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Determination of the content of vitamin A in compound feed

Proficiency Testing Report FAC-22/01

Stroka, J., Dehouck, P., Bouten, K., Serano, F., Stroh, A., Von Holst, C.

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Determination of the content of vitamin A in compound feed

Proficiency Testing Report
FAC-22/01

J. Stroka, P. Dehouck, K. Bouten, F. Serano, A. Stroh and C. von Holst

268-PT Accredited by the
Belgian Accreditation Body (BELAC)

This proficiency test report has been authorised by Ursula Vincent.

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Abstract

The European Union Reference Laboratory for Feed Additives Control (EURL-FA Control) organised a proficiency testing round (PT) for the determination of vitamin A (3a672a) in feed to support Regulation (EU) 2017/625. A commercial rabbit compound feed was purchased from a local market, processed and dispatched to participants. Fourteen National Reference Laboratories (NRLs) and three official control laboratories (OCLs) from 11 EU Member States and Switzerland registered to the exercise and reported results. Most of them (ca. 75 %) showed satisfactory performance according to the z score, which confirms their ability to control vitamin A at authorised levels in compound feed to support Commission Implementing Regulation (EU) 2015/724.

Acknowledgements

The EURL-FA Control wishes to thank colleagues from the JRC in Geel for their valuable contributions during the execution of this PT.

The laboratories listed hereafter are kindly acknowledged for their participation in this PT round.

Organisation	Country
ÚKZÚZ	Czech Republic
Veterinary and Food Laboratory	Estonia
Service Commun des Laboratoires DGCCRF-DGDDI	France
German Federal Institute for Risk Assessment (BfR)	Germany
Landeslabor Berlin-Brandenburg	Germany
Thüringer Landesamt für Landwirtschaft und Ländlichen Raum	Germany
Landesbetrieb Hessisches Landeslabor (LHL)	Germany
LUFA Speyer	Germany
LTZ Augustenberg	Germany
AGROLAB LUFA GmbH	Germany
Institute of Marine Research (IRM)	Norway
Agroscope	Switzerland
Ruokavirasto	Finland
Feeding Stuffs Quality Control Laboratory	Cyprus
Ústredný kontrolný a skúšobný ústav poľnohospodársky	Slovakia
University of Ljubljana, Veterinary Faculty, National Veterinary Institute	Slovenia
Eurofins Vitamin Testing	Denmark

Authors

J. Stroka, P. Dehouck, K. Bouten, F. Serano, A. Stroh and C. von Holst

Executive summary

The European Union Reference Laboratory for Feed Additives Control (EURL-FA Control) organised a proficiency testing round (PT) for the determination of the mass fractions of vitamin A in rabbit compound feed to support the implementation of Regulation (EU) 2017/625 on *official controls* and Commission Implementing Regulation (EU) 2015/724 *concerning the authorisation of various vitamin A forms as feed additives for all animal species*. This proficiency testing round was open to National Reference Laboratories (NRLs) and to official control laboratories (OCLs).

A commercial compound feed was purchased at a local market and processed. The material was indicated to contain vitamin A (3a672a).

The assigned value was derived from the reported results obtained using the EU official method described in Commission Regulation (EC) No 152/2009. The homogeneity of the test items was evaluated before dispatching to the participants, while the stability was assessed after the reporting of results deadline, according to the requirements set by the ISO 13528:2022.

Fourteen NRLs and three OCLs from 11 EU Member States and Switzerland registered to the exercise and reported results.

Laboratory results were assessed using z and zeta scores in accordance with ISO 13528:2022. The relative standard deviation for proficiency assessment (σ_{pt}) was set to 20 % of the assigned value, based on the reproducibility standard deviations determined during the validation study.

The majority of participants (75 %) showed satisfactory performance (according to the z score). This confirms the ability of most participating NRLs in monitoring the labelled content on rabbit compound feed fortified with vitamin A.

1 Introduction

The European Union Reference Laboratory for Feed Additives Control (EURL-FA Control) hosted by the Joint Research Centre (JRC) of the European Commission, organised a proficiency testing round (PT) for the determination of the content of vitamin A (3a672a) in rabbit compound feed to support the implementation of Regulation (EU) 2017/625 [1] on official controls and Commission Implementing Regulation (EU) 2015/724 [2] concerning the authorisation of retinyl acetate, retinyl palmitate and retinyl propionate as feed additives for all animal species.

This PT was agreed with the Directorate General for Health and Food Safety (DG SANTE) as part of the EURL-FA Control annual work programme 2021-2022, thus complying with the mandate set in Regulation (EU) 2017/625 [1]. The PT round was open to National Reference Laboratories (NRLs) and to Official Control Laboratories (OCLs) willing to participate.

This report summarises the outcome of this PT round.

2 Scope

The present PT aims to assess the performance of NRLs and OCLs in the determination of the content of Vitamin A in rabbit compound feed. The PT was mandatory for the NRLs and open to OCLs under certain conditions.

This PT, organised in line with ISO 17043:2010 [3], is identified as "FAC-22/01".

3 Set up of the exercise

3.1 Quality assurance

The JRC Unit hosting the EURL FA is accredited according to:

- ISO/IEC 17025:2017 (certificate number: BELAC 268-TEST); and
- ISO/IEC 17043:2010 (certificate number: BELAC 268-PT, as proficiency test provider)

The reported results were evaluated following the relevant administrative and logistic procedures.

3.2 Confidentiality

The procedures used for the organisation of PTs guarantee that the identity of the participants and the information provided by them is treated as confidential. The participants in this PT received a unique laboratory code used throughout this report. However, the laboratory codes of NRLs appointed in line with Regulation (EU) 2017/625 [1] may be disclosed to DG SANTE upon request for the purpose of an assessment of their (long-term) performance. Similarly, laboratory codes of appointed OCLs may be disclosed to their respective NRL upon request.

3.3 Time frame

The PT round FAC-22/01 was announced by an invitation letter to NRLs and OCLs on June 13, 2022 (Annex 1). The registration deadline was set to July 30, 2022. The samples were sent to participants on September 06, 2022. The dispatch was monitored by the PT coordinator using the online messenger's parcel tracking system. The deadline for reporting of results was set to September 30, 2022.

3.4 Distribution

Each participant received:

- One vacuumed sachet containing circa 70 g of material;
- The "Test item accompanying letter" (Annex 2); and
- A "Confirmation of receipt form" to be sent back to the PT coordinator after receipt of the test item (Annex 3).

Test items were sent in styrofoam isolated packages with cooling packs to avoid excess heat conditions during transport.

