Tampereen ammattikorkeakoulu

Commissioning and Validation of a Volumetric KF Titrator

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HAKKARAINEN, MINKA:
Volumetrisen KF-titraattorin käyttöönotto ja validointi

Opinnäytetyö 49 sivua, joista liitteitä 0 sivua
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Opinnäytetyön aiheena oli volumetrisen Karl Fischer -titraattorin käyttöönotto sekä menetelmän laadun varmentaminen eli validointi. Tavoitteena oli suorittaa käyttöönotto ja sen yhteydessä menetelmän validointi. Tarkoituksena oli tehdä validointisuunnitelma ja suorittaa validointi sen pohjalta sekä suorittaa muita käyttöönottoon liittyviä käytännön toimenpiteitä. Opinnäytetyö suunniteltiin ja toteutettiin yhteistyössä Neste Oyj:n kanssa. Laite haluttiin lisätä olemassaolevan kulometrisen KF-titraattorin yhteyteen, jotta eripitoisten näytteiden analysointimahdollisuudet monipuolistuvat.

Työssä perehdyttiin syvällisesti KF-titrauksen teoriaan, näytteiden matriisiin ja matriisien vaikutuksiin analyysissa. Lisäksi perehdyttiin validoinnin teoriaan, itse validoinnin suoritukseen sekä tulosten laskentaan, raportointiin ja analysointiin. Perehtymisen jälkeen itse validointi toteutettiin suunnitelman mukaisesti käyttäen valittuja testinäytteitä ja referenssimateriaaleja. Validoinnin painopiste oli selektiivisyyden, tarkkuuden, oikeellisuuden sekä havaitsemis- ja määritysrajojen määrittämisessä. Käyttöönoton osalta tehtiin menetelmäohje sekä lisättiin menetelmä laboratorion tiedonhallintajärjestelmään (LIMS).

Johtopäätöksissä pohdittiin työn tuloksia, haasteita ja kehityskohteita. Näytteiden valmistukseen, erityisesti näytteiden asianmukaiseen homogenisointiin ja veden haihtumiseen, liittyviä haasteita kartoitettiin ja pohdittiin mahdollisia virhelähteitä. Ajanpuutteen vuoksi työ jäi osittain kesken, ja menetelmän luotettavuuden ja laajemman käytettävyyden varmistamiseksi ehdotettiin jatkotoimenpiteitä. Näitä olivat lineaarisuuden ja uusittavuuden määritys, eri näytematriisien tutkiminen eri lämpötiloissa sekä suorainjektion verifiointi. Prosessin kehittämiseksi ehdotettiin uuden vaa’an hankkimista ja kalibrointitarpeen määrittämistä. Tulevaisuudessa laite pääsee käyttöön kulometrisen KF-titraattorin ohella.

Asiasanat: Karl Fischer -titraus, validointi, volumetrinen KF-titraattori, öljynäyte, vesipitoisuus

ABSTRACT

Tampereen ammattikorkeakoulu
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HAKKARAINEN, MINKA:
Commissioning and Validation of a Volumetric KF Titrator

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May 2025

This thesis focused on the commissioning and validation of a volumetric Karl Fischer (KF) titrator, and it was completed in collaboration with Neste Oyj. The objective was to conduct the commissioning process, including method validation and other related procedures, to enhance analytical capabilities by complementing the existing coulometric KF titrator. The aim was to develop a validation plan and perform the validation according to it, encompassing selectivity, precision, trueness, limit of detection, and limit of quantification parameter tests.

The commissioning included creating operating instructions and adding the method to the Laboratory Information Management System (LIMS). The study also involved a comprehensive review of KF titration theory, sample matrices, and validation principles.

The commissioning and validation of the volumetric Karl Fischer titrator were successfully completed, meeting the objectives of the thesis. The addition of this device enhances the laboratory’s ability to reliably analyse samples with higher water content. While the core validation goals were achieved, further work is recommended, including linearity testing, temperature profile testing, and verification of the direct injection method.

Improvements to the process, such as installing a scale in the fume hood and assessing calibration frequency, were also identified. Challenges with sample preparation, particularly with solidifying oil samples, were noted, and potential sources of error were considered. Overall, the validation process was successful, enabling the reliable use of the volumetric KF titrator, but further refinements and research are suggested for optimal performance and future studies on water content consistency.

Key words: Karl Fischer titration, volumetric KF titrator, validation, oil sample, water content

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ABBREVIATIONS AND TERMS

KF	Karl Fischer
LOD	Limit of detection
LOQ	Limit on quantification
LIMS	Laboratory Information Management System
ppm	Parts per million
RSD	Relative standard deviation
wt-%	Weight percentage

1 INTRODUCTION

The accurate determination of water content is critical in numerous industrial applications, most notably within the petrochemical sector, where it impacts processes such as filterability and purification. Inaccurate water content measurement can lead to inefficiencies, product quality issues, and increased operational costs. To ensure the reliability of analytical results, the validation of new analytical methods is a fundamental requirement, confirming that a device functions as expected and meets predefined performance criteria.

This thesis addresses the commissioning and validation of a new volumetric Karl Fischer (KF) titrator at Neste Oyj. Neste Oyj, established in Finland in 1948, is a company focused on renewable and circular solutions. Its mission is to combat climate change through refining of waste, residues, and innovative raw materials into high-quality, lower-emission products. The company's strategic focus on this area has driven the need for robust analytical methods to support its operations. (Neste n.d.)

The aim of this thesis is to validate a newly acquired volumetric KF titrator and perform practical measures related to the commissioning of the device. The purpose is to create a validation plan, and accordingly test selected validation parameters with the KF titrator. Besides the validation plan and testing, the work includes creating a method manual and adding the validated method to the laboratory's Laboratory Information Management System (LIMS).

The existing laboratory at Neste Oyj has historically relied only on coulometric KF titration. However, the limited water content range of this technique necessitates the introduction of a volumetric KF titrator to enable the reliable analysis of samples with higher water concentrations.

The scope of this work is limited to oil sample matrices. This is because other sample types analysed in the laboratory generally do not exhibit the elevated water content needed for proper validation of the volumetric KF titrator. Method development itself is also excluded, with the primary focus on the validation process and its practical implementation.

2 KARL FISCHER TITRATION

2.1 KF titration in various fields

KF titration plays an important role in various fields due to its accuracy and specificity in determining water content. It ensures that products meet specific water content standards, which is vital for maintaining consistency and quality in different kinds of products. Water content is important to determine across various fields such as pharmaceutical and cosmetic industry, chemistry, biology, and the food and beverage industry. (Rivera-Quintero 2024.) Water content in different kinds of products can significantly affect their shelf life and stability. Water can act as an unwanted reactant or catalyst in chemical reactions and that can affect the yield and purity of the final product. With KF titration the water levels can be controlled to optimize reactions. (Thomas 2023.)

In the petrochemical industry, elevated water content poses a significant risk of corrosion to storage tanks and pipelines (Zerust n.d.). Furthermore, excessive water can diminish fuel efficiency, lead to the formation of emulsions capable of obstructing pipelines and foster microbial growth that can compromise fuel quality. Given the sensitivity of numerous refinery processes to water contamination, accurate water content analysis using KF titration is a needed step in ensuring both operational safety and efficiency. (Jax 2023.) Water content is also a significant parameter on the pretreatment processes, as it impacts on the oil quality and the efficiency of subsequent processing steps (Ahmad 2024, 2).

