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Determination of diaveridine, trimethoprim and ormetoprim in feeds

饲料中二甲氧苄氨嘧啶、三甲氧苄氨嘧啶和二甲氧甲基苄氨嘧啶的测定

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1 Scope, normative references and terms

1 The document describes the liquid chromatography-tandem mass spectrometry method and the high performance liquid chromatography method for the determination of diaveridine, trimethoprim and ormetoprim in feeds, and applies to the determination of these three substances in compound feeds, concentrated feeds, concentrate supplements, additive premixes and feed materials of animal origin. For the liquid chromatography-tandem mass spectrometry method the limit of detection is 2.5 μg/kg and the limit of quantification 5.0 μg/kg; for the high performance liquid chromatography method the limit of detection is 0.25 mg/kg and the limit of quantification 0.50 mg/kg.

2 The normative references are GB/T 6682 on water for analytical laboratory use and GB/T 20195 on the preparation of test samples of animal feeding stuffs.

3 The document states that there are no terms and definitions to be defined.

4 Liquid chromatography-tandem mass spectrometry (referee method)

4.1 The analytes are extracted from the test portion with ethyl acetate under weakly alkaline conditions, defatted with n-hexane, cleaned up on a mixed-mode strong cation exchange cartridge, determined by liquid chromatography-tandem mass spectrometry and quantified by the internal standard method.

4.2 Unless otherwise stated the reagents are analytically pure: grade one water to GB/T 6682; chromatographic grade methanol, acetonitrile, formic acid and ammonium acetate; ammonia solution; ethyl acetate. Solutions prepared are sodium acetate solution at 82 g/L from 8.2 g of sodium acetate made up to 100 mL; 5 % acetic acid solution from 5 mL of glacial acetic acid made up to 100 mL with water; 1 % acetic acid in acetonitrile from 1 mL of glacial acetic acid made up to 100 mL with acetonitrile; acetonitrile in 5 % acetic acid from 10 mL of acetonitrile made up to 100 mL with the 5 % acetic acid solution; n-hexane saturated with acetonitrile, obtained by shaking 80 mL of n-hexane with 10 mL of acetonitrile vigorously for 5 min in a separating funnel and discarding the acetonitrile layer after separation; 8 % ammonia in methanol from 8 mL of ammonia solution added to 50 mL of methanol and made up to 100 mL with methanol; 0.2 % formic acid solution from 2 mL of formic acid added to 500 mL of water and made up to 1000 mL; ammonium acetate in 0.2 % formic acid from 0.154 g of ammonium acetate dissolved in the 0.2 % formic acid solution and made up to 1000 mL; and the reconstitution solution from 150 mL of acetonitrile made up to 1000 mL with the ammonium acetate in 0.2 % formic acid.

4.2.17 to 4.2.23 The stock standard solutions at 1 mg/mL are prepared from 10 mg, weighed to 0.01 mg, of diaveridine (CAS 5355-16-8), trimethoprim (CAS 738-70-5) and ormetoprim (CAS 6981-18-6), each of purity not below 98 %, dissolved and made up to 10 mL with the 1 % acetic acid in acetonitrile, and are kept below -18 °C for 6 months. Mixed intermediate solution I at 10 µg/mL is made by taking 1 mL of each stock solution to 100 mL, and is kept below -18 °C for 3 months; mixed intermediate solution II at 1 µg/mL is made by taking 1 mL of solution I to 10 mL and is prepared immediately before use. The internal standard stock solution at 1 mg/mL is prepared from 10 mg of d9-trimethoprim (CAS 1189460-62-5, purity not below 98 %) in 10 mL and kept below -18 °C for 6 months; the internal standard intermediate solution at 10 µg/mL and the internal standard working solution at 1 µg/mL follow by successive dilution. The mixed calibration series is prepared in 10 mL flasks from suitable volumes of mixed intermediate solution II plus 100 µL of internal standard working solution, made up with the reconstitution solution, at mass concentrations of 0.5, 1.0, 2.0, 10.0, 50.0 and 100.0 ng/mL, the internal standard being at 10 ng/mL in

each, and is prepared immediately before use. A mixed-mode strong cation exchange cartridge of 60 mg per 3 mL, or equivalent, and a 0.22 μm organic membrane filter are used.

4.3 The apparatus comprises a liquid chromatograph-tandem mass spectrometer with an electrospray ionisation source, analytical balances reading to 0.1 mg and 0.01 mg, a vortex mixer, a refrigerated centrifuge reaching at least 10 000 r/min, an ultrasonic cleaner, a solid phase extraction manifold and a nitrogen evaporator.

4.4 The test sample is prepared to GB/T 20195, at least 200 g being ground to pass entirely through a 0.425 mm test sieve, mixed evenly and kept in a closed container.

