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PREPARATION AND CHROMATOGRAPHIC ANALYSIS
OF BETULIN-BASED FEED ADDITIVE EMULSION

F. O. Adepoju,¹ A. V. Buehler,² V. A. Shevyrin,¹ E. G. Kovaleva¹

¹*Ural Federal University of the first President of Russia B. N. Yeltsin, Institute of Chemical Technology,
19 Mira St., Yekaterinburg, 620002, Russia*

E-mail: besec010@gmail.com

Abstract. Feed additives are products for livestock, intended mainly for preventive purposes, to prevent the occurrence of disease by supporting and improving the immune system to fight infections. Many feed additives can improve the growth and metabolism of livestock, including— a feed additive based on betulin. Betulin is a triterpenoid that is a major component of the birch bark extract (*Betula* spp.) and have been reported to possess antioxidant, anti-inflammatory, antiviral, antitumor, and antidiabetic effects.¹ This study was carried out to evaluate the shelf-life of a feed additive (an emulsion) by conducting stability tests and also to develop a HPLC conditions for the determination of betulin in the emulsion. The emulsion developed consists of betulin, water, ethanol, citric acid, TWEEN-80 emulsifying agent, NaCl, and agar. We used nanofluid experimental design for preparation of the nanoemulsion using compensation method and flow stabilization.

The chromatographic separation of the extract was conducted using the Agilent 1290 Infinity chromatographic system in an isocratic mode on a reversed stationary phase of ZORBAX Eclipse Plus C-18 RRHD (2.1 mm × 50 mm × 1.8 μm) with a pre-column ZORBAX Eclipse Plus C-18 (2.1 mm × 5 mm × 1.8 μm) and a mobile phase of water (eluent A) and acetonitrile (eluent B) of 20:80 (v/v) with a flow rate of 0.35 ml/min and the run time was 3 mins.² The temperature of the chromatographic column was maintained at 35 °C and detection was done at 210 nm and a slit width of 4 nm. A comparison of the results of the study obtained when determining the mass concentration of betulin before, during and after completion of the test using Student's criterion ($t_{95.4}=2.78$) showed no significant difference between the results obtained. Thus, the mass concentration of betulin in the emulsion remained constant during the stability tests as seen in Table 1.

Table 1. Results of analysis of the emulsion before, during and after stability tests

Samples	Exposure time at 55 °C, hours	Results of parallel determinations, mass concentration of betulin, g / dm ³			Final result, g / dm ³	Emulsion layering, yes/no
		1	2	3		
Emulsion	0	4.06	4.13	4.18	4.12 ± 0.11	no
Bottle 1	180	4.05	4.08	4.04	4.0606 ± 0.0404	no
Bottle 2	360	4.04	4.05	4.11	4.0707 ± 0.0707	no
Bottle 3	5.40	4.05	4.16	4.07	4.0909 ± 0.11	no
Bottle 4	720	4.17	4.13	4.22	4.17 ± 0.0808	no

References

1. Amiri S., Dastghaib S. et al. *Biotechnology Advances*, 38, (2020).
2. Singh P. A., Brindavanam N. B. et al. *International Journal of Pharmaceutical Sciences and Research* 7(2), 719 (2016).

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