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BSI Standards Publication

Animal feeding stuffs — Determination of fluoride content after hydrochloric acid treatment by ion-sensitive electrode method (ISE)

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Animal feeding stuffs - Determination of fluoride content after hydrochloric acid treatment by ion-sensitive electrode method (ISE)

Aliments des animaux - Détermination de la teneur en fluorure, après traitement à l'acide chlorhydrique, selon la méthode utilisant une électrode sélective d'ions (ISE)

Futtermittel - Bestimmung des Fluoridgehaltes nach Salzsäure-Behandlung mit ionensensitiver Elektrode (ISE)

This European Standard was approved by CEN on 17 May 2012.

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Foreword

This document (EN 16279:2012) has been prepared by Technical Committee CEN/TC 327 “Animal Feeding stuffs – Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

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Introduction

Fluorine (F) is one of the most abundant elements in the environment. Animals are exposed to the ionic form of the element, fluoride (F^-), which may be present in feeding stuffs. The toxicity of fluoride has already been established by EFSA [6]; and its availability greatly depends on the solubility of fluoride compounds.

In particular, fluoride compounds with low solubility are poorly absorbed while fluoride ions released from readily soluble compounds are almost completely absorbed from the gastrointestinal tract by passive diffusion in monogastric species. The extraction procedure of this method involves a mild acid treatment with hydrochloric acid solution of 1 mol/l which should reflect the gastric hydrochloric acid concentration of 0,1 mol/l– 0,3 mol/l.

1 Scope

This European Standard specifies an Ion-Selective Electrode method (ISE) after hydrochloric acid treatment for the determination of fluoride from animal feeding stuffs. The content of fluoride (F^-) corresponds to that of fluorine (F) specified in Commission Regulation (EU) 574/2011[3].

This European Standard is strictly based on several conventions such as those contained in the following example:

EXAMPLE 0,5 g test portion for extraction of fluoride from animal feeds by means of an acid treatment with 20 ml of 1 mol/l hydrochloric acid solution at ambient temperature (20 °C to 25 ºC) for 20 min. The pH is controlled and adjusted to 5,5 in the buffered test solution before determination of fluoride by ISE using standard addition technique.

The method was successfully tested in an interlaboratory study in concentrations between 100 mg/kg up to 500 mg/kg. If this method is followed strictly, then theoretically all concentrations from 40 mg/kg up to 4 000 mg/kg can be analysed within the linear calibration function.

Only for concentrations lower than 40 mg/kg is the use of an interpolation technique required instead of standard addition Annex C.

The quantification limit for fluoride using the conventions of the method including the standard addition technique is 40 mg/kg or lower than 2,5 mg/kg when using interpolation Annex C.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods*

EN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation*

3 Principle

For the determination of fluoride, a test portion of 0,5 g of the sample is treated with 20 ml of 1 mol/l hydrochloric acid solution for 20 min at ambient temperature (20 °C to 25 °C).

The amount of fluoride extracted from the sample is determined by means of a fluoride selective electrode.

NOTE This ISE method is based upon a potentiometric technique. This means that it is based upon the measurement of a concentration of an analyte present in solution, by means of an ion selective electrode. This electrode has a linear response within a working range of analyte concentrations, which is provided by the calibration curve. Thus, in general, the operator should take an adequate amount of sample to ensure that the final concentration of the analyte is within this working range given by the calibration step. To ensure satisfactory reproducibility of the method however, this extraction procedure as a convention method fixes the ratio of the amount of the test portion to the volume of the extraction solvent for all kind of feeds.

WARNING — The use of this European Standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

4 Reagents

Use only reagents of recognised analytical grade, unless otherwise specified.

4.1 Water, complying with grade 2 in accordance with EN ISO 3696.

4.2 Element stock solution, fluoride (NaF); Fluoride *c* = 1 000 mg/l**.**

4.3 Hydrochloric acid (HCl), mass fraction of 37 %, having a density of approximately ρ (HCl) ~ 1,185 g/ml.

4.4 Perchloric acid ($HClO_4$), mass fraction of 70 % to 72 %, having a density of approximately ρ ($HClO_4$) ~ 1,68 g/ml.