2.2 Principle of the volumetric KF titration

KF titration is a classical chemical titration method, and it was invented by a German chemist named Karl Fischer in 1935. In KF titration the water is titrated using iodine, sulfur dioxide, a solvent and a basic buffer. KF reaction is based on the elementary reaction, called the Bunsen reaction. This method was founded upon the reaction documented by R.W. Bunsen in 1853. (Korotcenkov 2019.) In this reaction sulfur dioxide is oxidated by iodine as follows (Thomas 2023.):

$$2H_2O + SO_2 + I_2 \rightarrow H_2SO_4 + 2\,HI \quad (1)$$

The current process involves the reaction of an alcohol (ROH) with sulfur dioxide to form a monoalkyl ester of sulfurous acid, a crucial intermediate for the subsequent KF reaction. A base (R'N) is used to neutralize this ester, facilitating the reaction of the resulting alkyl sulfite anion within the KF solution. (Rivera-Quintero 2024.) The reaction goes as follows (Thomas 2023):

$$ROH + SO_2 + RN \rightarrow [RNH]SO_3R \quad (2)$$

Under the influence of water in the sample and the iodine added to the solution, the alkyl sulfite intermediate is oxidized to alkyl sulfate as shown in the equation below (Thomas 2023):

$$H_2O + I_2 + [RNH]SO_3R + 2RN \rightarrow [RNH]SO_4R + 2\,[RNH]I \quad (3)$$

The reaction is performed in an alcohol solution containing a base. The base acts as a buffering agent, neutralizing the acidic by-products. The acidic HI will cause a reversible reaction if it is not neutralized, which will then again form H_2O. That is why the base is needed in this reaction. (GMP Insiders 2025.) With each mole of I_2, one mole of H_2O is consumed. Iodine is added to the solution in excess until the endpoint is marked, which can be detected potentiometrically. The endpoint is reached when all water from the analyte is consumed. (Thomas 2023.)

The reaction was originally made with pyridine. The required base was later changed to imidazole due pyridine's toxicity. Imidazole also sped up the reaction and improved its accuracy. It was eventually noticed that pyridine was not directly even a part of the reaction. (Thomas 2023.)

2.3 Volumetric KF titrator in practice

A volumetric KF titrator includes a titration vessel with a stirrer, a burette and a double platinum electrode for the endpoint determination, which the titrator detects potentiometrically. The first step of the determination is a pre-titration of the working medium with the KF reagent. It removes moisture from the working medium and makes it easier to reach a stable endpoint. After the pre-titration a sample is introduced to the titration cell, where the solvent dissolves the sample and a burette dispenses the KF reagent. (Rivera-Quintero 2024.)

KF titration utilizes dual platinum electrodes to detect the endpoint. A small current is applied, and the voltage difference between the electrodes remains low as long as water is present and reacts with the added iodine. Once all water is consumed, the increasing concentration of free iodine causes a rapid voltage surge, which the titrator identifies as the endpoint, halting reagent addition. In the endpoint determination, two platinum electrodes measure the voltage between the electrodes and trigger the endpoint when the voltage reaches a constant value. The water content is calculated automatically by the device, and it is directly proportional to the volume of the consumed titrant. (Korotcenkov 2019.)

The endpoint is calculated as follows:

$$\%\text{Water} = \frac{\text{B.R.}*\text{F}*100}{\text{WT}*1000} \quad (4)$$

Where:
B.R. = Volume of KF reagent consumed (ml)
F = KF reagent factor (titer) (mg/ml)
WT = Weight of the sample (g)
(Choudhary 2018.)

KF titration, which is sensitive to water, requires careful execution. The method is especially sensitive to errors from atmospheric moisture, poor sample handling, especially for hygroscopic and volatile substances, and degraded reagents (Bruttel 2006, 22). Strategies to preventing these errors include using airtight titration cells, working in low-humidity environments, rapid sample introduction, proper

reagent storage with desiccants, and regular reagent checks (Bruttel 2006, 8). Most instruments utilize desiccant-filled drying tubes to protect the reagents from moisture (Bruttel 2006, 13). Ensuring sample homogeneity through thorough mixing before performing the analysis is also essential for accurate results (Bruttel 2006, 24).

KF titration involves an error called drift, caused by unwanted water entry that appears as a false water results. The primary source of this error is atmospheric humidity, which can contaminate the sample, titrant, and titration equipment. This is especially problematic in humid regions, as vessel walls can absorb moisture and release it slowly. To minimize the drift, the titration stand must be tightly sealed. The vessel should be carefully dried before the analysis, and a 10-to-30-minute dry run should be performed to measure the drift rate. The drift rate is subsequently subtracted from the result. (Korotcenkov 2019.)

Volumetric KF titration can be performed using either a single-component or a two-component reagent system to determine water content. The single-component method pre-mixes all reagents in a solvent, usually methanol, in a burette, while the two-component method separates iodine in the burette from sulfur dioxide and base in the titration cell. Both methods involve pre-titration to remove existing water before the sample analysis. The single-component method is versatile and can be adapted to many workflows, while the two-component method offers faster titration and greater polarity. (Thomas 2023.) The choice between single-component and two-component reagents can be strategically made based on the specific sample matrix and the analytical requirements. (Mettler Toledo n.d.)

2.4 Oven method, direct injection and titer determination

The oven method for water determination involves heating a sample in an external oven. The water evaporates in the oven and is carried into a titration cell by an inert carrier gas, such as nitrogen or air. (Larsson 2008, 22.) A heated transfer tube ensures all moisture is transferred to the titration cell. The samples are transferred into the oven in closed vials which prevents water evaporation and excess

moisture from encountering the sample. The oven method allows fully automated determination, which frees up valuable time and increases laboratory productivity. Automation can increase reproducibility and reduce sample analysis failures which can happen due to improper handling. (Bruttel 2006, 16.)

Since KF titration is highly sensitive to water, the oven method is essential because it maintains a closed titration cell, effectively preventing atmospheric moisture absorption (Bruttel 2006, 8). This reduces conditioning time and enhances safety by eliminating direct contact with toxic reagents. Conditioning removes surface water from the equipment and any water present in the reagent. (Bruttel 2006, 12-13.)

The oven method is suitable for samples that do not release their water until a higher temperature has been reached, or for samples that release their water slowly (Bruttel 2006, 15). Other suitable cases are with samples that react with the KF reagent, or samples that have poor solubility, like non-polar oil samples (Larsson 2008, 1). Interference with the KF reagents, leading to inaccurate results, can occur due to some substances releasing water or consuming iodine. The separation in the oven method avoids direct contact between the sample and the KF reagent, thus preventing unwanted side reactions, matrix effects, and contamination of the titration cell. Separation also reduces risk of carryover and memory effect. (Bruttel 2006, 15-16.)

For accurate water content determination, especially in complex samples like oil (Larsson 2008, 1), establishing the optimal heating temperature using a device-generated heating curve is necessary. This ensures complete water release into the titration cell for analysis while preventing thermal degradation of the sample, which could spuriously release additional water and skew results. The KF oven method's advantage lies in minimizing matrix interferences and overcoming solubility limitations, often providing more reliable measurements than direct titration. Analysing the water release curve reveals the temperature-dependent kinetics, allowing precise identification of the ideal heating temperature and understanding of water release rates (Bruttel 2006, 16).

