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Review

Insights into Analytical Precision: Understanding the Factors Influencing Accurate Vitamin A Determination in Various Samples

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Abstract: The accurate assessment of vitamin A in animal feed and tissues is vital for safeguarding animal well-being and ensuring high-quality nutritional feed. However, challenges in achieving precise results persist, necessitating a comprehensive understanding of the influencing factors. This review delves into the historical progression of analytical techniques, from colorimetric assays and spectrophotometry to advanced chromatographic methods and non-destructive spectroscopic approaches. Factors influencing analytical precision are scrutinized, encompassing sample preparation, storage conditions, interfering substances, and human errors. The crucial role of quality control and standardized protocols in ensuring the reproducibility and reliability of results is emphasized. Moreover, this review highlights the need for tailored analytical approaches to account for the complexities of sample matrices and the significance of cutting-edge technologies, including on-site analysis and data science integration, in enhancing analytical precision. By acknowledging the challenges and prospects in vitamin A analysis, this paper provides insights for optimizing analytical methodologies and promoting animal welfare and product safety.

Keywords: vitamin A; analysis; determination; premix; feed; animal

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1. Introduction

Ensuring that animals receive a well-balanced diet and appropriate feed formulation can significantly enhance animal productivity, improve the quality of their products, and promote better animal welfare [1]. Vitamin A, also known as retinol, is a vital micronutrient crucial for maintaining optimal health and well-being in livestock [2,3]. It plays a central role in various physiological functions, including immune response regulation, vision maintenance, and cellular differentiation [4]. Consequently, the accurate determination of vitamin A levels in animal feed and premixes is essential for ensuring animal health and production. Beyond preventing deficiencies and associated health issues, it forms the foundation for their overall growth and performance [5–7].

In the realm of animal nutrition, ensuring the precise measurement of vitamin A content is of utmost importance. It is not merely a matter of scientific accuracy, but a critical factor in guaranteeing the safety and quality of the end products consumed by livestock [8]. With the increasing emphasis on food safety standards, the precise determination of vitamin A underscores the commitment to providing nutritious and safe feed for animals, safeguarding their well-being, and subsequently ensuring the quality of animal-derived products [9]. Likewise, evaluating the retinol levels in animal tissues is crucial for assessing nutritional status, preventing illnesses, and improving the general health and productivity of livestock [10].

However, the pursuit of accurate results in vitamin A determination faces several challenges. Researchers and analysts encounter various obstacles that impede the attainment of reliable outcomes, presenting a significant predicament in the analysis of animal feed and other samples [9,11]. Challenges range from intricate sample preparation and extraction

procedures to the complexities associated with analytical methodologies and the absence of standardized regulatory guidelines [12]. Achieving precision in vitamin A determination necessitates a comprehensive understanding of these challenges and their implications.

Therefore, this review paper aims to explore the multifaceted challenges confronted by researchers and analysts in accurately determining vitamin A in premixes, feed, and biological tissues. The focus of the synthesis will be specifically on the following key aspects:

1. Historical progress in vitamin A analysis techniques.
2. Quantitative vitamin A analysis: diverse analytical approaches.
3. Deciphering the factors: unveiling the complexities of analytical precision.
4. The crucial role of quality control: navigating the path to reliable results.
5. Reflection and future prospects: charting the course for enhanced analytical precision.

Through comprehensive analysis of these five subjects, this review underscores the imperative need for standardized protocols and advanced scientific methodologies. These measures are crucial in guaranteeing the precision and dependability of vitamin A determination, thereby fostering the overall improvement of animal health and productivity.

2. Historical Progress in Vitamin A Analysis Techniques

Vitamin A has been a focal point of significant interest within the realm of analytical chemistry. From its initial detection endeavors to the current sophisticated methodologies, the progression of analytical methods for vitamin A determination has been an evolution marked by challenges, breakthroughs, and paradigm shifts [8,13–15]. This section offers an overview of the historical development of these techniques, from their inception to the contemporary state-of-the-art methodologies.

