

Protocol No: 5129.1
Date: 3/8/2015

To: Phytofil – N. Stavrakis
Cc: Dr N. Stavrakis

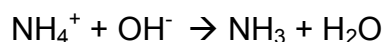
Information in laboratory methods: AMINOACID INDEX TEST

Dear Dr. Stavrakis,

We would like to inform you concerning the AMINOACID INDEX tests that are referred in certificate of analysis of Entomella 50 and Entomella 75.

Principle of method:

Formol reacts with free aminogroups of a-amino acids causing the amino acid to loose a proton which can then be titrated. Free ammonia is also titrated. The full equation for calculating nitrogen (NH_4^+ and a-amino N):



According to AOAC 965.31:

Total Amino acids = $0,4 \cdot V_{\text{NaOH } 0,1\text{N}}$

$n \text{ (mol)} = N \text{ (eq)}/f \text{ (valent)} \rightarrow (f=1) \text{ mol} = \text{eq}$

$n \text{ acid} = n \text{ base} \rightarrow C_s \cdot V_s \text{ (ml)} = C \text{ (meq/ml)} \cdot V_{\text{NaOH } 0,1\text{N}} \text{ (ml)}$

$V_{\text{sample}} = 25\text{ml}$
 $\rightarrow C_s = 0,1(\text{meq/ml}) \cdot V_{\text{NaOH } 0,1\text{N}} / 25\text{ml} \cdot 1000\text{ml/L} \rightarrow C_s = 4 \cdot V_{\text{NaOH } 0,1\text{N}} \text{ meq/L or}$

$C_s = 0,4 \cdot V_{\text{NaOH } 0,1\text{N}} \text{ meq/100ml}$
--

Instead of 25ml that it is referred in AOAC method for juices it was weighted 25g of sample.

$n \text{ acid} = n \text{ base} \rightarrow n \text{ acid} = C(\text{meq/ml}) \cdot V_{\text{NaOH } 0,1\text{N}}(\text{ml}) \rightarrow n \text{ acid} = 0,1(\text{meq/ml}) \cdot V_{\text{NaOH } 0,1\text{N}} \text{ (ml)}$
 $(f=1) \text{ mol} = \text{eq}$

$0,1 \cdot V_{\text{NaOH } 0,1\text{N}} \text{ (meq)}$ from 25g of sample. Then from 100g of sample:

$0,1 \cdot V_{\text{NaOH } 0,1\text{N}} \text{ (meq)} \cdot 100\text{g}/25\text{g} = 0,4 \cdot V_{\text{NaOH } 0,1\text{N}} \text{ (meq)}/100\text{g of sample}$

Total amino acids = $0,4 \cdot V_{\text{NaOH } 0,1\text{N}} \text{ (meq)}/100\text{g of sample}$
--

In a previous method, aminoacid index was referred as :

Aminoacid index = 0.5 x ml used in last titration

Indeed,

$$n_{\text{acid}} = n_{\text{base}} \rightarrow n_{\text{acid}} = C(\text{meq/ml}) \cdot V_{\text{NaOH } 0,1\text{N}}(\text{ml}) \rightarrow n_{\text{acid}} = 0,1(\text{meq/ml}) \cdot V_{\text{NaOH } 0,1\text{N}}(\text{ml})$$

$$(f=1) \text{ mol} = \text{eq}$$

$0,1 \cdot V_{\text{NaOH } 0,1\text{N}}(\text{meq})$ from 2g of sample. Then from 10g of sample:

$$0,1 \cdot V_{\text{NaOH } 0,1\text{N}}(\text{meq}) \cdot 10\text{g}/2\text{g} = 0,5 \cdot V_{\text{NaOH } 0,1\text{N}}(\text{meq})/10\text{g of sample}$$

amino acids index = $0,5 \cdot V_{\text{NaOH } 0,1\text{N}}(\text{meq})/10\text{g of sample}$

There is a difference between two methods, at the end point of titration. That it is explained as different amino acids ratio is expected in two methods and thus a different pH is selected as the best end point.

In HELLASCHEM laboratories Entomella 75SL was tested in accordance with the two methods. The results are referred below:

In accordance with the old method:

mass (g)	V (ml)	mq/10g
2,0009	2,00	1,00
2,0036	2,10	1,05
2,0025	2,10	1,05
2,0028	2,20	1,10
2,0018	2,20	1,10
2,0026	2,15	1,08
average		1,06
stdev		0,038

In accordance with the AOAC 965.31 method:

Mass (g)	V (ml)	meq/100g	meq/10g
25,0002	28,00	11,20	1,12
25,0004	28,40	11,36	1,14
25,0006	28,60	11,44	1,14
25,0003	28,30	11,32	1,13
25,0009	28,50	11,40	1,14
25,0001	28,20	11,28	1,13
average			1,13
stdev			0,01

As it was expected, AOAC 965.31 seems to be more accurate, but both methods have a strong repeatability.

Mr. Nikiforos Iliopoulos
 Chemist -Environmental M.Sc.
 HELLASCHEM Manager