3.5 Instructions to participants

Detailed instructions were given to participants in the "Test item accompanying letter" mentioned above. The measurand was defined as "the total content of vitamin A in rabbit compound feed, expressed in IU kg^{-1}".

Participants were asked to check whether the sample arrived undamaged, and to return the "Confirmation of receipt form" within 3 days after receipt. They were also instructed to store the test items refrigerated (ca. 4 °C) until analysis, far from any possible contamination.

The participating laboratories were asked to perform measurements according to the method they use for official control, to report their result (x_i) and the associated expanded measurement uncertainty ($U(x_i)$) together with the coverage factor (k) and the analytical technique used for analysis.

Results had to be reported relative to a feed with a moisture content of 12 % in line with the above mentioned Regulation authorising the vitamin A feed additive. For the determination of the moisture content the laboratory had to apply the official method established by Regulation (EC) No 152/2009 [4].

Laboratory codes were given randomly and communicated to the participants by e-mail. The individual code had to be used to access the on-line reporting interface in order to report the measurement results and to complete the related questionnaire. This dedicated questionnaire was used to gather additional information related to measurements and laboratories (Annex 4).

4 Test item

4.1 Preparation

A commercial compound feed for rabbit (Aveve Rabbit Basic Pellet) was purchased by the JRC from a local farm supply shop in Dessel (Belgium). The material was declared to contain 10000 UI kg^{-1} of vitamin A (3a672a). The producer also reported the following composition on the label:

Small pellets
Analytical Constituents: Crude proteins 15.5 %; Crude fat 3.0 %; Crude fibre 16.0 %; Crude ash 8.2 %; Calcium 1.00 %; Phosphorus 0.55 %; Sodium 0.17 %; a-Linolenic acid 0.26 %.
Composition: Wheat grits, Lucerne, Dried beet pulp, Oat, Palm kernel flakes, Grass flour, Milled sunflower seeds, Oat flakes, Cane molasses, Barley, Milled rape seeds, Corn, Wheat, Linseed flakes, Calcium carbonate, Sodium chloride, Soybean oil.

About 10 kg of the material (extruded pelleted form) was milled with a Retch ZM200 centrifugal mill at 8000 rpm using a 0.5 mm sieve. The pelleted material was blended with dry ice (at a ratio of around 1 part dry ice to 10 parts material) to avoid excess heat during high speed milling.

The material was collected in a barrel flooded from the bottom with a slight stream of nitrogen to exclude oxygen and allow to blow out any CO_2 and eventually accompanied humidity overnight. This material was then further mixed by hand and barrel rolling.

The final material was then filled by hand into aluminium coated plastic sachets in portions of approx. 70 grams per sachet. The sachets were sealed under vacuum, using a MultiVac A300/16 and stored at -20° C until dispatch or analysis. The final material had a water content of 11 % m/m.

The sachets were dispatched in styrofoam isolated packages filled with 2 freeze packs to guarantee a temperature below 4 °C.

4.2 Homogeneity and stability

Measurements for the homogeneity and for stability were carried out by a sub-contractor (SGS Germany, Hamburg) while the treatment of data were performed by the EURL-FA Control (Annex 5).

The assessment of homogeneity was performed after the test items were packed in their final form and before distribution to participants. Ten sachets of each test item were randomly selected and analysed in duplicates. Results were evaluated according to ISO 13528:2022 [5]. The test items proved to be adequately homogeneous for the purpose of this PT.

The stability study confirmed that the test items are adequately stable at 4 °C over the whole period of the PT (from homogeneity testing till the deadline for reporting of results).

5 Assigned values and corresponding uncertainties

5.1 Assigned values

In this PT the assigned value (x_{pt}) for the total content of vitamin A in rabbit compound feed, relative to a moisture content of 12 %, was calculated as the consensus value of the reported results obtained using the European Union method specified by Regulation (EC) No 152/2009 [4]. According to the "method cascade" established by Regulation (EU) 2017/625 [1], this method is the only one currently approved within the European Union to enforce legal limits set by European legislation. This procedure for setting x_{pt} is aligned with chapter 7.7.1.1 of ISO 13528:2022 [5].

The assigned value was calculated applying the "algorithm A" [6],[7] described in ISO 13528:2022 and implemented via an EXCEL Add-in for Robust Statistics developed by UK AMC [8].

5.2 Associated uncertainties

The standard uncertainty of the assigned value $u(x_{pt})$ was derived from the robust standard deviation (s*) calculated using the "algorithm A" mentioned earlier [5], as follows:

$$u(x_{pt}) = 1.25 \times \frac{s^*}{\sqrt{n}} \quad \text{Eq. 1}$$

where "*n*" is the number of participants using the official method described in Regulation (EC) No 152/2009 [4].

$u(x_{pt})$ was then benchmarked against σ_{pt} applying the following criterion [5]:

$$u\left(x_{pt}\right) < 0.3\sigma_{pt} \quad \text{Eq. 2}$$

As this criterion is met, the standard uncertainty of the assigned value is considered sufficiently small to ensure an unbiased evaluation of the participants' performance. Table 1 summarises the results of the statistical evaluation.

Table 1: Assigned value and its standard uncertainty. The assigned value x_{pt}, the standard deviation s*, the standard uncertainty $u(x_{pt})$ and the standard deviation for proficiency assessment (σ_{pt}) are expressed in IU kg^{-1}.

x_{pt}	Assigned value	4535
n	Number of participants in the subset	11
s*	Robust standard deviation of the results	569
$u(x_{pt})$	Standard uncertainty of the assigned value	215
σ_{pt}	Standard deviation for proficiency assessment absolute and relative (expressed in IU kg^{-1} and %)	907 (20%)
$\frac{u(x_{pt})}{\sigma_{pt}}$	Ratio of $u(x_{pt})$ to σ_{pt} (unitless)	0.24

5.3 Metrological traceability of the assigned value

According to ISO 13528:2022 [5], metrological traceability of the consensus value cannot be established.

5.4 Standard deviation for proficiency assessment, σ_{pt}

The relative standard deviation for proficiency assessment ($\sigma_{pt,rel}$) was set to 20 % of the assigned value, based on the reproducibility standard deviation provided in Commission Regulation (EC) No 152/2009.

6 Evaluation of results

6.1 Scores and evaluation criteria

The individual laboratory performances are expressed in terms of z and ζ scores:

$$z = \frac{x_i - x_{pt}}{\sigma_{pt}} \quad \text{Eq. 3}$$

$$\zeta = \frac{x_i - x_{pt}}{\sqrt{u^2(x_i) + u^2(x_{pt})}} \quad \text{Eq. 4}$$

Where: x_i is the measurement result reported by a participant;
$u(x_i)$ is the standard measurement uncertainty reported by a participant;
x_{pt} is the assigned value;
$u(x_{pt})$ is the standard uncertainty of the assigned value;
σ_{pt} is the standard deviation for proficiency assessment.