The journey commenced in the early 20th century, coinciding with the identification of vitamin A's chemical structure by Paul Karrer in 1932 [16] (Figure 1). Pivotal studies during this period focused on the isolation and purification of vitamin A from various natural sources, laying the foundation for subsequent analytical pursuits [17,18]. Elmer McCollum and Marguerite Davis' groundbreaking work in the early 1910s, emphasizing the vital role of vitamin A in human health, catalyzed the interest in analytical methodologies [16].

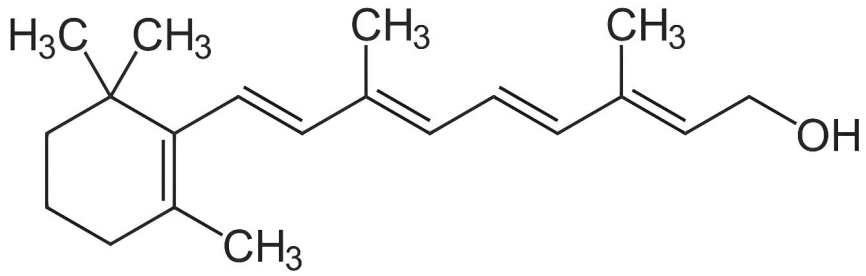

Figure 1. Structural formula for all-trans-retinol.

The development of analytical methods faced challenges, primarily due to the intricate nature of vitamin A's chemical structure and the existence of multiple isomers [19–21]. These isomers encompass various forms, such as all-trans-, 13-cis-, 11-cis-, 9-cis-, 9,13-dicis-, and 11,13-dicis-retinol [22]. Initial techniques, including colorimetric assays and spectrophotometric analyses, provided a starting point, but were restricted by issues related to specificity and sensitivity [23,24]. The interference posed by other dietary components further complicated the accurate quantification of vitamin A in complex matrices, such as food products, feed, and biological samples [25].

Overcoming these challenges required a collective effort from the scientific community. One critical hurdle was the development of robust extraction methods capable of efficiently isolating vitamin A from complex matrices, minimizing loss and cross-contamination [26]. The introduction of chromatographic techniques in the 1960s improved the precision and specificity of vitamin A determination [27]. However, the analytical landscape contin-

ued to face obstacles, particularly in quantifying trace amounts of vitamin A in diverse samples [28].

The integration of advanced spectroscopic methods, such as high-performance liquid chromatography (HPLC) and gas chromatography (GC), in the late 20th century addressed the need for heightened sensitivity [29–33]. These innovations facilitated the reliable detection and quantification of vitamin A at previously unattainable levels, ushering in a paradigm shift in the analytical approach to vitamin A analysis. Additionally, high-performance liquid chromatography has made it feasible to separate and determine the six cis–trans isomers of vitamin A, even in the form of commercially available esters like acetate, palmitate, and propionate [22].

The modern era has witnessed a renaissance in the field, characterized by the integration of mass spectrometry with chromatographic separations, providing unparalleled resolution and specificity in the determination of various vitamin A isomers [34–36]. Tandem mass spectrometry (MS/MS) and high-resolution mass spectrometry (HRMS) have further enhanced analytical capabilities, enabling the precise identification and quantification of vitamin A and its metabolites in intricate biological systems [37].

Furthermore, non-destructive analytical methods, such as nuclear magnetic resonance (NMR) spectroscopy and near-infrared spectroscopy (NIRS), have expanded the horizons of retinol analysis, allowing for the non-invasive monitoring of vitamin A dynamics within living systems as well as in premixes and feed [38,39]. These advancements have not only fostered a deeper comprehension of the physiological roles of vitamin A, but have also paved the way for tailored nutritional interventions and personalized healthcare strategies [40].

In conclusion, the journey through analytical techniques for vitamin A analysis highlights the resilience of the scientific community in unraveling the complexities of this essential micronutrient. The historical evolution, marked by challenges and refinements, has led to a sophisticated analytical landscape where modern methodologies have transcended the limitations of the past.

3. Quantitative Vitamin A Analysis: Diverse Analytical Approaches

In the field of nutritional science, the precise evaluation of vitamin A plays a pivotal role in understanding dietary sufficiency and identifying potential deficiencies. Similarly, the assessment of retinol levels in animal tissues is indispensable for gauging nutritional well-being, preventing diseases, and enhancing overall animal health and productivity. This chapter explores the intricate landscape of analytical techniques employed in the quantification of vitamin A, offering a succinct explanation of the fundamental principles that underlie these methodologies.