Direct injection is generally a straightforward and rapid way to introduce liquid samples into the titration cell. With direct injection, the sample is titrated in its entirety, and other substances in the sample matrix can interfere with the analysis. The sample may also contaminate the titration chamber. For those reasons, the oven method is better suited for samples that are reactive, insoluble or release water only at high temperatures. The oven method also ensures that all the water is evaporated making the analysis more reliable. (Rivera-Quintero 2024.)

In the oil analysis, different results obtained from both direct injection and heating methods, might stem from the influence of oil additives and aging byproducts. These substances can interact differently depending on the introduction method. For instance, some additives, like aldehydes or ketones (Thomas 2019), might release water or react with the KF reagent at elevated temperatures used in the heating method, leading to higher results compared to direct injection at ambient temperature. Conversely, certain aging byproducts could interfere with the direct titration reaction, potentially causing lower results. (Koch 2007, 1.)

Liquid samples are easily introduced to the cell with a clean pipette or a syringe. For the most accurate sample weight, the syringe should be weighed before and after injection. To prevent liquid loss during this process, drawing a small amount of air into the syringe after dispensing the sample is recommended. In non-polar liquids like oil, where water distribution can be uneven, thorough shaking before injection into the titration cell is essential. Volatile liquid samples should be refrigerated before sampling, but the syringe itself should remain at room temperature to avoid condensation. For highly viscous samples, warming the syringe can reduce viscosity and ease injection. (Rivera-Quintero 2024.)

Determination of the titer, which stands for titrant concentration, is necessary for accurate water content determination. Titer is expressed as mg of water per ml of titrant. Due to the inherent instability of KF reagents, regular titer checks are necessary for reliable results. The frequency of titer checks depends on the specific reagent and the titration cell’s sealing effectiveness. Reagent manufacturers often formulate titrants with a slightly higher initial titer to compensate for potential decreases during storage and use, ensuring it remains within an acceptable range. The frequency of titrant concentration determination in KF titration varies

based on several factors: the type of titrant, environmental conditions, and the required accuracy. Under stable laboratory conditions, weekly checks are usually sufficient. (GMP Insiders 2025.)

Leading reagent suppliers offer certified water standards for accurate titer determination (Bruttel 2006, 13). While water itself can be used, it requires expertise in handling very small volumes and can be initially challenging. Therefore, using certified standards is recommended. With certified standards, the added amount can be precisely determined by back-weighing, eliminating the need for volume dispensing. Solid standards, like di-sodium tartrate dihydrate, are also an option, but complete dissolution must be ensured. The recommended procedure for using the calibration standards involves rinsing the syringe, filling it without air bubbles, taring the syringe, injecting a portion into the KF solution, either submerged or with the last drop drawn back, and then back-weighing the syringe. That way the exact amount added can be determined, and it is used by the KF titrator to calculate the titer value. (Bruttel 2006, 13.) Volumetric titrants can degrade over time, necessitating frequent standardization through titer determination. Regular titer checks ensure the titrant solution's concentration remains stable. (Bruttel 2006, 12.)

The titer value is calculated with the weight of the titrated water and consumption of the titrant reagent. The formula goes as follows (Kossakowska 2016, 13.):

$$\text{Titer (mg/ml)} = \frac{\text{Weight of titrated water (mg)}}{\text{Consumption of reagent (ml)}} \quad (5)$$

2.5 Advantages, limitations and comparison of the volumetric and coulometric titration methods

KF titration offers several advantages, including wide applicable range of water content, minimal sample preparation, and a requirement of small sample amounts. Other advantages include speed, selectivity, ease of operation, and accuracy. (Korotcenkov 2019.) It is a versatile technique, suitable for determining water in solids, liquids, and gases. Modern software enhances its capabilities by

simplifying result recording, offering diverse method options, and streamlining data archiving. Automated KF systems minimize human error by precisely controlling procedures, improving reliability and consistency of the results. (Thomas 2019.)

Careful solvent selection is essential. Some samples are unsuitable for direct KF analysis due to poor solubility or unwanted chemical reactions with the KF reagents. Key solvent properties to consider include stability, conductivity, potential for side reactions, reaction rate, and how well the sample dissolves in the reagent. (Rivera-Quintero 2024.) For instance, ketones and aldehydes can interfere by reacting with methanol to produce water, leading to inaccurate results. This problem can be solved using methanol-free reagents. (Bruttel 2006, 19.)

One limitation of the method is the optimal range of pH in the reaction media. Maintaining an optimal pH between 5 and 7, typically achieved with a suitable base, is necessary for accurate results. A pH that is too low prevents reaching the titration endpoint, while a pH that is too high can cause non-stoichiometric side reactions. (Rivera-Quintero 2024.)

Furthermore, because KF titration involves a redox reaction, any redox-active substances in the sample, such as dimethyl sulfoxide, can interfere by reacting with the iodine reagent and lead to inaccurate results. Similarly, ketones, aldehydes, boric acid, metal peroxides, silanols, and strong acids require modifications. These substances can react with methanol generating water, which can cause a false endpoint and overestimation of the water content. Compounds with tightly bound water of hydration, such as lithium or chloride, can also cause issues when present in the solvent. Additionally, carbonates, oxides, and hydroxides undergo side reactions, making them incompatible with standard KF titration. (Thomas 2019.)

KF titration, used for determining water content, employs two main techniques: coulometric and volumetric (Rivera-Quintero 2024). Coulometric titration is employed for lower water content, typically from 1 parts per million (ppm) to 5%, and volumetric titration is used for higher water content ranging from 100 ppm to 100%. (Korotcenkov 2019.)

The primary difference between coulometric and volumetric KF titration lies in how the titrant is introduced and subsequently how water is quantified. Coulometric KF titration generates its own titrant, iodine, within the titration cell using an electrochemical reaction. The amount of water is then calculated from the electrical current used to produce the iodine. This method is highly beneficial for samples with very low water content due to its better sensitivity, faster analysis, and greater efficiency, as it eliminates the need for frequent calibration or solvent changes. (Thomas 2023.) In contrast, volumetric KF titrator uses a burette to add a pre-prepared titrant solution, and the volume of this reagent consumed during the titration directly indicates the amount of water in the sample. Coulometry is particularly suited for measuring significantly lower water concentrations compared to volumetric titration. (Rivera-Quintero 2024.)

Ultimately, while both the coulometric and the volumetric KF titration serve to determine the water content, they offer distinct advantages. Conversely, volumetry provides greater versatility through varied sample introduction techniques and solvent options, making complete replacement of one method by the other uncommon. (Xylem n.d.)

3 VALIDATION THEORY

Validation of an analytical method involves a comprehensive evaluation to establish its reliability and accuracy in quantifying the intended analyte. This systematic process provides assurance and proof that the method is suitable for its intended purpose. Typically, validation is conducted on methods that have already been developed. When a method is validated, information is gained about the certainty of the measurements it provides. Utilizing validated analytical methods is a key strategy for improving the dependability of analytical findings. Validation essentially demonstrates that a specific method meets quality standards. (Mäkinen 1996, 6.)