The interpretation of the *z* and *ζ* performance scores is done according ISO 13528:2022 [5]:

$\lvert score \rvert \leq 2$	satisfactory performance	(green in Annex 6)
$2 < \lvert score \rvert < 3$	questionable performance	(yellow in Annex 6)
$\lvert score \rvert \geq 3$	unsatisfactory performance	(red in Annex 6)

The *z* scores compare the participant's deviation from the assigned value with the standard deviation for proficiency assessment (σ_{pt}) used as common quality criterion.

The *ζ* scores state whether the laboratory's result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value $u(x_{pt})$ and of the measurement uncertainty as stated by the laboratory $u(x_i)$. The *ζ* score includes all parts of a measurement result, namely the expected value (assigned value), its measurement uncertainty in the unit of the result as well as the uncertainty of the reported values. An unsatisfactory *ζ* score can either be caused by an inappropriate estimation of the content, or of its measurement uncertainty, or both.

The standard measurement uncertainty of the laboratory $u(x_i)$ was obtained by dividing the reported expanded measurement uncertainty by the reported coverage factor, *k*. When no uncertainty was reported, it was set to zero ($u(x_i)$ = 0) by the PT coordinator.

The relative standard measurement uncertainty from the laboratory $u_{rel}(x_i)$ is most likely to fall in a range between a minimum and a maximum allowed relative uncertainties (case "a": $u_{rel,min} \leq u_{rel}(x_i) \leq u_{rel,max}$). $u_{rel,min}$ is set to the relative standard uncertainty of the assigned value $u_{rel}(x_{pt})$. It is unlikely that, a laboratory carrying out the analysis on a routine basis, would determine the measurand with a smaller measurement uncertainty than the uncertainty of the assigned value. $u_{rel,max}$ is set to the relative standard deviation accepted for the PT assessment ($\sigma_{pt,rel} = \sigma_{pt}/x_{pt}$). Consequently, case "a" becomes: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,rel}$.

If $u_{rel}(x_i)$ is smaller than $u_{rel}(x_{pt})$ (case "b") the laboratory may have underestimated its measurement uncertainty. Such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the measurement uncertainty associated with the assigned value also includes contributions for homogeneity and stability of the test item. If those are large, measurement uncertainties smaller than $u(x_{pt})$ are possible and plausible.

If $u_{rel}(x_i)$ is larger than $\sigma_{pt,rel}$ (case "c") the laboratory may have overestimated its measurement uncertainty. An evaluation of this statement can be made when looking at the difference between the reported value and the assigned value: if the difference is smaller than the expanded uncertainty $U(x_{pt})$ then overestimation is likely. If the difference is larger but x_i agrees with x_{pt} within their respective expanded measurement uncertainties, then the measurement uncertainty is properly assessed, resulting in a satisfactory performance expressed as a *ζ* score, though the corresponding performance, expressed as a *z* score, may be questionable or unsatisfactory.

6.2 General observations

A total number of 17 laboratories participated in the proficiency test, reported results, and applied the methods listed in Table 2.

Since the ratio of u(x_{pt}) to σ_{pt} is below the critical criterion of 0.3, the standard uncertainty of the assigned value is considered negligible for the purpose of this proficiency test. Hence, z score was used for performance evaluation of the laboratories.

The robust mean and the relative robust standard deviation of the results from all laboratories (4737 IU kg^{-1} and 17%, respectively) are in good agreement with the assigned value and the standard deviation for proficiency assessment. Visual inspection of the data showed that the data are distributed around the assigned value with a single extreme value of 12587 IU kg^{-1}.

Table 2: Analytical methods applied by the participating laboratories

Identification number	Title	Number of laboratories
Regulation 152/2009	Methods of analysis to control the level of authorised additives in feed: Determination of vitamin A	11
EN 17547	Animal feeding stuffs: Methods of sampling and analysis – Determination of vitamin A, E and D content	3
EN 12823	Foodstuffs - Determination of vitamin A by high performance liquid chromatography	2
Modified EN 12823		1

6.3 Laboratory results and scorings

6.3.1 Performances

Annex 6 presents the table of reported results and the corresponding graph. The Kernel density plot - produced using the Excel add-in available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [10] – is also included.

Figure 1 presents the performance of the participating laboratories expressed as z and ζ scores. A satisfactory performance above 75 %, expressed as z score, was obtained for the quantification of vitamin A in compound feed.

The majority of laboratories reported realistic relative measurement uncertainties, ranging from 7 to 25 %. Only one laboratory (L021) reported no measurement uncertainty, even though it was requested, while two laboratories (L003 and L011) reported seemingly underestimated measurement uncertainties.

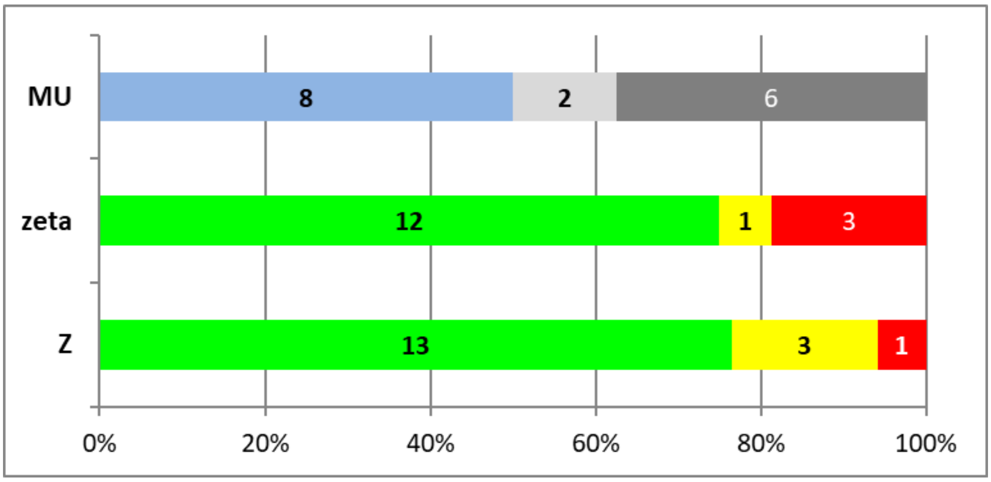

Figure 1: Overview of laboratory performance according to z and ζ scores, and measurement uncertainty evaluation. The corresponding number of laboratories are included in the graph. Satisfactory (S), questionable (Q) and unsatisfactory (U) performances are indicated in green, yellow and red, respectively. Similarly case "a" (realistic), "b" (probably underestimated), and "c" (overestimated) measurement uncertainties are presented in blue, light and dark grey, respectively.