3.1. Colorimetric Assays

Colorimetric assays have long been employed as a dependable means of quantifying vitamin A or retinol within various samples [41]. The methodology involves introducing a chromogenic reagent into a soluble fortified food or feed sample, resulting in a reaction with retinol and the development of a distinctive color complex [42]. The intensity of the resultant color is directly proportional to the concentration of vitamin A in the sample. The fundamental principles of colorimetric assays used for measuring vitamin A are represented by the following methods:

1. The Carr and Price assay: This method involves the quantitative evaluation of retinol utilizing antimony trichloride ($SbCl_3$) as a crucial component [43].
2. The Sobel and Werbin assay: This assay employs activated 1,3-dichloro-2-propanol to react with vitamin A, as initially proposed by Sobel and Werbin [44] and later expounded upon by Blake and Moran [23].
3. Trifluoroacetic acid-based colorimetric determination: This technique relies on the interaction of a vitamin A solution in food or feed materials with several Lewis acids, resulting in the transient manifestation of a blue color [41].

Colorimetric assays offer several advantages. They are a cost-effective and straightforward alternative to complex methodologies such as high-performance liquid chromatography (HPLC) [45]. Additionally, their versatility enables the measurement of vitamin A in diverse samples, ranging from foods and feeds to biological fluids [46,47]. Nevertheless, utilizing colorimetric assays for measuring vitamin A or retinol does have its drawbacks. They tend to be less sensitive when compared to more sophisticated techniques like HPLC [48]. Moreover, the presence of interfering substances within the sample can significantly impact the accuracy of the results [49]. Lastly, the lack of specificity for vitamin A or retinol means that these assays can inadvertently identify other compounds forming stable color complexes with the reagent, leading to potential inaccuracies [50].

3.2. Spectrophotometric Analyses

Spectrophotometric analysis represents a widely utilized approach for the determination of retinol levels in food and feed samples [43,51]. This technique operates on the fundamental principle that all-trans-retinol in isopropanol exhibits maximal absorption at 325 nm [43,51]. The analytical methods for quantifying vitamin A rely on the effective dispersion of the fortified food, premix, or feed matrix to liberate vitamin A, including its various esters [42]. To facilitate this process, a UV-light irradiation system is employed, which serves to degrade the retinol. The setup for this system may include a simple configuration, such as employing a UV lamp along with a protective curtain to safeguard operators from potential exposure to light [52]. Key to the success of this approach is the meticulous determination of the optimal duration of irradiation and the precise positioning of the UV light in relation to the solutions under examination [42].

Spectrophotometric analysis for assessing retinol offers both advantages and challenges. It is lauded for its simplicity, cost-effectiveness, and high sensitivity to trace amounts of retinol [43]. Additionally, its non-destructive nature allows for sample reusability, contributing to resource conservation [45]. Extensive validation across various food and feed matrices underscores its reliability, yet the method is susceptible to yielding inaccurate results in the presence of interfering substances [43]. Ensuring accurate analysis requires meticulous sample preparation and the effective extraction of vitamin A from complex food/feed matrices [40]. The lack of specificity in differentiating retinol from other compounds with similar light absorption characteristics presents a challenge [40]. Notably, it is less suitable for the simultaneous determination of retinol and its esters, necessitating alternative analytical approaches in such cases [53].

3.3. Chromatographic Techniques

Chromatographic techniques for the analysis of vitamin A are grounded in the fundamental principle of differentiating components based on their distinct interactions with a stationary phase and a mobile phase [54]. These methodologies capitalize on the varying affinities exhibited by the different forms of vitamin A towards the stationary phase, facilitating their separation and eventual quantification [55,56]. Several types of chromatography have been employed for the purpose of vitamin A analysis.

a. High-performance liquid chromatography (HPLC)