4.5 Acetic acid (CH_3COOH), glacial, 99 %.

4.6 Sodium acetate trihydrate ($CH_3COONa\ 3H_2O$), 99 %.

4.7 Trisodium citrate dihydrate ($C_6H_5Na_3O_7\ 2H_2O$), 99 %.

4.8 Hydrochloric acid solution of 1 mol/l.

To prepare: pipette 83 ml hydrochloric acid (4.3) into a 1 000 ml volumetric flask (5.5) and fill to the mark with water (4.1).

4.9 Sodium acetate solution of 3 mol/l.

To prepare: dissolve 408 g of sodium acetate trihydrate (4.6) in 750 ml of water (4.1). When the solution reaches room temperature (20 °C to 25 °C), adjust the pH to 7,0 with acetic acid (4.5), transfer to a 1 000 ml volumetric flask (5.5) and dilute to the mark with water (4.1).

4.10 Sodium citrate solution.

To prepare: dissolve 222 g of trisodium citrate dehydrate (4.7) in 250 ml of water (4.1), add 28 ml perchloric acid (4.4), transfer to a 1 000 ml volumetric flask (5.5) and dilute to the mark with water (4.1).

4.11 Intermediate fluoride solution of 100 mg/l.

To prepare: pipette 10 ml from the 1 000 mg/l fluoride stock solution (4.2) into a 100 ml plastic volumetric flask (5.7) and dilute to the mark with water (4.1).

4.12 Intermediate fluoride solution of 10 mg/l.

To prepare: pipette 10 ml from the 100 mg/l intermediate fluoride solution (4.11) into a 100 ml plastic volumetric flask (5.7) and dilute to the mark with water (4.1).

4.13 Sodium hydroxide solution of 5 mol/l.

To prepare: transfer NaOH pellets, weighing 200 g, to a 1 000 ml volumetric flask (5.5), which is about 2/3 filled with water (4.1) and contains a magnetic stirring bar (5.4). Stir until dissolved; after cooling down take away the magnet stirrer and fill up to the mark with water (4.1) and shake.

NOTE The use of sodium hydroxide solution of 1 mol/l might be more applicable for a precise pH adjustment.

5 Apparatus

Use typical laboratory apparatus with preference for plastic instead of glass for recipients, in order to avoid undesired reactions of fluoride with silicon from glass in the case of fluoride standard solutions and the test sample solutions.

5.1 Laboratory grinder

5.1.1 Laboratory grinder, capable of grinding to a particle size of less than or equal to 0,5 mm.

5.1.2 Laboratory grinder, capable of grinding to a particle size of less than or equal to 0,1 mm.

5.2 Analytical balance, capable of weighing to an accuracy of 0,1 mg.

5.3 Freeze drying equipment, capable of freeze-drying liquid animal feeding stuffs.

5.4 Magnetic stirrer, with polytetrafluoro-ethylene-coated stirring bar.

5.5 One-mark volumetric flasks, of capacities 100 ml, 500 ml and 1 000 ml.

5.6 Beakers, of capacities 100 ml and 250 ml.

5.7 One-mark plastic volumetric flasks, of capacities 100 ml and 200 ml.

5.8 Plastic beakers, of capacities 50 ml, 100 ml and 200 ml.

5.9 Fluoride selective electrode.

NOTE The use of combined electrodes (i.e. selective and reference in one) is possible.

5.10 pH combined electrode.

5.11 Reference electrode for fluoride.

5.12 Ion-pH-meter, capable of measuring to an accuracy of 0,1 mV.

6 Sampling

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497 [2].

It is important that the laboratory receives a sample that is truly representative and has not been damaged or changed during transport or storage.

7 Preparation of test sample

7.1 General

Prepare the test sample in accordance with EN ISO 6498.

The grinding must be performed in conditions such that the substance is not appreciably heated.

The operation is to be repeated as many times as is necessary and it shall be executed as quickly as possible in order to prevent any gain or loss of constituents (such as water).

The whole ground product is placed in a flask made of e.g. polypropylene, which can be stoppered and stored in such way to prevent any change in composition.

Before any weighing is carried out for the analysis, the whole test sample shall be thoroughly mixed for reasons of homogeneity.

7.2 Animal feeding stuffs which can be ground as such

Grind the laboratory sample (usually 500 g), using a grinder (5.1.1) or mortar, until a particle size of 0,5 mm or less has been reached. Use e.g. a knife mill or equivalent.

7.3 Liquid animal feeding stuffs

7.3.1 General

Liquid feeding stuffs shall be pre-dried according to the procedure described in 7.3.2 or freeze-dried according to the procedure described in 7.3.3.