Validation encompasses planning, experimentation, statistical analysis of results, and documentation. Laboratory tests identify critical steps in the method. Experimental data is accurately documented and analysed statistically. The findings determine if revalidation is necessary and establish procedures to ensure the method functions correctly. Validation is an ongoing process, and its effectiveness is tracked throughout the method's application. (Mäkinen 1996, 6.) The fundamental basis of validation is to determine the desired accuracy of laboratory results. Assessing certain validation parameters requires the use of replicate samples. These replicates work as a representative portion of a sample, which is analysed independently within the complete measurement process. (Eurachem 2014, 44.)

Analytical literature presents various approaches to evaluating validation parameters. The necessary validation parameters depend on the method's range and the properties of the analyte, to ensure reliable results. Systematic measurements determine if the method's performance characteristics meet the requirements for its intended use. Validation is always conducted using calibrated equipment. (Mäkinen 1996, 6-7.) The validation process generally follows a similar structure. The required accuracy of the method is established, with less stringent requirements for screening methods compared to quantitative methods. The purpose and scope of the validation should be clearly defined, including the equipment to be validated, and the parameters to be determined, such as precision, repeatability, and linearity. (Hägg 2016, 14.) Test series are then performed by

measuring these parameters using test samples, blanks, or standard reference materials, or possibly all of them (Mäkinen 1996, 7).

The evaluation process is straightforward for standard methods, where in-house validation results are compared against standard requirements. For internally developed methods, acceptance criteria must be chosen considering the application's needs, with analytical accuracy often being the most critical. Requirements set for a method must be realistic. Higher uncertainty may need to be accepted when analysing concentrations near the LOD or LOQ, when the sample matrix presents specific challenges, or when sample handling involves critical steps. (Mäkinen 1996, 64.)

Results obtained during a method validation are regularly monitored through quality control measurements. Method validity is tracked, for example, by daily measurements of synthetic control samples and by monitoring the dispersion of replicate results from actual samples. Control sample results ensure the continued validity of the initial validation outcomes. In addition, regular participation in proficiency tests is also essential, while it is potentially related to calibration verification. Revalidation is needed in situations such as when a new matrix is introduced, or when there are personnel changes. (Mäkinen 1996, 65.)

3.1 Selectivity

Selectivity describes a method's ability to specifically measure a target analyte without interference from other compounds in the sample matrix (Eurachem 2014, 19). To determine selectivity, especially when interferences are not obvious, the method's analyte measurement can be compared to independent methods. Furthermore, a method based on a different principle can be used for verification. (Eurachem 2014, 19.) These tests aim to identify systematic errors caused by background factors and ensure the method truly measures the intended analyte. Matrix components can interfere by enhancing or suppressing the analyte signal, even without producing a signal themselves, highlighting the need for systematic experiments and statistical analysis. Ultimately, validation results help

users judge the method's suitability for a specific analysis. (Mäkinen 1996, 13-15.)

To evaluate the method's selectivity, its results from a confirmatory technique can be compared to those of the candidate method. A statistical two-tailed t-test is used to analyse the means of the datasets. If the calculated t-value is less than the critical t-value, at a 95% confidence level for a two-tailed test, the candidate method is deemed selective for the analyte. (Mäkinen 1996, 13)

A t-test is a key statistical tool used to compare the means of two groups, helping researchers determine if observed differences are statistically significant or likely due to random chance. It exists in three main forms, including independent samples, paired samples and one sample. Each t-value is linked to a p-value, which indicates the probability of obtaining the results by chance; a common threshold for this probability is 0.05, meaning there is a 5% chance of the observed difference occurring randomly. Lower p-values suggest greater significance. The general procedure involves starting with a null hypothesis, which assumes there is no difference or effect between the groups being compared, then stating an alternative hypothesis, setting a significance level (often 0.05), calculating the t-statistic, and comparing it to a critical value to decide about whether to reject the null hypothesis. (Statistics How To n.d.)

3.2 Trueness

The term "measurement accuracy" should not be used interchangeably with "trueness", nor should "measurement precision" be used to describe "accuracy", although accuracy encompasses both. Accuracy is defined as trueness plus precision. Accuracy is a qualitative concept that is quantitatively expressed as the combination of systematic error (bias) and random error (repeatability). (Hägg 2016, 31.) Trueness describes how close the result is to the true value and the extent of systematic error. As trueness and precision are interconnected, they are often derived from the same data set. Limits are set according to factors like instrument limitations. (Mäkinen 1996, 27.)

For analytical results to be considered reliable and truthful, they must exhibit minimal systematic error and the smallest possible random error. Determining trueness requires knowing the sample's true value, often through certified reference materials. Relative bias is quantified as the percentage difference between the measured value and the true or expected value, using the following formula (Mäkinen 1996, 33.):

$$\text{Relative bias } \% = \frac{100 * (X - \mu)}{\mu} \quad (6)$$

where μ represents the true value and X represents the measured value. Furthermore, accuracy can be evaluated by comparing results with alternative methods and through participation in interlaboratory comparisons. It is important to note that random errors also contribute to the overall accuracy of the results. (Mäkinen 1996, 33-34.)

3.3 Precision

Precision describes the consistency of results when a measurement is repeated multiple times. It is a general term for the dispersion of results, typically evaluated using standard deviation. In practice, two measures of precision are needed: repeatability and reproducibility. When discussing precision, the specific type should be indicated. Repeatability refers to the agreement of measurement results when measurements are conducted in short intervals, using the same method, by the same or different analyst, with the same or different equipment, within the same laboratory. (Mäkinen 1996, 40.)

Reproducibility, on the other hand, refers to the agreement of measurement results when individual measurements are performed using the same or different method, with different equipment, in different laboratories, by different analysts, over time intervals that are long compared to the duration of a single measurement. Reproducibility variation is generally greater than repeatability variation. Reproducibility is primarily used in the context of interlaboratory comparison studies, where results from multiple laboratories are compared. (Mäkinen 1996, 40.)

Repeatability often depends on the concentration of the analyte and should be determined at several different concentrations whenever possible. If repeatability measurements for a given method are performed in a single batch, the repeatability of the results is usually better than if the measurements are taken over a longer period. Generally, testing the repeatability of standards alone is insufficient, as the dispersion in samples may differ from that in standards. (Mäkinen 1996, 41-42.)

Repeatability can be assessed by performing multiple replicate analyses on various sample types across different concentration ranges. These samples can include actual samples, internal laboratory controls, reference materials, or recovery samples. From these analyses, the average concentration (mean), standard deviation (s), and relative standard deviation (RSD) are calculated. (Hägg 2016, 32.)

RSD is calculated as follows:

$$\mathrm{RSD} = \frac{s*100\%}{x} \tag{7}$$

Where s is the standard deviation of the group data, and x is the mean of the group data. (Six Sigma 2016.)

Based on the standard deviation (s), a ‘precision limit’ is also a useful calculation. This limit helps analysts determine if the difference between duplicate analyses of a sample, performed under specific conditions, is statistically significant at a chosen confidence level. The repeatability limit (r) is calculated as follows (Eurachem 2014, 36.):

$$r = 2.8 * s \tag{8}$$

For a relatively large number of degrees of freedom, the t-factor is approximately 2 at the 95% confidence level. This allows for a common approximation of the repeatability limit. (Eurachem 2014, 36.)