HPLC is a widely used analytical technique that employs high-pressure pumps to propel the mobile phase, comprising the sample, through a column containing the stationary phase [57]. The methodology for determining retinol in food, premix, feed, and biological samples using HPLC commonly involves an isocratic, aqueous solvent system consisting of a mixture of methanol and water as the mobile phase, enabling the isolation of retinol from the extracted sample [58,59]. The column is a crucial element in the HPLC setup, serving as the site for the separation of sample components [60]. The fundamental principle behind HPLC separation relies on the distribution of the analyte (sample) between the mobile phase (eluent) and the stationary phase (packing material) [61]. In the case of retinol, a reverse-phase column is commonly employed as the stationary phase, facilitating the segregation of retinol from other sample constituents based on variations in their

hydrophobicity [62]. Prior to HPLC analysis, the retinol must be extracted from the sample and prepared for injection into the HPLC system. This typically involves a lipid extraction step aimed at eliminating interfering lipids and other components [63]. Subsequent to the separation of retinol by the HPLC system, identification and quantification become imperative. This task is typically accomplished using a UV detector, which gauges the absorbance of retinol at a specific wavelength [64].

b. Gas–liquid chromatography (GLC)

GLC is an established analytical technique employed for the separation and analysis of volatile compounds within a given sample [65]. Prior to the chromatographic process, the sample undergoes an extraction and purification procedure to eliminate any potentially confounding substances [65]. The utilization of a glass column, packed with meticulously chosen glass beads, is imperative to enable the effective separation of retinol from other compounds within the sample [66].

A crucial aspect of GLC revolves around the selection of an inert carrier gas that does not interact with the sample constituents. Helium, known for its inert nature, has emerged as a preferred choice for the carrier gas in GLC [67]. Furthermore, precise regulation of the column temperature is paramount to ensure optimal separation of the compounds within the sample. For instance, the introduction of elevated column temperatures and extended retention times can facilitate the generation of anhydro-retinol during gas chromatography, thereby serving as an effective assay for retinol analysis [66].

To accurately detect the effluent from the column, the implementation of an appropriate detector, such as a mass spectrometer, is fundamental in the GLC process. This detector aids in the identification and quantification of the separated compounds, thereby facilitating comprehensive analysis and interpretation [65].

c. Liquid–liquid chromatography (LLC)

LLC is a separation technique in which the stationary phase comprises a liquid supported on a solid, while the mobile phase is also a liquid. LLC amalgamates the principles of liquid–liquid extraction and chromatography [68]. Similar to chromatography, one of the phases involved remains stationary during the separation process. However, several significant distinctions differentiate LLC from traditional chromatography utilizing solid stationary phases, such as HPLC [68].

In the extraction of retinol from the sample, a suitable solvent is employed. The selection of the solvent is contingent upon the characteristics of the sample and the specific type of liquid chromatography utilized. Within this method, the compounds are segregated based on their distribution between the two immiscible liquid phases [62,69].

d. Waters UltraPerformance Convergence Chromatography (UPC)

UPC represents a modern separation technique that harnesses compressed carbon dioxide as the primary mobile phase [70]. This method capitalizes on the utilization of sub-2 µm particle chromatography columns, taking advantage of the low-viscosity properties of CO_2 and a sophisticated chromatography system. This approach distinguishes itself from conventional HPLC and notably enhances the sensitivity of the assay. Moreover, UPC demonstrates a significant reduction in solvent waste generation in comparison to traditional liquid chromatography [70].

e. Ultra-high-performance liquid chromatography–tandem triple quadrupole mass spectrometry (UHPLC-MS/MS)

UHPLC, an advanced form of HPLC known for its enhanced capabilities in terms of resolution, analysis speed, and sensitivity [71], has paved the way for more sophisticated analytical methodologies. One such prominent application is the utilization of UHPLC-MS/MS for the precise determination of vitamin A levels in biological tissues, particularly in blood samples [72]. The approach demonstrates rapidity, accuracy, and heightened sensitivity, coupled with straightforward preprocessing procedures. Mass spectrometric

analysis is carried out in the positive ion mode using the multiple reaction monitoring mode, while quantification is facilitated through the utilization of the internal standard method [72].

