 and standard of Chinese pharmacopeia is introduced [1,2].

For the nitrogen determination according to Kjeldahl the samples are digested using the SpeedDigester K-436 followed by steam distillation using the KjFlex K-360 and titration.

The nitrogen content in bovine serum albumin was determined by the standard- and micro-Kjeldahl methods and compared. The determined nitrogen contents correspond well to the labelled values.

1. Introduction

For many pharmaceutical products, the nitrogen content is determined to evaluate the production process and the product quality. In order to save chemicals, the micro-Kjeldahl method can be applied to samples with a high nitrogen content.

2. Experimental

Equipment: SpeedDigester K-436 / KjFlex K-360 with Metrohm 877 Titrino plus / Scrubber K-415 TripleScrub^{ECO}.

Samples: The sample bovine serum albumin was provided from Tanjin CanSino Biotechnology Inc. China. The nitrogen content of sample was 1.6 mg/mL and it also contains 10 % glycerin.

Determination: The samples were homogenized and added directly into a sample tube as described in Table 1. One Kjeldahl Tablet and sulfuric acid (conc. 98 %) were added.

Table 1: Method parameters for micro-, and standard-Kjeldahl.

Method	Sample volume [mL]	Sample tube size [mL]	Kjeldahl Tablet	H ₂ SO ₄ [mL]
micro	0.5	100	Titanium Micro	3
standard	1	300	Titanium	10

The digestion was performed using the SpeedDigester K-436 applying the parameters specified in Table 2. The method was verified by measuring 0.05 g glycine as the reference substance.

Table 2: Temperature profile for digestion with the K-436.

Step	Standard-Kjeldahl		Mirco-Kjeldahl	
	Heating level	Time [min]	Heating level	Time [min]
1	5	30	5	15
2	9	90	9	45
Cooling	--	30	--	30
Digestion time	--	120	--	60

After digestion, the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid (see Table 3).

Table 3: Method parameters for the distillation and titration with the KjFlex K-360 according to the standard-Kjeldahl and the micro-Kjeldahl method.

KjFlex K-360		Metrohm 877 titrino plus	
H ₂ O volume	50 mL(standard-) 25 mL(mirco-)	Titration solution	H ₂ SO ₄ 0.01 mol/L H ₂ SO ₄ 0.05 mol/L (for glycine)
NaOH volume	60 mL (standard-) 30 mL (mirco-)	Sensor type	pH electrode
H ₃ BO ₃ volume	50 mL	Measuring mode	Endpoint pH=4.65
Reaction time	5 s	Titration rate	Optimal
Steam Power	100 %	Stop Criterion	Drift
Distillation time	240 s	Stop Drift	20 µl/min
Titration Start	240 s	Stop Time	Off
Titration type	Boric acid	Filling Rate	max. ml/min

3. Results

The glycine recoveries are presented in Table 4. The determined nitrogen contents of the pharmaceutical sample are listed in Table 5.

Table 4: Results of the recovery of nitrogen in glycine(n=3, in standard-Kjeldahl; n=4, in micro-Kjeldahl).

Glycine	Standard-Kjeldahl	Mirco-Kjeldahl
Average recovery [%]	100.1	101.0
RSD [%]	0.35	0.56

Table 5: Determined nitrogen contents (the result is averaged, n=4).

Sample	Standard-Kjeldahl	Mirco-Kjeldahl
Average N content [mg/mL]	1.63	1.59
RSD [%]	1.86	1.57

4. Conclusion

The determination of nitrogen in bovine serum albumin using the SpeedDigester K-436 and KjFlex K-360 provides reliable and well reproducible results. All measured results correspond well to the labelled values with low relative standard deviations.

Digestion took 120 minutes for the standard-Kjeldahl process and only 60 minutes for the micro-Kjeldahl method.

Besides reducing the process time compared to the standard Kjeldahl method, the micro-Kjeldahl method helps to reduce the amount of chemicals needed for the nitrogen determination, thereby saving money and resources.

5. References

- [1] AOAC 960.52 Microchemical Determination of Nitrogen (Micro-Kjeldahl Method)
 - [2] Standard of Chinese pharmacopeia (version 2015): Nitrogen determination in Medicine
- For more detailed information and safety considerations please refer to the Application Note no. 230/2016.