3.4 LOD & LOQ

Definitions and evaluation methods for the limit of detection (LOD) and limit of quantification (LOQ) vary in literature. LOD is the lowest concentration at which the presence of an analyte can be reliably detected and is significantly different from a blank. LOQ is the lowest concentration that can be determined with acceptable accuracy and precision, statistically distinguishable from zero with a certain probability. LOQ should be verified using an appropriate standard or sample. Quantitatively, LOD and LOQ can be estimated by calculating the standard deviation (s) of replicate measurements of a blank or a very low-level sample. Ideally, a matrix-matched sample should be used, but a blank is often chosen if a suitable matrix is unavailable. If the blank yields no signal, a standard near the LOQ can be used for LOQ estimation, with caution to avoid overly optimistic values. Both LOD and LOQ are generally assessed at a 95% probability level. (Mäkinen 1996, 29-30.)

LOD and LOQ are usually calculated as follows:

$$\mathrm{LOD} = \mu_B + 3 * s \tag{9}$$

$$\mathrm{LOQ} = \mu_B + 10 * s \tag{10}$$

Where:
μ_B = mean of the results
s = standard deviation of the results
(Mäkinen 1996, 30.)

4 SAMPLES

4.1 Sample matrix

Vegetable oils and animal fats are primarily composed of triglycerides, with varying fatty acid profiles that influence their physical state and chemical behavior (Patterson 2009, 1). Their non-polar nature and the presence of minor components like free fatty acids, unsaturated fatty acids, and potential oxidation products can cause issues in the KF titration (Merck n.d.).

Both vegetable oils and animal fats are classified as non-petroleum oils by the EPA. They share properties with petroleum oils, like water insolubility and emulsion formation. (EPA 2025.) Their viscosity and density vary based on the specific oil or fat and their fatty acid profile (Tulcan 2008, 3). Vegetable oils are liquid at room temperature due to their higher proportion of unsaturated fatty acids, while animal fats are typically solid, containing more saturated fatty acids (Kansas State University n.d.). Fatty acids vary in chain length and saturation. Besides triglycerides, they contain minor components like mono- and diglycerides, free fatty acids, phosphatides, sterols, tocopherols, and carotenoids with free fatty acid concentration being a key quality factor. (Dunford 2016.) The level of fatty acid saturation influences their chemical behavior, with vegetable oils being more prone to oxidation. Polyunsaturated fatty acids can also potentially react with the KF reagent's iodine. (Kansas State University n.d.)

4.2 Potential challenges with the sample matrix

Volumetric KF titration of vegetable oils and animal fats faces challenges primarily due to their properties (Larsson 2008, 1). Limited solubility in polar solvents like methanol, commonly used in KF reagents, hinders efficient water release and reaction, leading to inaccurate results. Side reactions between KF reagents and unsaturated fatty acids, free fatty acids, or antioxidants present in these matrices can also lead to errors in the water content results. (Merck n.d.) Endpoint detection can be problematic due to the inherent color and potential turbidity of the

samples, making visual detection difficult. High viscosity can also slow down iodine diffusion, affecting potentiometric detection, and low electrical conductivity can impede the detection system's functioning. (Grünke 2003.) Oxidized oils may contain aldehydes and ketones that react with methanol, releasing water and causing falsely elevated results (Metrohm n.d.).

The oven method aims to address solubility and direct interference issues by separating the sample matrix from the KF reagent. However, matrix-related challenges still exist. (Metrohm n.d.) Thermal stability is a primary concern; the oven temperature must be optimized to release water without decomposing the sample (Staub-Jubb n.d.). Overheating can lead to breakdown of triglycerides, or oxidation, affecting water content measurement (Dunford 2016). Efficiency of water vapor transfer from the oven to the titration cell is crucial (Stern n.d.). Insufficient carrier gas flow or leaks can cause underestimation of water content (Noria Corporation n.d.). Carryover from previous samples can also affect accuracy, necessitating proper cleaning procedures (Stern n.d.). While the oven method minimizes direct matrix interference, volatile co-evaporated components may still reach the titration cell and interfere with the KF reaction (Dunford 2016). Finally, the selection of the KF reagent remains important, as specialized reagents might be needed to minimize side reactions (Rivera-Quintero 2024).

5 SAFETY, LIMS AND NMS

Strict adherence to safety protocols is paramount and using appropriate personal protective equipment (PPE) in a laboratory is mandatory. In addition to standard laboratory PPE, such as gloves, eye protection, and a laboratory coat, specific supplementary equipment, addressed in the next paragraph, and cleaning solutions are required for this procedure. Furthermore, adherence to general laboratory safety guidelines and established best practices, such as working in a fume hood, is required. Prior to commencing work, a thorough understanding of potential hazards is compulsory. (Trapotsis 2022.)

The KF reagents contain hazardous components, including methanol, sulfur dioxide, and organic bases (Rivera-Quintero 2024). When exchanging titration chemical bottles, it is imperative to wear additional Viton gloves (Honeywell 2022, 10). Liquid standard reference materials are also toxic, and caution must be exercised during both handling and disposal (Honeywell 2023, 2). Waste generated from these chemicals must be managed through a designated waste disposal company, and in the meanwhile, the chemicals must be stored in a specified waste cabinet (Honeywell 2018, 11). All toxic reagent containers must be labeled according to relevant regulations (Trapotsis 2022).

Some of the chosen test samples are classified as biosafety hazard category 1, a factor that necessitates specific considerations during processing, like appropriate cleaning. Category 1 samples are potentially contaminated with low-risk pathogens. While the infection risk is minimal, precautions are required to mitigate any potential exposure. (Trapotsis 2022.) In the event of a sample spillage, liquid disinfectant must be used for decontamination (Stanford University n.d.).

The Laboratory Information Management System (LIMS) is a software platform used for the comprehensive management of laboratory samples, analyses, and processes. Within LIMS, laboratory equipment can be integrated into the system, and associated analytical methods can be defined for each piece of equipment. (Mäkelä 2022.) Quality control is maintained through Statistical Process Control (SPC) charts, which are linked to each method and define acceptable control

limits based on validation data. SPC control charts serve to monitor process performance, enabling the identification of deviations from normal operating parameters that may necessitate corrective actions. (Six Sigma 2024.)

NMS is an internal site at Neste Oyj. There is a wide range of guidance, from validation procedure to equipment and method instructions. NMS guidelines are usually methodological guidelines applied to the needs of the laboratory where the work is performed. A method instruction is usually written under the following headings: Purpose of the method, principle, responsibilities, equipment, reagents and safety issues, preparations, performance check, analysis and calibration processes, calculations, repeatability and reproducibility of the method. In this work, NMS is used for validation guidance, and it is also used for the method instruction addition.

6 VALIDATION PERFORMANCE

6.1 Validation details

The method was validated according to internal guides of Neste Oyj, found in NMS. Validation was performed in the context of method modification and parameters included selectivity, trueness, precision, LOD, and LOQ. Range was also a parameter in the guideline, but it was agreed not to perform it at this time, due to time limits.

The method validation was carried out in around a week and the measurements were done on two separate days. Selectivity was determined on day one and precision on day two. The last run was left overnight, and the results were checked first thing in the morning on both days. After the validation measurements were done, the results were gathered in an excel sheet and analysed. Precision results were used for the RSD calculations (Equation 7) as well as LOD & LOQ calculations (Equation 9-10). Selectivity results were used for the selectivity t-test and bias calculations (Equation 6).