Generally, chromatographic techniques offer a multitude of benefits in the assessment of vitamin A, including superior sensitivity and specificity, adaptability across various sample types, and reduced sample preparation requirements [73,74]. Despite these advantages, chromatography methodologies also exhibit certain drawbacks, such as prolonged analysis time, significant financial investment, and susceptibility to matrix effects [45,73].

3.4. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a powerful analytical technique employed in the determination of retinol, leveraging the magnetic properties of hydrogen nuclei [75]. Grounded in the principle of resonance, it involves the matching of electromagnetic radiation frequencies to specific energy transitions of the nuclei, thereby facilitating the comprehensive analysis of the chemical structure and environment of retinol [76]. The phenomenon of chemical shift, denoting the displacement of the resonance frequency relative to a standard reference, plays a critical role in discerning the functional groups and molecular constituents of retinol [75]. Additionally, the integration of NMR signals allows for quantitative insights into the relative abundance of distinct types of hydrogen atoms within various retinol molecules [77].

NMR spectroscopy offers several advantages for the precise quantification of vitamin A in samples, owing to its high sensitivity, versatility, and dependable analytical capabilities [78]. However, it comes with certain drawbacks, including significant financial costs and the requirement for specialized equipment and expertise [79].

3.5. Near-Infrared Spectroscopy (NIRS)

NIRS is a non-invasive analytical technique widely used for studying the molecular structure, composition, and concentration of various substances [80]. It functions on the basis of the unique light absorption properties of different molecules at specific wavelengths, enabling the identification and quantification of compounds of interest. Near-infrared light, falling within the 800- to 2500-nanometer wavelength range, is particularly favored for its ability to penetrate biological samples effectively, making it a suitable tool for analyzing complex biological materials [81].

In practical applications, NIRS involves the transmission of near-infrared light through a sample, followed by the measurement of the absorbed or reflected light [82]. Through the analysis of absorption and reflection patterns, researchers can discern the presence and concentration of specific compounds or functional groups within the sample. Notably, NIR spectroscopy has also found utility in the examination of retinol within vitamin compositions [38].

NIRS is recognized as a non-destructive, expedient, and cost-effective technique for determining retinol in diverse samples [83,84]. Nevertheless, its sensitivity and accuracy are relatively restricted compared to standard reference methods such as HPLC, necessitating calibration with these methods [85]. It is imperative to note that NIRS might not be universally applicable across all sample types, as certain samples could potentially introduce interference during the analysis process [83].

3.6. Enzyme-Linked Immunosorbent Assays (ELISAs) for Biological Tissues

ELISA kits are used to measure the amount of a specific substance, such as vitamin A, in a sample. The kits use antibodies to detect and measure the substance, and there are different types of ELISA kits available depending on the specific needs of the experiment [86]. For example, the competitive EIA ELISA kit uses a competitive inhibition method to measure the amount of vitamin A in a sample [87,88], while the sandwich ELISA kit uses a sandwich method [89]. ELISA kits can be used to measure vitamin A in a variety

of biological tissues, including plasma, serum, tissue homogenates, cell lysates, and cell culture supernatants [90].

ELISA kits for measuring vitamin A in biological tissues offer the advantages of high sensitivity and user-friendliness for processing multiple samples simultaneously, but they can be time-consuming and may lack sensitivity for detecting low levels of vitamin A [91]. Cross-reactivity with other substances in the sample can also lead to occasional false positives.

4. Deciphering the Factors: Unveiling the Complexities of Analytical Precision

Historically, the analysis of vitamin A has been recognized for its challenging reproducibility and repeatability [8]. For instance, AAFCO [92] states that there is an accepted analytical fluctuation of 30% in the measurement of vitamin A within feed samples. This deviation is typically encountered when analyzing a sample on two separate occasions, corresponding to twice the coefficient of variation or relative standard deviation [1]. Additionally, VDLUFA [93] provides a detailed reference, highlighting the allowable range for different concentrations of vitamin A within a sample (Table 1). Despite this acknowledged permissible fluctuation, the precise measurement of vitamin A in biological tissues, feed, and premixes remains essential for upholding the optimal nourishment and overall health of livestock and pets [4]. Numerous factors intricately influence the accuracy of this determination, rendering the analytical process complex [11]. In this section, we explore the various elements that significantly impact the accurate determination of vitamin A, shedding light on the difficulties encountered by analysts in maintaining analytical precision within this domain.