7 Conclusion

The proficiency testing round "FAC-22/01" was organised to assess the analytical capabilities of EU NRLs and OCLs to determine the content of vitamin A in rabbit compound feed. The overall performance of the laboratories was satisfactory, confirming their analytical capabilities to control vitamin A at authorised levels in compound feed to support Commission Implementing Regulation (EU) 2015/724 [2].

References

[1] Commission Regulation (EU) No 2017/625 of The European Parliament and of The Council of 15 march 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. Official Journal of the European Union, L 95/1 (2017).

[2] Commission Implementing Regulation (EU) 2015/724 of 5 May 2015 concerning the authorisation of retinyl acetate, retinyl palmitate and retinyl propionate as feed additives for all animal species. Official Journal of the European Union L 115/2 5- L115/31.

[3] ISO/IEC 17043:2010 "Conformity assessment – General requirements for proficiency testing", International Organisation for Standardization, Geneva, Switzerland.

[4] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union L54/1 – L54/130

[5] ISO 13528:2022 "Statistical methods for use in proficiency testing by interlaboratory comparisons". International Organisation for Standardization, Geneva, Switzerland.

[6] Analytical Methods Committee, Analyst, 1989, 114, 1489.

[7] Analytical Methods Committee, Analyst, 1989, 114, 1693.

[8] AMC software: https://www.rsc.org/membership-and-community/connect-with-others/join-scientific-networks/subject-communities/analytical-science-community/amc/software/, assessed on 28.10.2022

[9] EN 17547:2020 "Animal feeding stuffs: Methods of sampling and analysis - Determination of vitamin A, E and D content - Method using solid phase extraction clean-up and High Performance Liquid Chromatography". European Committee for Standardization, Brussels, Belgium.

[10] Analytical Methods Committee Technical Brief - "Representing data distributions with kernel density estimates", AMC Tech. Br. 4 (2006). http://www.rsc.org/images/brief4_tcm18-25925.pdf.
The Excel add-in to calculate/plot kernel densities is available from https://rsc.li/3dGc3PG , accessed on 28.10.2022.

List of abbreviations and symbols

DG SANTE	Directorate General for Health and Food Safety of the European Commission
JRC	Joint Research Centre of the European Commission
EURL-FA Control	European Union Reference Laboratory for Feed Additives Control
NRL	National Reference Laboratory
OCL	Official Control Laboratory
ISO	International Organization for Standardization
PT	Proficiency Test
HPLC-UV	High performance liquid chromatography coupled with ultra-violet detection
LC-DAD	Liquid chromatography coupled with diode array detection
k	coverage factor (associated with an expanded measurement uncertainty)
σ_{pt}	standard deviation for proficiency assessment
$u(x_i)$	standard measurement uncertainty reported by participant "i"
$u(x_{pt})$	standard uncertainty of the assigned value
$U(x_i)$	expanded uncertainty reported by participant "i"
$U(x_{pt})$	expanded uncertainty of the assigned value
x_i	mean value reported by participant "i"
x_{pt}	assigned value
z	z score
ζ	zeta score

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Annex 1: Invitation letter

EUROPEAN COMMISSION
Joint Research Centre
Directorate F – Health, Consumers & Reference Materials
European Union Reference Laboratory for Feed Additives Control

Ref. Ares(2022)4197738 - 07/06/2022

EURL
European Union Reference Laboratory
Feed Additives

Geel, 07 June 2022

(sent by e-mail)

Subject: Invitation to participate in the Proficiency Test round "FAC-22/01"

Dear Colleagues from the National Reference Laboratories for feed control,

I hereby invite you to participate in the Proficiency Test (PT) round EURL-FAC-22/01 concerning the **"Determination of the content of vitamin A in rabbit feed".**

This PT fulfils the EURL-FA Control mandate under Regulation (EC) No 2017/625.

According to Regulation (EC) No 2017/625 it is your duty as NRL to participate in PTs organised by the EURL-FA Control if you hold a mandate for this type of matrix or to mandate a laboratory to represent you in the exercise.

Please note that as NRL under Regulation (EC) No 2017/625 you are allowed to invite Official Control Laboratories (OCLs) in your Member State to participate to the PT. In this instance, please inform the respective OCLs that their performance results may be communicated to you by the EURL upon your request. Please inform us how many OCLs would be interested in participating, together with their addresses, contact number and responsible person. They should register electronically by using the URL below. The total number of participating laboratories in this PT may not exceed 55. The EURL has therefore the right to accept or refuse the participation of OCLs during the registration period; you will be informed about the participation of OCLs in your Member State after the registration deadline.

The participation to the PT is free of charge.

The main objective of this study is to assess the proficiency of the participating laboratories to correctly determine the content of vitamin A in rabbit feed. For this, participants will be asked to analyse a single sample (+50 g sachet). The proficiency test also includes evaluating the capability of the laboratories to carry out the requested analysis within a defined time frame.

Please register electronically by copying the URL below, pasting it in your navigator and following the instructions on the screen (or in the attached guidelines):

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=2801

Please fill in the form comprehensively. These details will be used for the dispatch of the test material and for any further correspondence. Do not forget to "**submit**" your registration form.

Once you have submitted your registration electronically, you will have to:

- Print your registration form, as indicated on the screen
- Sign it, date it and send it to us by e-mail (jrc-eurl-feed-additives-control@ec.europa.eu)

The deadline for registration is 30 July 2022 *(may be modified)*.

Samples are foreseen to be dispatched in September 2022 *(may be modified)*.

The deadline for submission of results is foreseen 4 weeks after dispatch *(may be modified)*.

The exercise will be completed by the organisation of the annual workshop open to all participants and to all NRLs for Feed additives. The target period for the workshop is mid November 2022 *(may be modified)*. The workshop is free of charge. Travel and accommodation costs will be reimbursed for one representative of each National Reference Laboratory for Feed Additives defined according to Regulation (EC) No 2017/625 or Regulation (EC) No 1831/2003 (if no NRL is nominated according to Regulation (EC) No 2017/625 for a given Member State).