7.3.2 Pre-drying

Pre-dry the laboratory sample at a temperature of (60 ± 5) °C during at least 16 h to reduce the moisture content to a level of 8 % to 12 %. Determine the mass of the sample before and after pre-drying using an analytical balance (5.2) and calculate the moisture loss on pre-drying. Grind the pre-dried sample in accordance with 7.1.

7.3.3 Freeze-drying

Freeze-dry the laboratory sample following the instructions of the freeze-drying equipment (5.3). The mass of the sample before and after the freeze-drying is to be determined using an analytical balance (5.2). Grind the freeze-dried sample in accordance with 7.1.

7.4 Feed materials

Feed materials shall be ground using a grinder (5.1.2) or mortar until a particle size of 0,1 mm or less has been reached. Use e.g. a ball mill or equivalent.

8 Procedure

8.1 Extraction: wet extraction – 1 mol/l HCl

Weigh 0,5 g of the homogenised and (to a particle size of ≤ 0,5 mm) ground test sample as test portion to an accuracy of 1 mg into a plastic beaker of e.g. 200 ml volume (5.8).

Pipette in (20,0 ± 0,1) ml of 1 mol/l of hydrochloric acid solution (4.8) and stir for (20 ± 0,25) min at constant speed, fast enough to provide an adequate homogeneous mixing on the magnetic stirrer at ambient temperature (20 °C to 25 °C).

If the test portion is not completely dissolved, check that the pH of the solution is still acid (pH ≤ 4) after the wet extraction to verify that there has been an adequate acid extraction and identify possible problems with very alkaline samples that could significantly reduce the fluoride acid extraction, particularly with higher test portions. The risk is much reduced using 0,5 g test portion and 20 ml volume of 1 mol/l HCl for extraction as conventions, as has been observed in the last inter-laboratory study (Annex A). If the pH of the solution is > 4, the amount of HCl might have not been enough to release all the fluoride; and therefore a remark needs to be included in the report along with the analysis result stating when a smaller test portion or a higher volume of 1 mol/l HCl has been used.

Add (50,0 ± 0,1) ml sodium acetate solution (4.9) and (50,0 ± 0,1) ml sodium citrate solution (4.10). Adjust the pH at exactly 5,5 ± 0,1 using a sodium hydroxide solution (4.13) or a hydrochloric acid solution (4.8). Transfer quantitatively to a 200 ml volumetric plastic flask (5.7) and dilute to the mark with deionised water and mix.

When using 250 ml volumetric plastic flasks, the weighed test portion is (625 ± 1) mg to which is added (25 ± 0,1) ml of 1 mol/l hydrochloric acid solution (4.8), (62,5 ± 0,1) ml of sodium acetate solution (4.9) and (62,5 ± 0,1) ml sodium citrate solution (4.10) to fulfil the conventions of this method. Filter through an acid washed gravimetric filter paper into a 200 ml plastic flask and keep the test sample solution stoppered. This is the test solution to be measured by ISE.

Centrifugation instead of filtration is the preferred alternative for those samples that present filtration problems (for example, bentonites). Decant after centrifugation into another 200 ml volumetric plastic flask (5.7).

8.2 Calibration

The following working solutions are recommended:

Pipette 1 ml, 3 ml and 10 ml from the intermediate fluoride solution of 10 mg/l (4.12) and 3 ml and 10 ml from the intermediate fluoride solution of 100 mg/l (4.11) into five 100 ml plastic volumetric flasks (5.7). This is done in order to prepare 0,1 mg/l, 0,3 mg/l, 1,0 mg/l, 3,0 mg/l and 10 mg/l fluoride working solutions, respectively (see Table 1).

Table 1 — Recommended calibration solutions for the determination of fluoride

Calibration standards	**Volume of intermediate fluoride solution (4.12) resp. (4.11)** **(ml)**	**Final concentrations using 100 ml volumetric flasks (5.7)** **(mg/l)**
1	1 (0)	0,1
2	3 (0)	0,3
3	10 (0)	1
4	3 (0)	3
5	10 (0)	10

To each volumetric flask, add 10,0 ml 1 mol/l HCl (4.8), 25,0 ml sodium acetate solution (4.9) and 25,0 ml sodium citrate solution (4.10).

Dilute to the mark with deionised water (4.1) and mix.

Connect the fluoride and reference electrodes to the pH-meter, place the electrodes in a low concentration fluoride solution and wait for the pH meter to warm up.