Table 1. Vitamin A analysis leeway for premix and feed investigations [93].

Ascertained Level (c), IU/kg	Relative Leeway	Absolute Leeway	Extrapolated Leeway
2000–<3720	-	-	$2.1696 \cdot c^{0.8495}$ IU
3720–<7800	-	-	2340 IU
7800–<100,000	30%	-	-
100,000–<125,000	-	30,000 IU	-
125,000–<375,000	24%	-	-
375,000–<450,000	-	90,000 IU	-
450,000–<1,020,000	20%	-	-
1,020,000–<7,570,000	-	-	20%
7,570,000–≤460,000,000	-	-	$2.1696 \cdot c^{0.8495}$ IU
>460,000,000	-	-	$2309 \cdot c^{0.5}$ IU

The determination of vitamin A activity is not a straightforward process, and its accuracy is influenced by numerous complex factors:

1. Source of vitamin A: The susceptibility of various sources or commercial products of vitamin A to degradation can vary significantly due to differences in their formulation [94] (Figure 2). Factors such as light, oxygen, temperature, and moisture play crucial roles in the degradation process. Consequently, these variations can potentially influence the analytical outcomes, even if the initial activity of multiple vitamin A sources in an identical premix composition is similar. Furthermore, repeatability in retinol analysis is influenced by the physical properties of the vitamin A source (beadlet) utilized during the production of the premix or feed [8]. It is inversely correlated with the concentration of vitamin A present in the sample.

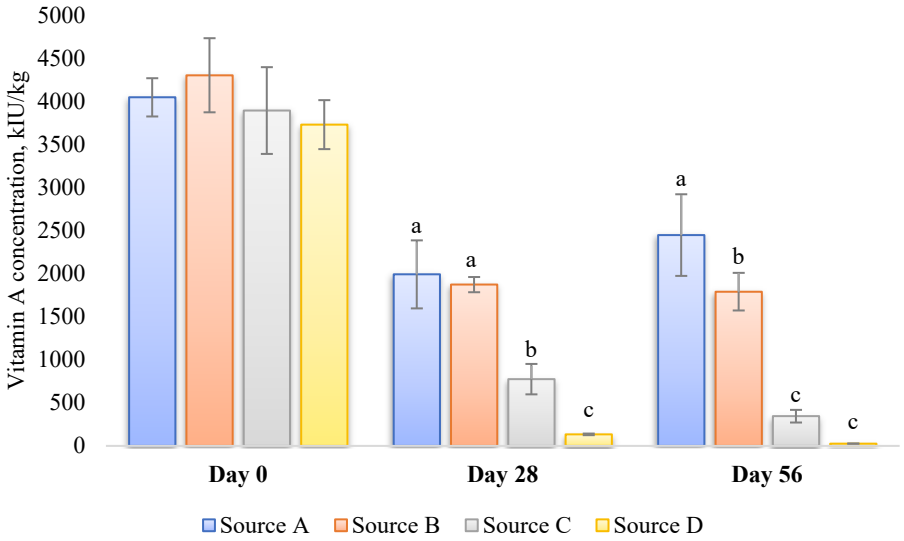

Figure 2. Stability of four different commercial vitamin A sources stored for up to 56 days in a broiler vitamin–mineral premix (incl., choline chloride) at 35 °C and 60–70% r.h. [94]. Values are presented as mean ± SD (n = 3). The premixes were prepared to contain 4.4 Mio IU vitamin A per kg. $^{a–c}$ within a time frame of 0, 28, or 56 d. Values not sharing a common superscript letter are significantly different ($p < 0.05$).

