



## DETERMINATION OF **FREE FORMS OF WATER-SOLUBLE VITAMINS** IN PREMIXES, VITAMIN ADDITIVES, CONCENTRATED PRODUCTS, AND FEED MIXES

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GOST R 52741-2007

### INTRODUCTION

To keep the animals healthy and feed them in a scientifically founded manner, all the nutrients, vitamins in particular, should be properly balanced. The vitamin deficiency or excess may cause undesirable changes in the physiological state of the animals, which reduces their productivity and breeding capacity. Therefore, an acute problem of fast and accurate quantitative determination of the vitamin content in the raw stock for production of fodder and feed mixes arises.

### MEASUREMENT METHOD

The measurement method is based on extracting water-soluble vitamins from the samples by an extractant solution (a mixture of sodium tetraborate and sodium sulfite); separation, identification, and determination of weight fractions of vitamins using the capillary electrophoresis (CE) technique. Depending on the composition of the analyzed sample and requirements to the measurement accuracy, two versions of the CE technique can be used, that are, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

The vitamins are detected by their intrinsic absorption at wavelengths of 200 and 240 nm (with programmable wavelength switching).

### MEASUREMENT RANGE

Vitamins	Range of measurable weight fractions*, g/kg	
	Premixes	Vitamin additives
B <sub>1</sub> (thiamine chloride)	0.1–5.0	0.5–25
B <sub>2</sub> (riboflavin)	0.1–5.0	0.5–25
B <sub>3</sub> (pantothenic acid)	1–25	5–125
B <sub>5</sub> (nicotinic acid)	2–100	10–500
B <sub>5</sub> (nicotinamide)**	0.1–5.0	0.5–25
B <sub>6</sub> (pyridoxine)	0.2–10	1.0–50
B <sub>c</sub> (folic acid)	0.1–5.0	0.5–25
C (ascorbic acid)	2–50	10–250

\* The sample weight is **1.0 g** for premixes and **0,2 g** for other types of samples.

\*\* Vitamin B<sub>5</sub> in the nicotinamide form is determined by the MEKC method only.

Along with the above-listed vitamins, quantitative determination of vitamin H (biotin), vitamin B<sub>6</sub> in the pyridoxal form, and vitamin P in the rutin and quercitine forms is also possible.

### EQUIPMENT AND REAGENTS

The "CAPEL<sup>®</sup>-105/105M" capillary electrophoresis system with a special capillary cassette for the vitamins analysis is used in measurements.

Data acquisition, collection, processing and output are performed using a personal computer running under "WINDOWS<sup>®</sup> 2000/XP" operating system with installed dedicated software package for acquisition and processing of chromatography data.

All reagents must be of analytical grade or higher.

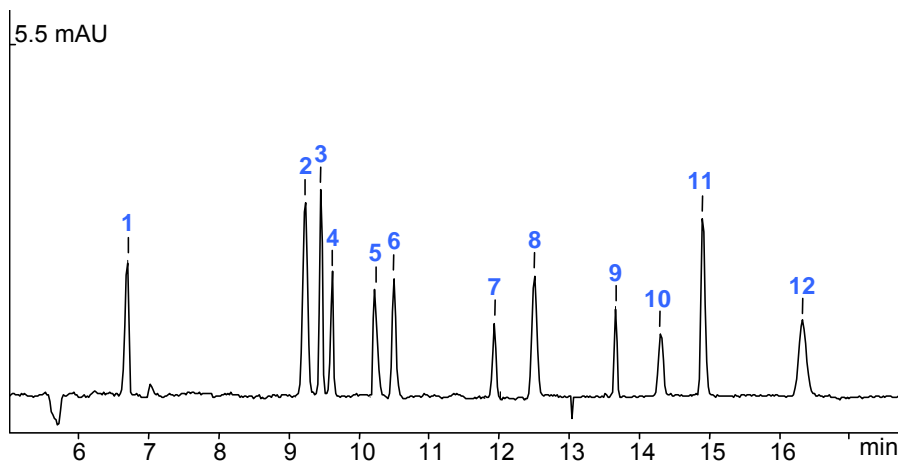


EXAMPLES OF REAL ANALYSES

**Buffer :** borate, with SDS  
**Capillary:**  $L_{\text{eff}}/L_{\text{tot}}$  65/75 cm; ID 50  $\mu\text{m}$   
**Injection:** 600 mbar x sec  
**Voltage:** + 25 kV  
**Pressure:** 0 mbar, 50 mbar  
**Temperature:** +30 °C  
**Detection:** 200, 240 nm

**Sample:** test solution

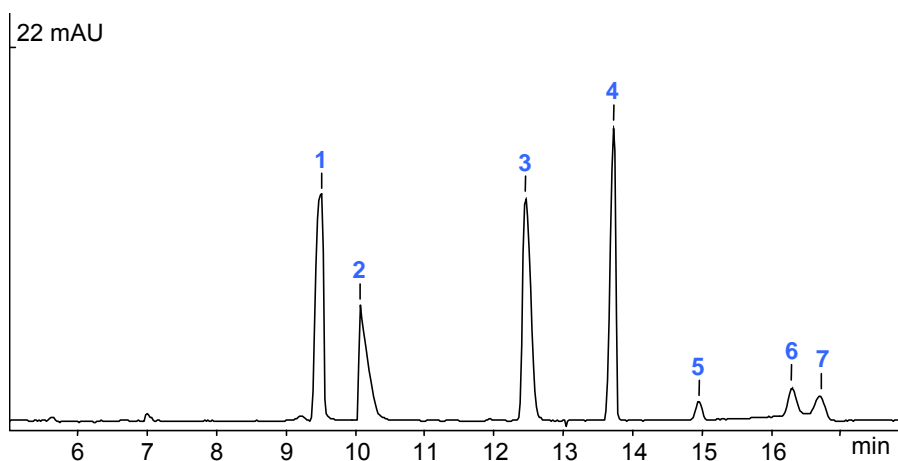
- 1 – nicotinamide (B<sub>5</sub>)
- 2 – pyridoxal (B<sub>6</sub>)
- 3 – pyridoxine (B<sub>6</sub>)
- 4 – biotin (H)
- 5 – vitamin B<sub>2</sub>
- 6 – rutin (P)
- 7 – vitamin C
- 8 – vitamin B<sub>3</sub>
- 9 – nicotinic acid
- 10 – quercetin (P)
- 11 – folic acid (B<sub>c</sub>)
- 12 – vitamin B<sub>1</sub>



**Sample:** vitamin concentrate

**Measurement results:**

- 1 – pyridoxine (B<sub>6</sub>) (15.8 g/kg)
- 2 – vitamin B<sub>2</sub> (20.1 g/kg)
- 3 – vitamin B<sub>3</sub> (56.2 g/kg)
- 4 – nicotinic acid (192 g/kg)
- 5 – folic acid (B<sub>c</sub>) (6.2 g/kg)
- 6 – vitamin B<sub>1</sub> (19.7 g/kg)
- 7 – component of the sample



The contents on this paper are subject to change without notice.