Calibrate the apparatus with the working standard solutions by constructing the standard curve. Place the electrodes in each standard while stirring with the magnetic stirrer at constant rate. Read *mV* when the measurement becomes stable[1)]. Rinse and wipe off the electrodes and stirring bar between solutions. Plot the electrode potential, in *mV*, as a function of the logarithm of the fluoride solution concentration, in *mg/l*.

[1)] All ISE instruments specify suitable criteria for assessing the stability of the signal, in order to register the appropriate value of the reading. Nevertheless, a good criteria is to assure that the reading does not vary more than 0,1 mV for 30 s.

Determine the slope value (S) in the linear region of the calibration curve by least squares linear regression fitting (R^2 > 0,99). This will be used in the calculation of the fluoride concentration in the test sample using the standard addition technique (Figure 1).

X (mg/l)	Log [X]	Y (mV)
0,1	-1,000	136,1
0,3	-0,523	108,6
1	0,000	78,4
3	0,477	50,2
10	1,000	20,1

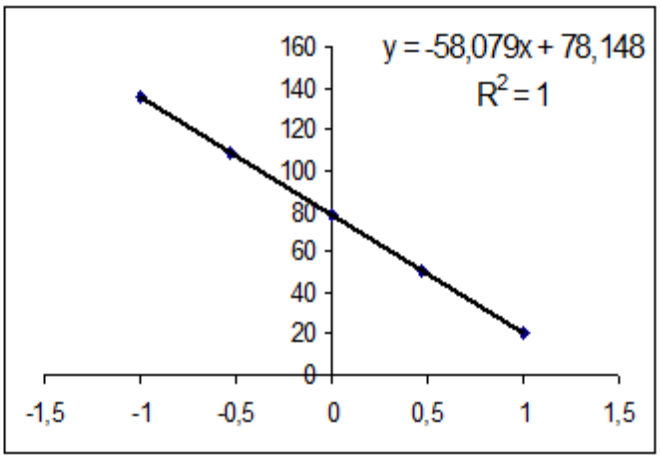


Figure 1 — Example of plotting of the electrical potential Y in (mV) of the fluoride concentration X in (mg/l) of the calibration standards (8.2) as a function of the logarithm (= Log [X]) and determination of the slope (e.g. 58,079) in the linear region of the calibration function

NOTE The response of the electrode can be improved by keeping it dipped in a diluted fluoride solution (10^{-6} mol/l) for 30 min prior to measuring the potential of the standard and sample solutions.

8.3 Determination by standard addition technique

Immediately after having calibrated the apparatus (8.2) and with the fluoride and reference electrodes connected to the warmed-up pH meter, pour 50 ml of each test sample solution (8.1) into separate 100 ml plastic beakers (5.8).

Place electrodes in each solution and, while stirring with magnetic stirrer at constant rate, read *mV* as the first potential (E_1) of unknown solutions. Register the reading.

Add 5 ml of a standard solution (8.2) of suitable concentration of the analyte to increase the reading by between 5 mV and 40 mV. Register the potential reading after adding the standard solution as the second potential (E_2). Rinse and blot off electrodes and stirring bar between solutions (Table 2).

The electrode potential reading of the sample solution shall be in the linear region of the calibration curve for the standard addition technique to be applicable. Otherwise, it is recommended to determine the fluoride concentration in the sample solution by interpolation of the potential reading in the non-linear region of the calibration curve. In this case, more standard solution points should be used to define more precisely this non-linear region at very low fluoride concentration (see Annex C).

9 Calculation and expression of results

Fluoride (F^-) concentration in the test solution, expressed in mg/l, is determined using the following formula:

$$c_s = c_A \frac{0{,}1}{1{,}1 \times 10^{\Delta E / S} - 1} \quad (1)$$

where

c_s is the fluoride concentration in the test solution (8.1), in mg/l;

c_A is the fluoride concentration of the added standard calibration solution (8.2), in mg/l;

ΔE is the potential difference $|E_1 - E_2|$ after adding 5 ml of the standard solution (8.2), in mV;

S is the slope of the calibration curve in the linear region (in absolute value), in mV.

Fluoride in the test sample, expressed in mg/kg of animal feeding stuff, is determined using the following formula:

$$c_F = \frac{c_s \times V_s}{m} \quad (2)$$

where

c_F is the fluoride concentration in the test (feed) sample, in mg/kg;

c_s is the fluoride concentration in the test solution (8.1), calculated with formula (1), in mg/l;

V_s is the total volume of the test solution, in l (due to this convention v_s = 0,200 l);

m is the mass of the test sample, in kg (due to this convention m = 0,0005 kg).