2. Type of sample: Different types of samples, such as premix, feed, blood, or other tissues, may necessitate distinct analytical methodologies [43].
3. Quality of the sample: The accuracy of analytical procedures can be significantly impacted by the quality of the sample. Contaminants or interfering substances within the sample can exert substantial influence on the physicochemical processes utilized during analysis [23].
4. Representativeness of the sample: Ensuring a representative sample is imperative. Ideally, the laboratory should only determine the amount of vitamin A present in the sample. If the sample does not accurately reflect the entire batch, the precision achieved is rendered ineffective.
5. Method of analysis: The precision of the outcomes can be influenced by the analytical approach employed [95]. Various methodologies may exhibit varied sensitivities to distinct configurations of vitamin A [23]. Furthermore, variations in the adherence of analysts to established and sanctioned protocols within a specific methodology may also exert an influence [96].
6. Laboratory: An empirical analysis reveals that the discrepancy in the precision of vitamin A analysis among different laboratories surpasses the variation attributed to differences in analytical methods [96]. Certain techniques or procedures can significantly contribute to substantial interlaboratory variation [96]. Examples of such techniques include the inconsistent reporting or calculation of results, particularly when comparing retinol palmitate with retinyl acetate. Furthermore, modifications made to the vitamin A analysis procedure, which lack validation through rigorous interlaboratory collaborative studies or statistically sound within-laboratory comparisons with validated test methods, can also be a source of significant variability. Additionally, within-laboratory sampling techniques may further compound this issue.
7. Storage conditions: The stability of vitamin A is known to be influenced by various storage conditions, including temperature, light exposure, and oxygen levels [97]. The improper storage of laboratory samples under such conditions can significantly impact the precision and reliability of the analysis.
8. Target tissue cellularity, integrity, and function (for biological tissues): Vitamin A status is characterized by the cellular structure, integrity, and functional capabilities of the target tissues. Unlike some biochemical indicators, any compromise in these

aspects may require several weeks of restoration following vitamin A repletion or depletion [98].

9. Sample preparation approaches: It is crucial to emphasize the importance of obtaining an adequately sized initial sample for the evaluation. Moreover, it is essential to refrain from presuming uniform dispersion of vitamin A throughout the sample during the analysis [11]. Following the grinding process, it is imperative to ensure comprehensive remixing of the ground sample and repeat this process before proceeding with the weighing of a test portion [11].
10. Sample quantity for analysis: The precision of the chemical analysis of vitamin A in feed or premix samples is significantly affected by the weight of the sample. Dry vitamin A supplements are composed of beadlets (Figure 3 and Table 2) that contain multiple units of retinyl acetate [99]. When assessing a small sample of the feed, there might be a limited number of particles per sample [100]. A recent study by Inerowicz et al. [8] indicated that the relative standard deviations for vitamin A determinations in feed varied between 10.5–24.7% and 2.26–10.7% for sample sizes of 10 g and 100 g, respectively (Table 3). The findings of the study suggest that the mass of the sample can considerably influence the accuracy of vitamin A testing in animal feed materials.

Table 2. Results of microscopic particle size measurements obtained through microscopic examination [8].

Vitamin A Source	Initial Mass, g	Density, g/cm^3 @ 21.9 °C [c]	Number of Particles Measured	Particle Size Measurements [a,b]				
				Average, mm	Median, mm	Minimum, mm	Maximum, mm	SD
1	221.0	0.60	2074 [a]	0.466	0.456	0.065	1.179	0.156
2	109.5	0.63	2415 [b]	0.333	0.323	0.047	0.738	0.102

[a] Particle size measurement by microscopic examination, $n = 20$. Test portion masses ranged approximately from 0.3 to 0.5 g for Source1. [b] Particle size measurement by microscopic examination, $n = 20$. Test portion masses ranged approximately from 0.3 to 0.5 g for Source 2 B. [c] $n = 3$; SD = standard deviation.