For the selectivity test, 10 replicates of 1% standard reference sample, and 10 replicates of a test sample were measured with the volumetric and the coulometric KF titrators with the oven method. For the precision test, 6 replicates of 0.01%, 0.1%, 1% and 5% standard reference samples, and 6 replicates of 3 different test samples with different water contents were measured with the volumetric KF titrator with the oven method.

All samples were analysed as reference samples after the samples were distributed in smaller sample bottles. Several different samples were taken from the smaller test sample bottles and the original sample bottles were not analysed more than once.

6.2 Setup for samples and the equipment

The procedure started with sample preparations. The test samples were melted in an oven (60 – 80 °C, depending on the sample). After the melting, they were weighed into separate vials. Sample amounts with the test samples were 0.25 g, with liquid standard reference samples 1 ml and with solid samples 0.8 g. Sample amounts were taken directly from the coulometric KF titrator procedure. Sample homogeneity was ensured by vigorous mixing at the beginning and between the weighings.

After the weighing, the samples were put into an automatic sample rack, which is a part of the oven. The rack included a conditioning bottle and a methanol rinse bottle. The nitrogen tap was switched on and the device's software program was filled with the sample information. The information needed for the program included sample names, their exact weights, and the running order of the samples. The program was run on the computer, and the titrator ran the samples automatically. The run was started after the drift had stabilized at 5 µl/min. The titrator calculated the results based on the titration consumption.

Standard reference samples and test samples were measured in different temperatures. Liquid standard reference samples were measured in 120 °C and solid standard reference samples in 160 °C. All test samples were measured in 140 °C.

The coulometric side of the KF titrator worked similarly to the volumetric side but it needed additional 3 empty vials. Empty vials were used as blanks, and they were changed in every determination. First, the determinations were measured with the volumetric side. After the measurements were done, the determinations were changed to the coulometric side. A gas tube was moved between the titration cells after the first measurements.

A titer determination was performed before the validation determinations. It was performed with 3 ion-exchanged water samples. Each water sample was weighed and injected directly into the titration cell. The sample amount was

around 1 ml, and it was weighed with a syringe. The sample mass in grams was added to the device's software program.

6.3 Instrument and measurement parameters

Instrument and measurement parameters are shown in table 1. The table covers sample processor, burette information, vial and sample size, oven temperature, carrier gas, cell solution, titrating reagent, software, test time, rinsing reagent, sample information and replicates.

TABLE 1. Volumetric KF titrator specifications

Sample processor	Metrohm 874 oven sample processor, OMNIS titrator, OMNIS solvent module
Electronic burette	Cylinder unit 10 ml
Vial and sample size	6 mL and 0.08 g/0.25 g/1 ml
Oven temperature	160°C/140°C/120°C
Carrier gas	N2 at 50 ml/min
Cell solution	Hydranal Medium K
Titrating reagent	Hydranal Composite 5K
Software	OMNIS software
Test time	300 s
Replicates per sample	10 in selectivity 6 in precision
Different samples in total	4 in selectivity 7 in precision
Rinse bottle	Methanol

6.4 Samples and chemicals for titration

The selected test samples for this work were all oil or fat samples. All the used test samples fall within the scope of renewable raw materials. The test samples were selected based on their water content, and it was desired that the samples would differ from each other. Each different type of test sample contained around 1% or more water. Samples were selected in collaboration with several researchers from available samples.

The following chemicals (table 2) were used in the validation process. Additionally, a molecular sieve was used to prevent moisture contamination and to maintain the stability of the titrant.

TABLE 2. Chemicals used in the volumetric KF titration

KF reagent and medium	**Standard reference materials**	**Additional materials**
Hydranal – Composite 5 K (Honeywell, Germany)	Standard Lactose 5% H_2O (VWR, Germany)	Molecular sieve (Metrohm AG, Schweiz)
Hydranal – Medium K (Honeywell, Germany)	Water standard oven 1% Aquastar (Sigma-Aldrich Pte Ltd, Singapore)	
	Hydranal Water Standard 1.0 (Honeywell, Germany)	
	Hydranal Water Standard 0.1 (Honeywell, Germany)	

6.5 Example calculations

Water content results from the KF titrator were calculated automatically by the software. Below is an example of how the software calculated the result (Equation 4). The example calculation was obtained from the precision test, more specifically from test sample 3, result 1:

$$\frac{0.0405485366814157\ \text{ml} * 5.20227401600346\ \frac{\text{mg}}{\text{ml}} * 0.1}{0.2619\ \text{g}} = 0.0805439476\%$$

All validation results were calculated with excel. Example calculation for the relative bias (b-%) (Equation 6) goes as follows:

$$\frac{1.0333 - 0.99}{0.99} * 100\% = 4.373737374$$

It was calculated with 1% standard reference sample. The measured mean value for the standard reference sample was 1.0333 wt-% and the real value according to the package was 0.99 wt-%.

The relative standard deviations were calculated with the means (=AVERAGE) and the standard deviations (=STDEV.S). Mean and standard deviation were first calculated with excel for each sample. Then, for the 1% standard reference sample, the RSD (Equation 7) was calculated as follows:

$$\frac{0.031677542}{1.041666667} * 100\% = 3.041044031$$

Precision limit (r) (Equation 8) for 1% standard reference sample was calculated as follows:

$$2.8 * 0.031677542 = 0.088697118$$

LOD and LOQ (Equation 9-10) for 0.01% standard reference sample was calculated as follows:

$$0.017 + 3 * 0.00275681 = 0.025270429$$

$$0.017 + 10 * 0.00275681 = 0.044568098$$

Selectivity's t-test was calculated in excel with a two-tailed, unpaired t-test (=T.TEST) comparing volumetric and coulometric KF titrators test results for one test sample.

7 VALIDATION RESULTS

7.1 Titer determination

The KF titration software automatically calculated the titer value, and the calculated value was 5.20 mg/ml. The titer measurements were performed before the validation measurements and all the results of the validation measurements were calculated with the same titer value. The titer determination values are shown in table 3.

TABLE 3. Titer determination values

Ion-exchanged H_2O sample	Titer value in mg/ml
1	5.1312
2	5.2473
3	5.2283
Mean:	5.202267
Rounded result:	5.20 mg/ml

7.2 Precision

The calculated RSD values for test samples were 2.82% (test sample 1), 2.12% (test sample 2), 35.44% (test sample 3). For the standard reference samples 16.22% (0.01% standard), 7.05% (0.1% standard), 3.04% (1% standard) and 2.31% (5% standard).

The precision results were calculated with excel. First the mean and the standard deviation (s) were calculated from the precision data, then their results were used to calculate the relative standard deviation (RSD). RSD was calculated with test samples 1-3 and standard reference samples 0.01%, 0.1%, 1% and 5%. Additionally, the precision limit (r) was calculated. The precision data from the tables 4-10 were obtained from replicate analyses of test samples and standard reference samples. The combined results (mean, standard deviation, precision limit and relative standard deviation) for each sample are shown in table 11 and 12.