Due to the convention that V_s = 0,200 l and m = 0,0005 kg, the calculation of c_F is as follows:

$$c_F = c_s \times 400 \quad (3)$$

NOTE Inserting as c_s the fluoride concentrations of calibration standards 1 and 5 into formula (3) gives a working range of 40 mg/kg to 4 000 mg/kg for the fluoride content of feed samples within the linear calibration function.

Results in the test report should be expressed in mg/kg as follows: Fluorine (measured as fluoride).

Table 2 — Example for calculation of all samples of the interlaboratory study (Annex A) using standard addition technique

Matrix	E_1 (mV)	c_A (mg/l)	E_2 (mV)	S (mV)	ΔE (mV)	c_s mg/l	c_F (mg/kg)
Fish feed	114,5	5	89,9	58,08	24,6	0,261	104,4
Mineral dairy cow feed	80,2	10	64,5	58,08	15,7	0,953	381,2
Mineral pig feed	100,0	5	83,4	58,08	16,6	0,445	178,0
Calcium carbonate	107,5	5	85,7	58,08	21,8	0,310	124,0
Sodium bicarbonate	186,4 [a]	0,5	154,7 [a]	58,08	31,7	0,017	6,8
Mineral cattle feed	83,1	10	66,4	58,08	16,7	0,883	353,2
Sepiolite	78,2	10	64,3	57,52	13,9	1,088	435,2

[a] Sodium bicarbonate with E_1 = 186,4 mV and E_2 = 154,7 mV is out of the linear range of the calibration function, starting from calibration standard solution 1 with 136,1 mV (see Figure 1).

10 Precision

10.1 General

An interlaboratory comparison as a follow-up study was organized by the Technische Universität München, Research Centre for Nutrition and Food Sciences, Bioanalytic Weihenstephan, in December 2010. Only the results of the method protocol using standard addition technique were given in Annex A. A previous collaborative study organized by Tolsa, S.A. in 2007 had identified a high variation between laboratories that related to the quantity of test portion used and pH level, which required more specific extraction conventions to be included in the method. Further details were given in the final report [8].

10.2 Interlaboratory study

Details of an interlaboratory study done in 2010/2011 on the precision of the method are summarized in Table 3 and Annex A. The values derived from this interlaboratory study may not be applicable to concentration ranges and matrices other than those given.

10.3 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same apparatus within the shortest feasible time interval, will in not more than 5 % of the cases exceed the repeatability limit r.

10.4 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of the cases exceed the reproducibility limit R.

Table 3 — Results of the interlaboratory study

Matrix	**Mean** (mg/kg)	r (mg/kg)	R (mg/kg)
Fish feed	105	13,4	33,9
Mineral dairy cow feed	386	54,0	78,1
Mineral pig feed	181	25,6	42,1
Calcium carbonate	126	10,3	19,2
Mineral cattle feed	341	36,1	46,4
Sepiolite	501	70,9	171
NOTE r is the repeatability limit; R is the reproducibility limit.			

11 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this European Standard;

d) all operating details not specified in this European Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained as fluorine (F) content, measured as fluoride (F^-);

f) clarifications due to deviations of the conventions of this European Standard concerning:

   1) test portion of 0,5 g;

   2) volume of 20 ml of 1 mol/l hydrochloric acid solution;

   3) extraction time of 20 min;

   4) ambient temperature of 20 °C to 25 °C;

   5) pH ≤ 4 after wet extraction

   6) adjustment after adding both buffers to pH of 5,5;

   7) use of standard addition technique within linear calibration function or otherwise interpolation.