4.5.1 Two portions are run in parallel. About 2 g of the sample, weighed to 0.1 mg, is placed in a 50 mL centrifuge tube with 100 μL of internal standard working solution, 10 mL of sodium acetate solution and 0.5 mL of ammonia solution, vortexed for 2 min, then 10 mL of ethyl acetate is added accurately, vortexed for 2 min and extracted with ultrasound for 10 min with one vortex mixing after 5 min, and centrifuged at 10 000 r/min at 4 °C for 5 min. The upper organic phase is transferred to a second 50 mL tube, the extraction is repeated with a further accurate 10 mL of ethyl acetate and the organic phases are combined and mixed. An accurate 2 mL of the organic phase is evaporated almost to dryness under nitrogen at 50 °C, the residue is dissolved in 3 mL of acetonitrile in 5 % acetic acid with 30 s of vortexing, 3 mL of n-hexane saturated with acetonitrile is added with a further 30 s of vortexing, and the tube is centrifuged at 10 000 r/min at 4 °C for 5 min. The upper n-hexane layer is discarded and the whole lower layer kept. Note: where the extract emulsifies badly, the defatting is repeated with a further 3 mL of n-hexane saturated with acetonitrile after the upper layer has been discarded.

4.5.2 The mixed-mode strong cation exchange cartridge is conditioned with 3 mL of methanol and 3 mL of acetonitrile in 5 % acetic acid, the whole retained solution is passed through, and the cartridge is washed with 3 mL of acetonitrile in 5 % acetic acid and 3 mL of methanol. After drying under vacuum the analytes are eluted with 5 mL of 8 % ammonia in methanol, the eluate is evaporated almost to dryness under nitrogen at 50 °C, an accurate 1 mL of the reconstitution solution is added with 30 s of vortexing, and the solution is passed through the membrane filter.

4.5.3.1 The liquid chromatographic conditions given for guidance are a C18 column 50 mm long with an internal diameter of 2.1 mm and a particle size of 1.8 μm, or equivalent; a column temperature of 35 °C; a flow rate of 0.3 mL/min; an injection volume of 3 μL; and a mobile phase of acetonitrile as A and ammonium acetate in 0.2 % formic acid as B, with the gradient of Table 1: at 0.00 min 15 % A and 85 % B; at 2.00 min 15 % and 85 %; at 5.00 min 30 % and 70 %; at 5.10 min 100 % and 0 %; at 6.00 min 100 % and 0 %; at 6.10 min 15 % and 85 %; at 7.00 min 15 % and 85 %.

4.5.3.2 The mass spectrometric conditions given for guidance are electrospray ionisation in

positive ion mode, multiple reaction monitoring, a capillary voltage of 4.0 kV, an ion source temperature of 350 °C, a desolvation gas temperature of 300 °C and a desolvation gas flow of 7 L/min. Table 2 lists, for diaveridine, trimethoprim, ormetoprim and the internal standard d9-trimethoprim, the qualifier ion pairs, the quantifier ion pair and the collision energies; the extracted text does not allow the rows and columns of that table to be re-paired reliably, so its values are not reproduced here.

4.5.3.4 For identification, the retention time of the analyte in the test solution is to agree with that in the calibration solution of comparable concentration within a relative deviation of +/-2.5 %. The relative ion abundance of the monitored ion pairs of Table 2 in the test chromatogram is compared with that of the calibration solution of comparable concentration; where the deviation stays within Table 3, the analyte is taken to be present. Table 3 allows +/-20 % for a relative ion abundance above 50 %, +/-25 % for above 20 % up to 50 %, +/-30 % for above 10 % up to 20 % and +/-50 % for 10 % or below.

4.5.3.5 For quantification, the ratio of the peak area of the analyte to that of the internal standard is plotted against the ratio of the mass concentration of the analyte to that of the internal standard, and the correlation coefficient of the calibration curve is to be not lower than 0.99. The responses of the test and calibration solutions are to lie within the linear range of the instrument, the determination being repeated where they do not. For single point calibration the mass concentration of the analyte in the test solution is not to differ from that of the calibration solution by more than 30 %.

4.6 The content is expressed as a mass fraction in micrograms per kilogram, one equation being given for multi-point calibration and one for single point calibration. The symbols are the mass concentration of the analyte in the test solution obtained from the calibration curve, in nanograms per millilitre; the total volume of ethyl acetate used for extraction, in millilitres; the volume of the extract taken for clean-up, in millilitres; the volume to which the cleaned-up test solution is reconstituted after evaporation under nitrogen, in millilitres; the mass of the test portion, in grams; and a conversion factor of 1000. The single point equation adds the peak areas of the analyte and of the internal standard in the test and calibration solutions and the mass concentrations of the analyte and of the internal standard in those solutions, all in nanograms per millilitre. The result is the arithmetic mean of the parallel determinations, kept to three significant figures.

4.7 Under repeatability conditions the absolute difference between each of two independent results and their arithmetic mean is not to exceed 15 % of that mean.

5 High performance liquid chromatography

5.1 The analytes are extracted from the test portion with ethyl acetate under weakly alkaline conditions, defatted with n-hexane, cleaned up on a mixed-mode strong cation exchange cartridge, determined by high performance liquid chromatography and quantified by the external standard method.