DETERMINATION OF HYDROCYANIC ACID IN FEEDINGSTUFFS BY HPLC

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Cyanide is a highly toxic compound; in feed it is mainly bound as cyanoglycosides. Cyanide is released from cyanoglycosides by enzymatic breakdown, which process is known as cyanogenesis. So far, the reference method as described in First Commission Directive 71/250/EEC was used in the EC to determine the content of cyanide (expressed as hydrocyanic acid) in animal feed and feed materials. However, the reference method proved to be not sufficiently sensitive and robust. In addition, thiocyanates are detected as well and false positive results could be obtained.

According to EC-guideline 2002/32, describing the maximum allowed concentration of hydrocyanic acid in feed and feed materials, detection at a low level of 10 mg HCN/kg should be possible. However, the method does not fulfill this requirement.

To overcome these disadvantages an improved method has been developed at RIKILT which is more reliable, sensitive and robust. The EC protocol consists of 4 steps: 1) extraction of cyanoglycosides, 2) formation of hydrogen cyanide by enzymatic degradation, 3) extraction of hydrogen cyanide by steam distillation, and 4) titrimetric detection of cyanide. The first two steps were improved by using a weak acid for extraction of cyanoglycosides (step 1) and applying commercially available enzyme instead of an almond suspension (step 2). Instead of the titrimetric method (step 4), cyanide detection was performed by derivatization with taurine and NDA (2,3 napthylene dicarboxy aldehyde), to form a fluorescence complex, and determination by HPLC with fluorescence detection. The detection of the cyanide-complex is selective and not interfered by other compounds.

The method has been selected for standardization by CEN (European Committee for Standardization) in the committee CEN/TC 327 Animal feedingstuffs – Methods of sampling and analysis. The method was initially investigated in a pre-trial by four European laboratories during autumn 2008. The performance and transferability of the method proved to be satisfactory, only a few modifications of the procedure were necessary for further improvement of the robustness.

In June 2009, an international collaborative study started. 19 Laboratories across Europe participate. The first results indicate that the method meets the following performance characteristics: (i) applicable to analyze cyanide in various feeds and feed materials, (ii) a limit of determination of 2 mg/kg or lower, (iii) applicable over a range of 2 – 500 mg/kg, and (iv) a recovery for spiked samples at a level of 10 - and 50 mg/kg of about 60-80%.
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