Annex A
(informative)

Results of the interlaboratory study

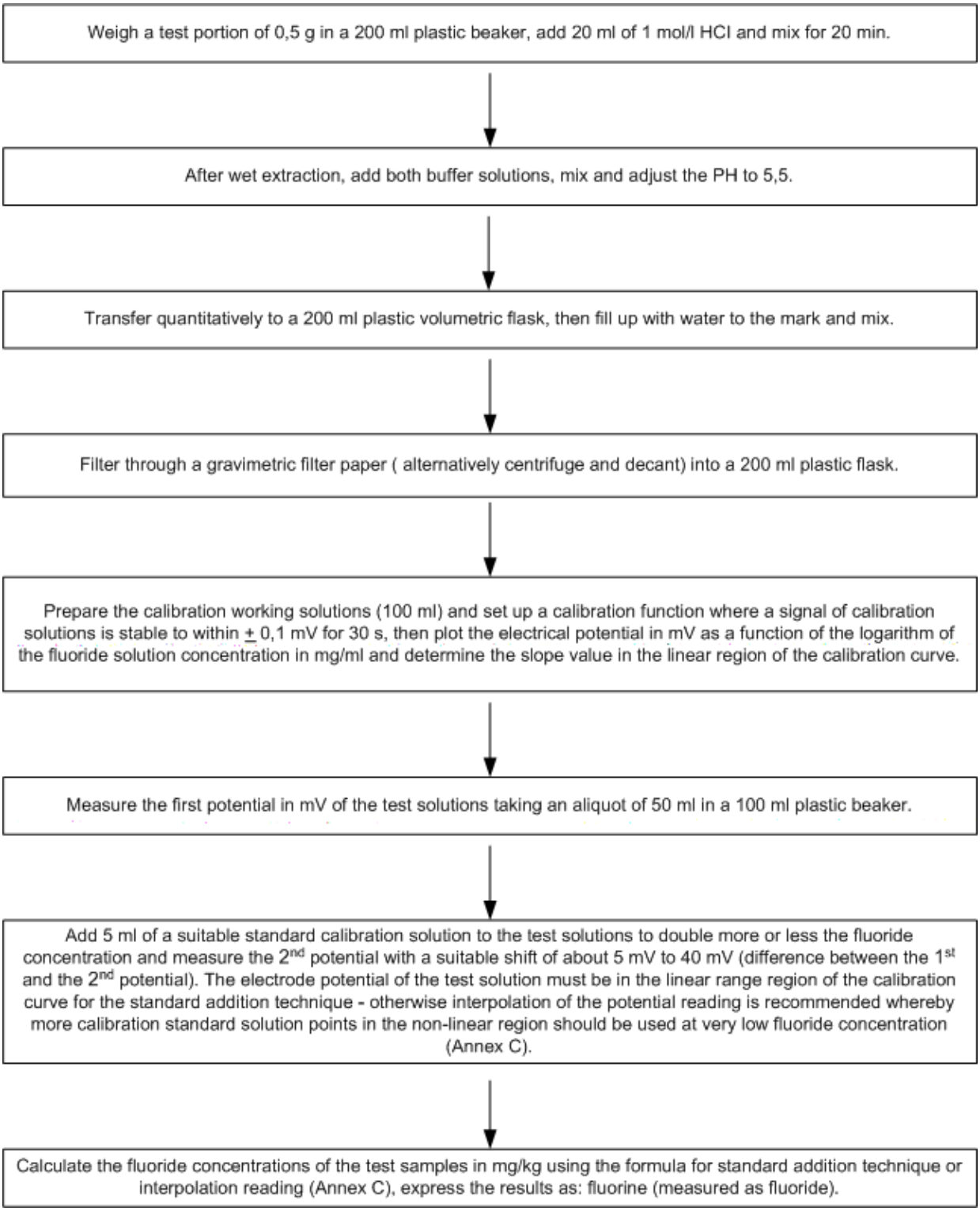
Table A.1 – Results of the interlaboratory study

Matrix tested	Fish feed	Mineral cow feed	Mineral pig feed	Calcium carbonate	Mineral cattle feed	Sepiolite
No. of labs	17	17	17	17	17	17
No. of outlier labs	1	2	1	3	2	3
No. of valid labs	16	15	16	14	15	14
Mean value, mg/kg	105	386	181	126	341	501
$s_{r,}$ mg/kg	4,8	19,3	9,1	3,7	12,9	25,3
r, mg/kg	13,4	54,0	25,6	10,3	36,1	70,9
CV_r, %	4,5	5,0	5,0	2,9	3,8	5,1
$s_{R,}$ mg/kg	12,1	27,9	15,0	6,8	16,6	61,0
R, mg/kg	33,9	78,1	42,1	19,2	46,4	171
CV_R, %	11,5	7,2	8,3	5,4	4,9	12,2
HORRATvalue (R)	1,5	1,1	1,1	0,7	0,7	1,9

NOTE All subsamples were fully prepared for direct weighing; r is the repeatability limit; s_r is the repeatability standard deviation; CV_r is the repeatability variation coefficient; R is the reproducibility limit; s_R is the reproducibility standard deviation; CV_R is the reproducibility variation coefficient; HORRATvalue is Horwitz–Ratio value.