Do not hesitate to contact us if you have any further questions at JRC-EURL-FEED-ADDITIVES-CONTROL@ec.europa.eu

Best regards,

/signed electronically in Ares/

Joerg Stroka

Cc: Ursula Vincent (Head of Unit, Food & Feed Compliance, JRC F.5)

Electronically signed on 07/06/2022 10:54 (UTC+02) in accordance with Article 11 of Commission Decision (EU) 2021/2121

Annex 2: Test item accompanying letter

Ref. Ares(2022)6157165 - 06/09/2022

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel)
Food & Feed Compliance

EURL
European Union Reference Laboratory
Feed Additives

Geel, 06 Sept. 2022
JRC F.5/JS/AS/Ares

Subject: **Participation in FAC-PT-22/01 – "Determination of the content of vitamin A in animal feed"**

Dear Participant,

Thank you for participating in our proficiency test**.** This PT is part of the execution of the EURL Feed Additives Control programme and supports the implementation of Regulation (EU) 2017/625.

The parcel you received contains:

- 1 sachet with at least 55 g of test material for vitamin A analysis (3a 672a).

Please keep this letter. You will need it to report your results.

Upon arrival of the parcel, please check whether the test item is undamaged after transport, and send us by email the "Confirmation of receipt" form within 3 days after receipt of the test item.

Upon reception, store the sample in a dark place at 4 °C or below. and until one hour before the analysis. One hour before the analysis keep the sample at room temperature.

The requested measurand is "**the mass fraction of vitamin A in poultry compound feed expressed as IU kg^{-1}".**

The procedure used for the analyses should resemble as closely as possible the one you use in routine analyses. The test item homogeneity is demonstrated at 10 g sample intake level:

DO NOT use a smaller test portion size for analysis.

The procedures used for the organisation of PTs are accredited according to ISO/IEC 17043:2010 and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, the lab codes of the NRLs that have been appointed in line with Regulation (EC) No 882/2004 and Regulation (EU) 2017/625 may be disclosed to DG SANTE upon request for the purpose of an assessment of their (long-term) performance and lab codes of appointed Official Control Laboratories may be disclosed to their respective NRL upon request.

Determine the **moisture** content and **correct the measurement** results for moisture content as prescribed in DIR 2002/32/EC.

1

Perform independent measurements and report:

- the result for the **moisture content** determination (in % w/w),
- the **mean** (when relevant) of your measurements results (in IU kg^{-1}),
- the associated expanded **uncertainty** (in IU kg^{-1}),
- the **coverage factor**, and
- the **analytical technique** used.

The results should be reported in the same form (e.g. number of significant figures) as you normally report to customers.

The reporting website is https://web.jrc.ec.europa.eu/ilcReportingWeb

To access the webpage you need a personal password key that will be sent to you in a separate e-mail.

The system will guide you through the reporting procedure. Then complete the corresponding questionnaire. **Do not forget to submit and confirm when required.**

Directly after submitting your results and the questionnaire information online, you will be requested to print the completed report form.

Please check carefully your report. In the case mistakes are detected contact the PT coordinator as soon as possible before the reporting deadline.

The deadline for submission of results is **30/09/2022**.

Remember that **collusion** is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike.

Please be aware of the existence of an **appeal procedure** in case you disagree with your performance scores.

Your participation in this project is greatly appreciated and do not hesitate to contact us for further information.

Kind regards,

/signed electronically in Ares/

Joerg Stroka
FAC-PT-19-01 PT Coordinator

Cc: Dr. Ursula Vincent (Head of Unit, JRC F.5, Food & Feed Compliance unit)

Electronically signed on 06/09/2022 13:29 (UTC+02) in accordance with Article 11 of Commission Decision (EU) 2021/2121

Annex 3: Confirmation of receipt form

Ref. Ares(2022)6157165 - 06/09/2022

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel)
Food & Feed Compliance

EURL European Union Reference Laboratory Feed Additives

Geel, 06 Sept. 2022
JRC F.5/JS/AS/Ares

Attn.: «Firstname» «Surname»
«Organisation»,
«Country»
Lab Code: «LCode»

Subject: "Confirmation receipt" form
FAC-PT-22/01 "Determination of the content of vitamin A in animal feed."

Please return this form within 3 days after reception of the parcel, to confirm that the package arrived well. If the sample is damaged, mention it under "Remarks" and contact us as soon as possible.

Date of package arrival ……………………………………………..

Remarks ……………………………………………..
……………………………………………..
……………………………………………..

Sample code ……………………………………………..

Signature ……………………………………………..

Thank you for returning this form by email to:

J. Stroka
FAC-PT-20/01 Coordinator
e-mail : jrc-eurl-feed-additives-control@ec.europa.eu

Europese Commissie, Retieseweg 111, 2440 Geel, BELGIQUE/BELGIË
Office: 010 L01/153 - Tel. direct line +32 (0)14 571 229

JRC-EURL-FEED-ADDITIVES-CONTROL@ec.europa.eu

Annex 4: Questionnaire

Milc questionnaire

Comparison for EURL-FAC-22/01

PT for the performance evaluation of the NRLs in the determination of the content of vitamin A in rabbit feed.

Submission Form

1. **Your laboratory is?**

a) National Reference Laboratory (NRL)

b) Official Control Laboratory (OCL)

1.1. If NRL to which legislation do you comply?

Reg. (EC) 1831/2003 Authorization of feed additives

Reg. (EU) 2017/625 Control of feed additives

☐

☐

2. **Considering your measurement result and given a stated (addition) of 12.000 I.U. vitamin A (3a672a) per kg, would you consider the test item as non-compliant with Commission Implementing Regulation (EU) 2019/849?**

a) Yes

b) No

2.1. Please specify why?

3. **What method did you use to perform the analysis?**

○ a) Official method as laid down in Commission Regulation 152/2009

○ b) EN 17547:2021 'Determination of vitamin A, E & D with SPE and HPLC'

- Page 1 of 6 -

c) Other

3.1. If "Other" please specify the method used.

5. **Which test portion did you use for analysis (g)?**

6. **Did you take measures to minimize analyte degradation by light or oxidation? If yes, please indcate how?**

7. **Which antioxidant was used?**

a) Ascorbic acid

b) Sodium ascorbate

c) Pyrogallol

d) sodium sulfite

e) sodium sulfide (acts also as chelating agent)

f) butylated hydroxytoluene

g) hexylresorcinol

h) Other

i) None

7.1. Which amount of antioxidant was used per analysis (g)?

7.2. If "Other" please specify.

8. **Was the analysis subject to any "over night" stops or equivalent? If yes, please state at what stage this step was made and how the sample was kept during the break.**

9. **Which extraction solvent was used?**

- a) Petrolether (e.g. 40/60)
- b) Diethyl ether
- c) Dichloromethane
- d) n-Hexane
- e) Ethylacetate
- f) Other

9.1. If "Other" please specify

10. **Did you identify any interference that were not baseline separated from the target peak?**

- a) Yes
- b) No

11. Please give us some details on the calibrant used, such as source, concentration of the stock solution (mother of all calibrants used), nature of solvent and for what period the stock solution was in use when performing the analysis. Did you check the stock solution in it's quantity (e.g. photometer) and possible degradation products (e.g. HPLC UV 220 nm).