TABLE 4. Precision data for test sample 1

Test sample 1	Water content in wt-%
1	1.188
2	1.190
3	1.211
4	1.190
5	1.117
6	1.200

TABLE 5. Precision data for test sample 2

Test sample 2	Water content in wt-%
1	2.478
2	2.460
3	2.352
4	2.364
5	2.439
6	2.412

TABLE 6. Precision data for test sample 3

Test sample 3	Water content in wt-%
1	0.081
2	0.063
3	0.053
4	0.044
5	0.051
6	0.025

TABLE 7. Precision data for 0.01% standard reference sample

0.01% Standard	Water content in wt-%
1	0.013
2	0.020
3	0.017
4	0.020
5	0.015
6	0.017

TABLE 8. Precision data for 0.1% standard reference sample

0.1% Standard	Water content in wt-%
1	0.085
2	0.073
3	0.086
4	0.085
5	0.088
6	0.090

TABLE 9. Precision data for 1% standard reference sample

1% Standard	Water content in wt-%
1	1.053
2	1.016
3	1.048
4	1.001
5	1.092
6	1.040

TABLE 10. Precision data for 5% standard reference sample

5% Standard	Water content in wt-%
1	4.776
2	4.704
3	4.583
4	4.902
5	4.832
6	4.746

TABLE 11. Combined results for test samples

Results	Test sample 1	Test sample 2	Test sample 3
Mean:	1.182666667	2.4175	0.052833333
Standard deviation (s):	0.033320664	0.051208398	0.018723426
Precision limit (r):	0.09329786	0.143383514	0.052425592
Relative standard devitation (RSD):	2.81741806	2.118237756	35.43866047
Rounded result (RSD):	2.82%	2.12%	35.44%

TABLE 12. Combined results for standard reference samples

Results	0.01% standard	0.1% standard	1% standard	5% standard
Mean:	0.017	0.0845	1.041666667	4.757166667
Standard deviation (s):	0.00275681	0.005958188	0.031677542	0.109742274
Precision limit (r):	0.007719067	0.016682925	0.088697118	0.307278367
Relative standard devitation (RSD):	16.21652794	7.051109638	3.041044031	2.306883099
Rounded result (RSD):	16.22%	7.05%	3.04%	2.31%

7.3 Selectivity

A statistical two tailed t-test was performed in excel to compare the volumetric and coulometric titration results on test sample 4. The calculated p-value was 0.00000023. Water content results from test sample 4 in wt-% and the calculated p-value are shown in table 13.

TABLE 13. Selectivity data and results for test sample 4

Test sample 4	Volumetric titrator (water content in wt-%)	Coulometric titrator (water content in wt-%)
1	0.960	0.8164
2	0.919	0.8047
3	0.897	0.8206
4	0.963	0.8109
5	0.937	0.7638
6	0.895	0.7007
7	0.828	0.7554
8	0.898	0.7444
9	0.935	0.7325
10	0.917	0.7334
Mean:	0.9149	0.76828
p-value:		2.31592E-07
Rounded result (p-value):		0.00000023

7.4 Trueness

For trueness calculations, the water content results were taken from the selectivity test data for 1% standard reference sample. Relative bias (b-%) for the 1% standard reference sample was 4.37%. Bias was calculated with the mean value of the water content results and the real value of the standard reference sample. Relative bias, absolute deviation and relative recovery results are shown in table 14.

Table 14 also shows the result from the bias calculation from volumetric versus coulometric KF titrators water content results with 1% standard reference sample. It was calculated with both means of the water content results. Relative bias for the comparison of the two methods was 2.45%.

TABLE 14. Trueness data and results for 1% standard reference sample

1% standard	Coulometric titrator (water content in wt-%)	Volumetric titrator (water content in wt-%)
1	1.0208	1.173
2	1.0102	1.019
3	1.0156	1.057
4	1.0075	1.047
5	1.0172	1.049
6	1.0157	1.072
7	1.0003	0.962
8	0.9942	0.843
9	1.0005	1.046
10	1.0035	1.065
Mean:	1.00855	1.0333
Relative bias (b-%):		4.373737374
Absolute deviation (b):		0.0433
Relative recovery (R-%):		4.33
Rounded result (b-%):		4.37%
Volumetric vs coulometric, relative bias with 1% standard		2.454018145
Rounded result (b-%):		2.45%

7.5 LOD & LOQ

LOD was calculated to be 0.025% and LOQ 0.045%. LOD and LOQ were calculated with excel from precision test results with 0.01% standard reference sample. Results were calculated using mean and standard deviation. The results are shown in table 15.

TABLE 15. LOD & LOQ results for 0.01% standard reference sample

LOD & LOQ from 0.01% standard	Result
LOD	0.025270429
LOQ	0.044568098
Rounded result LOD:	0.025%
Rounded result LOQ:	0.045%

8 VALIDATION TEST RESULT ANALYSIS

It is essential that the requirements established for an analytical method are achievable. Higher levels of uncertainty may be unavoidable when dealing with concentrations close to the method's limit of detection or quantification, when the sample matrix poses difficulties, or when critical stages are involved in sample preparation. To illustrate, the relative standard deviation between laboratories can rise from 5% to 50% as concentrations decrease from 100 µg/l to 5 µg/l. (Mäkinen 1996, 64.)

Due to the lack of a specific standard for the volumetric KF titration with the oven method, establishing direct validation result limits was not straightforward. While ASTM E203 (Standard Test Method for Water Using Volumetric Karl Fischer Titration) can serve as a guideline for these limits, it cannot be applied rigidly.

The selectivity tests involved comparing the results obtained from the candidate method, volumetric KF titration, with those from a confirmatory technique, the coulometric KF titrator. The means of the data sets from the confirmatory technique and the candidate method were investigated by a statistical two tailed t-test. A hypothesis was set that the devices would not differ from each other more than 0.05. The p-value was calculated with test sample 4 and with 1% standard reference sample. It was noticed that with the test sample, the results were clearly selective, but with the standard reference sample they were not. It was concluded that the student's t-test is unsuitable for the standard reference sample in this context. However, it yields accurate results with the test sample, as the outcome was significantly below the target.

The precision tests involved measuring numerous different test samples and standard reference samples. According to the standard (ASTM E203), the acceptance criteria for RSD is < 5%. Test samples 1 and 2, and standard reference samples 1% and 5%, passed the acceptance criteria. However, 0.01% and 0.1% standard reference samples did not pass the acceptance limit, most likely because their water content was below the reliable limit that the manufacturer has given for the volumetric KF titrators range. It is also possible that a larger sample amount should have been used for a reliable measurement. Test sample 3, which

had the least amount of water, was also not within acceptable limits. This is probably due to the same reason as in the lower standard reference samples. The lower standard reference samples and test sample 3 were mainly measured for the LOD and LOQ determination, because it was known that the volumetric KF titrator cannot measure such low water content reliably. However, it was beneficial to know that the other test samples and the higher standard reference samples gave better results. Because of the results, it is now known that the repeatability for the method is working reliably with higher water content. Reproducibility testing, even though it would have been useful, was not performed due to time constraints as it would have required the involvement of additional laboratory technicians.