Annex B
(informative)

Flowchart – Determination of fluoride content after hydrochloric acid treatment by ISE method



Annex C
(informative)

Interpolation reading

For the accurate determination of fluoride in animal feeding stuffs below 40 mg/kg, more calibration standards are necessary to describe the non-linear calibration function for interpolation reading using a 500 mg test portion (Figure C.1 and Table C.1).

$$Y = -3{,}9012\ X^2 - 58{,}149\ X + 29{,}114 \text{ with } R^2 = 0{,}9991$$

X (mg/l)	Log [X]	Y (mV)
0,01	-2,0000	128,2
0,05	-1,3010	100,3
0,1	-1,0000	85,2
0,2	-0,6990	67,8
0,3	-0,5229	57,7
1	0,0000	27,1
3	0,4771	-0,5
10	1,0000	-31,5

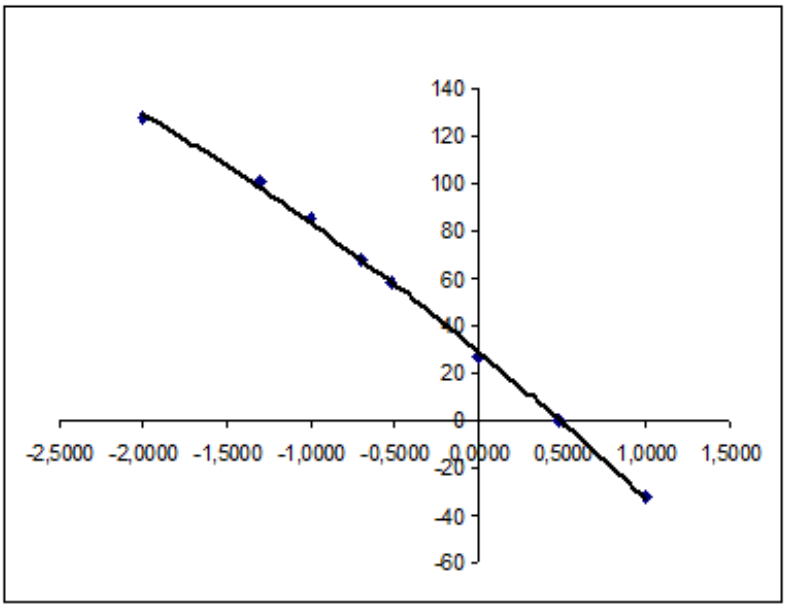


Figure C.1. — Example of plotting of the electrical potential Y in (mV) of the fluoride concentration X in (mg/l) of the calibration standards (8.2) as a function of the logarithm (= Log [X]) for interpolation reading in the non-linear region of the calibration function

Table C.1 – Example for calculation of all samples of the interlaboratory study (Annex A) using interpolation and comparison to standard addition technique

Matrix	E_1 (mV)	**Interpolated** c_s (mg/l)	**Interpolated** c_F (mg/kg)	**Standard addition** c_F (mg/kg)	**Difference (standard addition vs. interpolation)** (mg/kg)
Fish feed	59,1	0,2784	106,2	108,9	2,9
Mineral dairy cow feed	28,3	0,9870	377,5	377,6	-1,7
Mineral pig feed	46,0	0,4802	183,7	175,0	-2,9
Calcium carbonate	56,1	0,3157	120,8	123,4	-2,3
Sodium bicarbonate	134,2	0,00787	3,01	6,8	4,1
Mineral cattle feed	30,6	0,9428	362,0	350,3	-19,8
Sepiolite	19,4	1,4629	562,3	510,2	-51,4

NOTE Only standard addition technique was successfully tested within the interlaboratory study (Annex A) in all samples with the exception of sodium bicarbonate with low fluoride content where CV_R and corresponding HORRATvalue (R) of 3,8 were insufficient. The data and results given in Table C.1 are from one laboratory only as example and for illustration purposes.

Bibliography

[1] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[2] EN ISO 6497, *Animal feeding stuffs — Sampling (ISO 6497)*

[3] Commission Regulation (EU) No 574/2011 of 16 June 2011 on maximum levels for nitrate, melamine, Ambrosia spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto

[4] Regulation 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition

[5] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed

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