12. **Do you have any comments? Let us know!**

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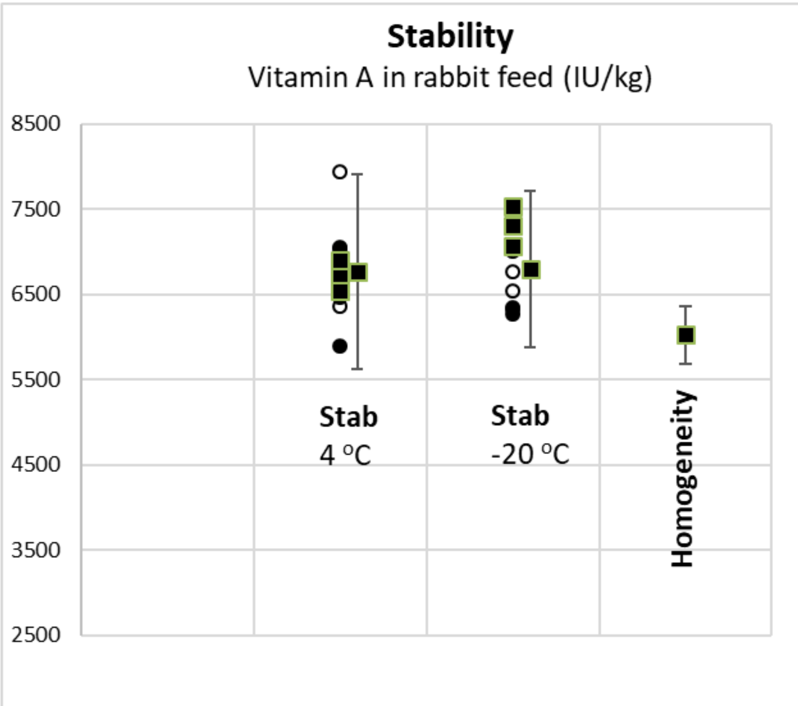
Annex 5: Homogeneity and stability studies (all values in IU kg[-1])

Homogeneity			Stability		
Sample	**Replicate 1**	**Replicate 2**	**Sample**	**4 °C days**	**-20 °C days**
1	6133	6166	1	6466	6350
2	5966	5833	2	7033	6266
3	6066	6599	3	5899	6330
4	6466	5866	4	7050	6766
5	5866	6099	5	6366	6533
6	5866	5833	6	7933	6999
7	5866	6366	8	6716	7300
8	6066	6199	9	6533	7533
9	5499	5499			
10	6133	6033			
mean	6021		mean	6766	6794
s_{samp}	168		Difference (Δ)	28	
σ_{pt}	1204		σ_{pt}	1356	
$0.3\ \sigma_{pt}$	361		$0.3\ \sigma_{pt}$	407	
$s_{samp} \leq 0.3\ \sigma_{pt}$	Adequately homogeneous		$\Delta \leq 0.3\ \sigma_{pt}$	Adequately stable	
According to	ISO 13528:2022 § B.2 [5]			ISO 13528:2022 § B.5 [5]	

Where: s_{samp} is the between-bottle standard deviation,
σ_{pt} is the standard deviation for proficiency assessment,
x_{st} is the mean value from the stability study.



Annex 6: Results for Vitamin A in rabbit compound feed

Assigned range: x_{pt} ± U $(k=2)$ = 4535 ± 430 and σ_{pt} = 907

All values are expressed in IU kg^{-1}

Lab	x_i	$U(x_i)$	k	Analytical method	Z score [a]	ζ score [a]	MU [b]
L001	4900	2000	2	EN 17547	0.40	0.36	c
L002	4822	2340	2	Regulation 152/2009	0.32	0.24	c
L003	4117	22.7	2	Regulation 152/2009	-0.46	-1.94	b
L004	5540	550	2	EN 17547	1.11	2.88	a
L006	4054	526	2	Regulation 152/2009	-0.53	-1.42	a
L009	5001.2	1200.3	2	EN 12823-1:2014 in-house method, modif.	0.51	0.73	a
L010	5328	1066	2	EN 12823-1: 2000	0.87	1.38	a
L011	12587	25	2	EN 17547	8.88	37.39	b
L013	6888	689	2	Regulation 152/2009	2.59	5.79	a
L014	4201	2340	2	Regulation 152/2009	-0.37	-0.28	c
L015	4810	1106	2	Regulation 152/2009	0.30	0.46	a
L016	4585	1150	2	Regulation 152/2009	0.06	0.08	a
L017	2520	760	2	Regulation 152/2009	-2.22	-4.62	a
L018	4572	2268	2	Regulation 152/2009	0.04	0.03	c
L019	5246	2340	1.96	Regulation 152/2009	0.78	0.59	c
L020	4412	2340	2	Regulation 152/2009	-0.14	-0.10	c
L021	2683	np		EN 12823-1:2014	-2.04	-8.61	np