The trueness of the method was evaluated using the data from the 1% standard reference sample that was used in the selectivity testing. According to Neste Oyj's internal procedure, an acceptable method bias result is typically ±20% from the reference value. The certified value for the 1% standard reference sample is 0.99% ± 0.02%. The calculated bias for this standard reference sample fell within the acceptable ±20% range, thus demonstrating acceptable trueness. It would be more reliable to assess the trueness at various concentrations within the working range, but the bias calculations using the 5% standard reference sample were deemed unusable due to unexpectedly low results, likely caused by insufficient sample dosing. Similarly, the 0.01% and 0.1% standard reference samples did not provide reliable bias results, probably due to their very low water content. However, the bias determined by comparing the volumetric and coulometric KF titrators using the 1% standard reference sample was satisfactory, remaining well below the established limit.

LOD and LOQ were determined using the 0.01% standard reference sample. The calculations were also explored with test sample 3, which had the lowest water content, but the higher RSD observed for test sample 3 likely led to a slightly higher LOD and LOQ estimates, compared to the 0.01% standard reference sample. The lack of readily available information on expected LOD and LOQ values presented a considerable challenge in choosing an appropriate blank for the measurements. However, Metrohm's Monograph suggested that a potential lower determination would be in the range of 50-100 ppm under ideal conditions

(Bruttel 2006, 8). Considering that the experimental setup was most likely not fully optimized, the resulting LOD of around 250 ppm was deemed adequate. For the analysis, the lowest available standard reference sample and a test sample with very low water content were used for the LOD and LOQ testing, with the former yielding more dependable results.

9 CONCLUSIONS AND DISCUSSION

9.1 Progress of the work

The aim of this work was to get a new volumetric KF titrator up and running and validated, including the practicalities, which consisted of making the internal instructions and adding the method to LIMS. There was also a lot of preliminary work that was done, including chemical risk assessments and ordering of the chemicals, examining which samples are the most suitable for the validation, making of the validation plan and protocol, and after the actual validation, recording and documenting the results. The purpose of this work was to develop a validation plan and based on it, perform several different validation parameter tests with the titrator. Both the aim and the purpose were completed successfully, as far as the time allowed. The addition of the volumetric side to the whole titration system diversified the range of reliable results on the higher water content range.

Due to lack of time some parts of the validation were left out. Consultation with one of Neste Oyj's engineers led to the recommendation to still assess linearity for confidence in the working range. Additionally, it was advised to conduct temperature profile testing on the titrator to enable the determination of the most suitable temperatures for different sample matrices. According to the manufacturer, the optimum oven temperature for every sample matrix can be determined with a temperature gradient (Bruttel 2006, 16).

A verification of the direct injection was also left out due to time limitation. However, there were already some test runs during the installation, which can act as a verification from the manufacturer. Direct injection method does not need a full validation process, since the method is the same as in the standard ASTM E203, but a verification is still recommended (Hägg 2016, 11).

9.2 Possible improvements

Overall, the validation process was quite successful although the results left some room for improvement in the implementation of the measures. Further clarifications include reproducibility tests for the whole laboratory team to ensure that the method is reliable regardless of the technician. The working range should be established using linearity testing to determine the upper limit of reliable water content. The equipment manufacturer mentions that with higher water contents (>40%), the sample should be diluted with an inert dry solvent, like methanol (Bruttel 2006, 22). Defining the dilution limit could be useful, even though it is likely not a current requirement in this laboratory due to typically low water levels. It would still be a good parameter to have on record for potential future needs.

There is also room for improvement in the process. Installing a scale in the fume hood would significantly improve testing efficiency. Currently, the scale's location on the opposite side of the laboratory adds time to the process. Furthermore, this setup presents safety risks, as rushing to the scale could lead to accidental falls or needle punctures. Placing a dedicated scale in the fume hood would enhance result reliability by minimizing the risk of the scale being inadvertently used by others during measurements. While clearly marking the current scale during measurements could mitigate this latter risk, a dedicated fume hood scale addresses both efficiency and safety more directly. If a new scale is acquired for this purpose, it is worth mentioning that with a connected balance, the sample weight can be directly input into the titration system (Bruttel 2006, 12). That would reduce the risk of human error and would speed up the process even further.

The calibration requirements should also be evaluated, specifically how frequently it is needed and whether titer determination with ion-exchanged water alone is sufficient, or if calibration standards should also be used for the calibration. The coulometric titrators calibration, performed monthly with calibration standards, raises the question of whether the volumetric side also necessitates this level of calibration. When results differ significantly, calibration alone is not enough. In such cases, the instrument solutions and dried components must be replaced (Rivera-Quintero 2024).

Additionally, it was observed that the coulometric side of the titrator provided better results with the 5% lactose standard reference sample. Consequently, an examination of the optimal sample size and oven temperature for this and also for other standard reference samples would be recommended to optimize the conditions for maximum result reliability.

9.3 Sources of errors

The validation process was affected by some device errors. Notably, a gas flow error was encountered during precision testing, which was addressed by replacing the conditioning bottle and cleaning the oven injection needle. The extent to which this error influenced the validation results remains unknown, as a repeat validation was impossible due to time constraints. Additionally, other factors such as human error, other possible device malfunctions, and contaminations could have introduced errors into the process. Atmospheric humidity, solubility, pH, and sample handling are among the most common sources or errors in KF titration (Rivera-Quintero 2024).

Furthermore, the validation process itself might have contained other errors. The constantly evolving and diverse nature of validation requirements (Rambla-Alegre 2012) made it challenging to determine the most accurate approach. The lack of a ready standard for volumetric KF titration with the oven method meant clear guidance on its execution was unavailable.

The requirements and design of an analytical procedure fundamentally determine its suitability, and therefore, the validation process must be specifically tailored. This requires identifying key performance parameters, defining suitable acceptance criteria, and appropriately designing validation studies. Analysts must understand the core meaning, calculations, and tests associated with these parameters in the context of their specific application. A lack of knowledge or a misunderstanding of concepts, like efficiency, will lead to validation results that do not accurately represent the procedure’s performance. (Rambla-Alegre 2012.)

Errors could also have arisen from the instrument or how the samples were handled. For instance, the titrant's stability changes over time, sample matrix effects might have been present, and issues with temperature, sample size, homogeneity, unstable drift, or air bubbles in the system could have been possible. (Rivera-Quintero 2024.)

9.4 Challenges in the process and future views

The challenging nature of these samples could have impacted the reliability of the results. Specifically, the rapid solidification of the oil samples made precise single-step dosing into vials rather difficult, especially with multiple samples. Maintaining the sample in a liquid state is critical for proper homogenization, necessitating melting the samples in an oven (Bruttel 2006, 24). However, oven heating can lead to water evaporation and potentially affect the results. Furthermore, repeated handling of the samples, including melting and cooling of the sample several times, can cause a decrease in their water content over time. This potential reduction in the water content due to sample handling should be considered when interpreting future water content results.

To ensure accurate water content determination in oil samples, sampling must be conducted before any preparation steps. Quick sampling is the best practice to minimize changes in water content during transport and storage, avoiding both contamination with additional water and loss due to excessive heat. (Bruttel 2006, 22.) For instance, water in crude oil is not evenly distributed, so thorough homogenization using methods like ultrasonic baths or high-frequency mixers is necessary before analysis, particularly for samples prone to poor homogenization (Bruttel 2006, 48).

Future research could possibly explore the challenges of maintaining consistent water content during the sample pretreatment. A key issue is that adding water to oil can exceed its solubility limit. While rapid mixing is needed to disperse the water, this process also risks significant water evaporation. Further investigation into optimizing this balance would be beneficial.

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