[a] Performance: satisfactory, questionable, unsatisfactory,

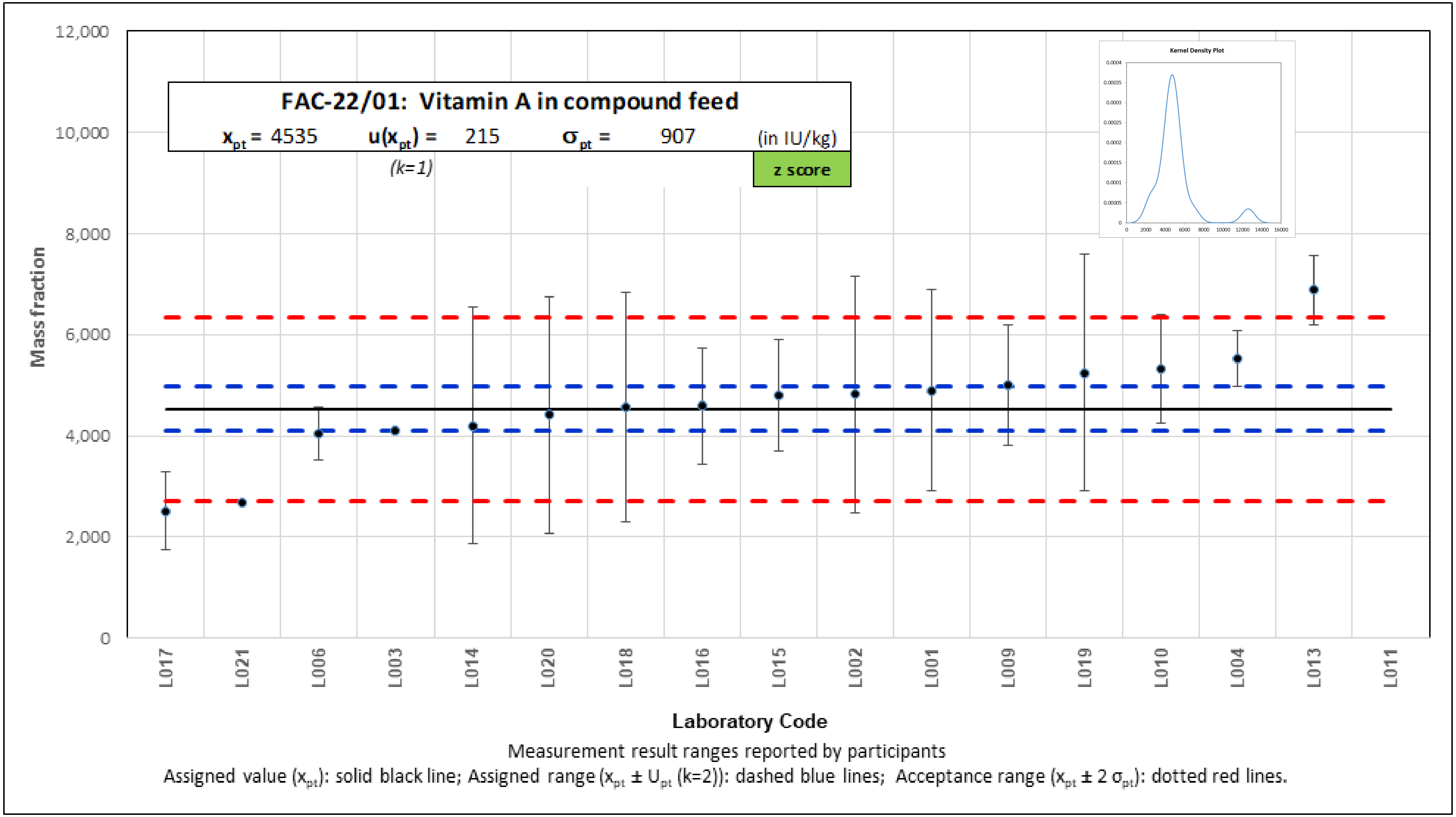
[b] Measurement uncertainty evaluation:

np: no measurement uncertainty was provided

a: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,rel}$;

b: $u_{rel}(x_i) < u_{rel}(x_{pt})$ and

c: $u_{rel}(x_i) > \sigma_{pt,rel}$


FAC-22/01: Vitamin A in compound feed
x_{pt} = 4535
$u(x_{pt})$ = 215
(k=1)
σ_{pt} = 907
(in IU/kg)
z score
Kernel Density Plot
Mass fraction
12,000
10,000
8,000
6,000
4,000
2,000
0
L017
L021
L006
L003
L014
L020
L018
L016
L015
L002
L001
L009
L019
L010
L004
L013
L011
Laboratory Code
Measurement result ranges reported by participants
Assigned value (x_{pt}): solid black line; Assigned range ($x_{pt} \pm U_{pt}$ (k=2)): dashed blue lines; Acceptance range ($x_{pt} \pm 2\,\sigma_{pt}$): dotted red lines.

Annex 7 – Details of the analytical methods applied by the participants

Laboratory code	Your laboratory is?	What method did you use to perform the analysis?
L001	National Reference Laboratory (NRL)	EN 17547:2021 Determination of vitamin A, E & D with SPE and HPLC
L002	National Reference Laboratory (NRL)	Official method as laid down in Commission Regulation 152/2009
L003	National Reference Laboratory (NRL)	Official method as laid down in Commission Regulation 152/2009
L004	Official Control Laboratory (OCL)	EN 17547:2021 Determination of vitamin A, E & D with SPE and HPLC
L006	National Reference Laboratory (NRL)	Official method as laid down in Commission Regulation 152/2009
L009		in-house method, modified 12823-1:2014
L010	National Reference Laboratory (NRL)	EN 12823-1: 2000 Determination of vitamin A by HPLC-DAD
L011	National Reference Laboratory (NRL)	EN 17547:2021 Determination of vitamin A, E & D with SPE and HPLC
L013	Official Control Laboratory (OCL)	Official method as laid down in Commission Regulation 152/2009
L014	National Reference Laboratory (NRL)	Official method as laid down in Commission Regulation 152/2009
L015	National Reference Laboratory (NRL)	Official method as laid down in Commission Regulation 152/2009
L016	Official Control Laboratory (OCL)	Official method as laid down in Commission Regulation 152/2009
L017	National Reference Laboratory (NRL)	Modified CR 152/2009
L018	Official Control Laboratory (OCL)	Official method as laid down in Commission Regulation 152/2009
L019	Official Control Laboratory (OCL)	Official method as laid down in Commission Regulation 152/2009
L020	Official Control Laboratory (OCL)	Official method as laid down in Commission Regulation 152/2009
L021	Official Control Laboratory (OCL)	EN 12823-1:2014

Laboratory code	Which test portion did you use for analysis (g)?	Did you take measures to minimize analyte degradation by light or oxidation? If yes, please indcate how?
L001	25	Refrigerator since openning
L002	20	brown glass, nitrogen
L003	10	dark and nitrogen
L004	25	
L006	10	use amber glassswear
L009	1	yes
L010	10	Sample is covered with aluminum foil. Yellow light in the lab. Use of pyrogallol
L011	30	Yes, dark glass and a dark room for both saponification and extraction.
L013	10	no
L014	20, 20, 15	Antioxidant, N2 by saponification, smoked windows
L015	10	Amber vials, no UV-light in the lab.
L016	10	
L017	10	UV protection foil on windows
L018	10 to 20	yellow light laboratory, antioxidant
L019	10	We kept the sample dry and dark at 4°C before analysis. One hour before analysis, we took the sample out of the fridge and stored at room temperature. The material was then inserted in a browny glass.
L020	10	yes, exclusion of sunlight, working under nitrogen
L021	10	Yes, added antioxidants (specified below) to the sample and UV-filter on lightning in the laboratory.

Laboratory code	Which antioxidant was used?	Which amount of antioxidant was used per analysis (g)?
L001	100mg B+B3:B19+C3:C19HT + 200mg Natriumascorbat	1 g ascorbic acid (+ 100 mg BHT)
L002	Sodium ascorbate	100mg BHT + 200mg Natriumascorbat
L003	butylated hydroxytoluene	0,1 g / 10 g
L004	butylated hydroxytoluene	0.1
L006	Ascorbic acid	150mg
L009	ascorbic acid and pyrogallol	1% pyrogallol solution + 0.2 g ascorbic acid
L010	Ascorbic acid	Spateltip Pyrogaloll
L011	Ascorbic acid	0.75
L013	Sodium ascorbate	0.25g
L014	0,2g sodium ascorbate, 0,078g sodium sulfit, 0,1g BHT	
L015	Ascorbic acid	1 ml of 10 % ascorbic acid solution 0,1 g + butylated hydroxytoluene 0,05 g + hydroquinone 0,1 g
L016	Ascorbic acid	150 mg ascorbic acid + 100 mg BHT + 1 mMol natrium sulfide
L017	Ascorbic acid + sodium ascorbate + BHT	0.1 g of each
L018	Ascorbic acid	0,2g
L019	Sodium ascorbate	2 ml sodium ascorbate; Additionally, we used 2 ml sodium sulfide
L020	according to Commision Regulation 152/2009 sodium ascorbate solution, sodium sulfite solution and BHT was applied	0,1 BHT, 0,2 sodium ascorbate, 0,05 sodium sulfite
L021	BHT, ascorbic acid, pyrogallol and sodium sulfide	BHT: 0.+C3:C19075g, ascorbic acid: 0.3g, pyrogallol: 0.1125g, sodium sulfide: 0.36g

Laboratory code	Was the analysis subject to any "over night" stops or equivalent? If yes, please state at what stage this step was made and how the sample was kept during the break.	Which extraction solvent was used?	Did you identify any interference that were not baseline separated from the target peak?
L001		Ethanol (with KOH and Na2S)	Yes
L002	no	Petrolether (e.g. 40/60)	No
L003	After extraction, stored at +4°C, in the dark, under nitrogen	Petrolether (e.g. 40/60)	No
L004		cyclohexane	No
L006		n-Hexane	No
L009	no	n-Hexane	Yes
L010	No	n-Hexane	Yes
L011	No stops, only over night saponification in a dark room.	n-Hexane	No
L013	no	n-Hexane	No
L014	No	Petrolether (e.g. 40/60)	Yes
L015	No	Petrolether (e.g. 40/60)	Yes
L016	no	Petrolether (e.g. 40/60)	No
L017	No.	Petrolether (e.g. 40/60)	No
L018	no	Petrolether (e.g. 40/60)	No
L019	The analysis was done without any stops.	n-Hexane	Yes
L020	No	Petrolether (e.g. 40/60)	Yes
L021	After weighing, the sample was left, purged with nitrogen, over night in refrigerator	hexane : ethyl acetate (85:15 w/w)	Yes

Laboratory code	Did you identify any interference that were not baseline separated from the target peak?	Give us some details on the calibrant used, such as source, concentration of the stock solution (mother of all calibrants used), nature of solvent and for what period the stock solution was in use when performing the analysis. Did you check the stock solution in it's quantity (e.g. photometer) and possible degradation products (e.g. HPLC UV 220 nm)
L001	Yes	Retinol in methanol, september 2022, photometer controlled
L002	No	Retinyl palmitate (Sigm, freshly prepared and UV checked every week, prepared by saponification of a 6,72 I.E./ml stock solution in 2-propanol like the samples
L003	No	Retinol-acetat, sigma, 1400 IE/ml 2-propanole, freshly prepared, checking concentration by photometer
L004	No	Check lambda max @325-327nm
L006	No	SUPELCO stock solln used retinyl acetate with certified activity (2851029 IU/g), std was hydrolysed, diluted and its conc was checked photometrically
L009	Yes	All-trans-Retinol standard from Acros. Calibration stock solution control by photometer.
L010	Yes	Retinol Acetat All Trans R-4632 Sigma, Check of stock solution photometer 325 nm
L011	No	The STD is from Sigma - Retinyl palmitate R1512. The concentration of the stock standard solution is approx. 60 mg/l in methanol. The period of validity is half a year. We check it by standardization on a spectrophotometer each month. We did not notice the degradation of Vitamin A, and we could not at 220 nm in the UV, we are measuring a different area of the spectrum.
L013	No	Retinyl acetate with activity 475 000 - 650 000 units/g (Sigma Aldrich). About 100 mg taken for hydrolysis and after extraction and evaporation reconstituted in 100ml of methanol. Stored in dark in freezer (-20C). Stock was prepared fresh couple days before PT. In routine stock is checked each 3 months using spectrophotometer.
L014	Yes	Retinol which is seponified to VIT A-acetat, concentration 1300IE/ml; Stock solution checked monthly using a photometer
L015	Yes	Mother stock solution: 10 mg/200 ml Meoh (about 163 iu/ml, done 9.9.2020, kept in the freezer.) The quantity of the standard solution (about 8 iu/ml) was checked by a spectrometer and done on the same day as analysis as well as the calibration standards.

Laboratory code	Did you identify any interference that were not baseline separated from the target peak?	Give us some details on the calibrant used, such as source, concentration of the stock solution (mother of all calibrants used), nature of solvent and for what period the stock solution was in use when performing the analysis. Did you check the stock solution in it's quantity (e.g. photometer) and possible degradation products (e.g. HPLC UV 220 nm)
L016	No	ca. 300 mg retinol-acetat (Sigma-Aldrich) in 100 ml ethanol, after saponification in use since 3 months; check of concentration with photometer (326 nm)
L017	No	Vit. A acetate, Fluka 95138, 2000 IU/mL, hexane, prepared fresh, concentration checked photometrically, no HPLC-UV degradation product test
L018	No	solution with Retinyl-Acetat standard (Sigm
L019	Yes	The concentration of the stock solution must be 560 IE/mL. Yes, we checked the stock solution with a photometer (UV).
L020	Yes	stock solution: Vitamin a palmitate from supplier Dr. Ehrenstorfer, Lot. 1147936, concentration 6,3232 IE/ml checked via photometer, prepared on day of measurement in isopropanol. calibration solution: prepared on day of measurement by hydrolysis of stock solution, concentration 5,945 IE/ml in methanol, checked via photometer
L021	Yes	The calibrant used is all-trans retinol (purity 98%, cas number = 68-26-8) dissolved in n-hexane. The concentration of the stock solution is measured with a photometer at 325nm.

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