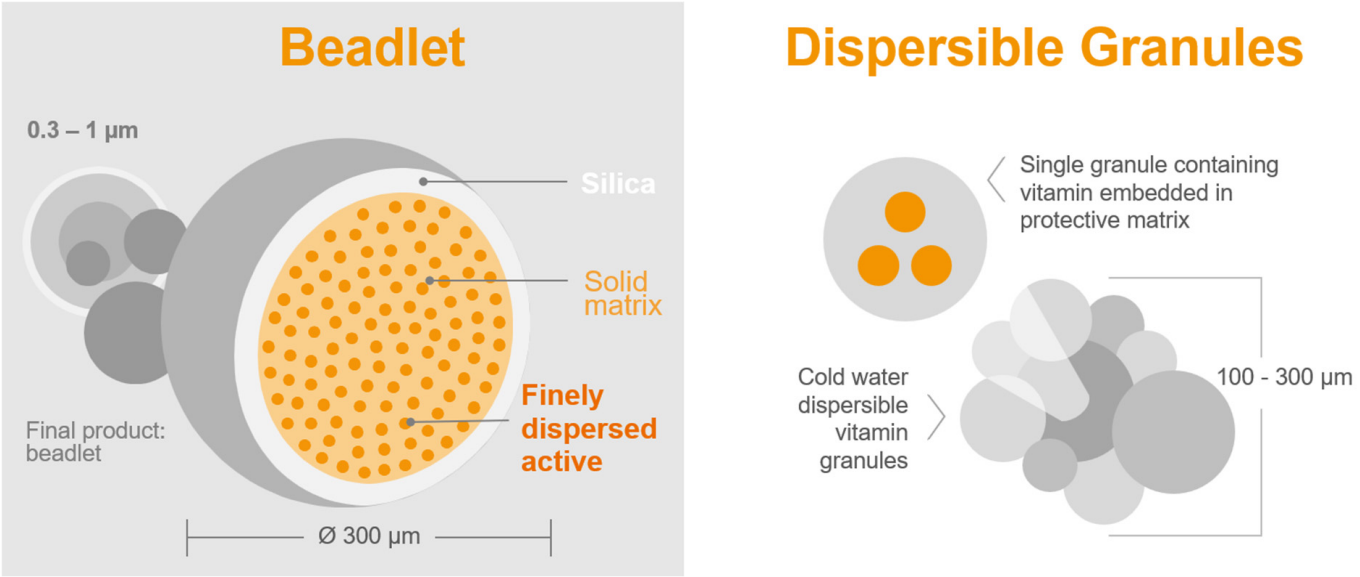

Figure 3. Microencapsulation: delivering a diverse range of products tailored to target applications [101].

Table 3. Vitamin A content in 10 g and 100 g test portions with various samples [8].

Sample	Vitamin A, IU/kg					
	Poultry Feed (Conditioner)		Poultry Feed (Texturized)		Mineral Mix	
Quantity, g	10	100	10	100	10	100
1	6112	5898	20,875	24,476	164,762	176,587
2	5230	5748	18,851	18,443	163,719	171,192
3	5352	5654	24,575	21,794	184,971	173,370
4	4875	5779	15,140	22,853	176,246	177,037
5	6736	6223	23,810	19,640	180,409	168,987
6	7801	6346	22,685	19,642	203,235	180,078
7	6575	6430	34,162	16,716	138,778	173,772
8	7294	5923	26,550	19,186	184,375	180,673
9	6818	4926	22,687	23,999	170,451	170,817
10	5768	5490	18,112	19,503	151,056	171,569
11	5682	5153	25,915	21,724	185,745	180,100
12	4646	5283	31,337	18,976	168,742	181,044
13	6904	6181	22,925	19,556	190,880	170,998
14	5907	5228	28,815	18,182	205,806	177,503
15	5746	5870	16,339	20,145	183,401	177,366
16	5389	5400	14,127	22,279	204,112	175,273
Average [1]	6052	5721	22,930	20,450	178,500	175,400
SD	893	448	5673	2181	18,670	3972
RSD, %	14.8	7.82	24.7	10.7	10.5	2.26

[1] Rounded to 4 significant figures; RSD = relative standard deviation; SD = standard deviation.

11. Analytical standards as benchmarks for the identification and quantification of retinol: The variability in the purity of these standards is a critical factor contributing to the observed inconsistencies among laboratories engaged in vitamin A analysis. In the comparison with the recognized US Pharmacopeia (USP) standard retinyl acetate, varying standards often display significant disparities in measurements, with values fluctuating between 50% and 140% of the officially stated value [11]. Regrettably, certain laboratories fail to validate the vitamin A content of the reference materials and exhibit insufficient quality control protocols for their analytical methods [96]. Laboratories exhibiting exemplary accuracy and precision continuously validate reference materials and incorporate in-house quality control samples, employing robust statistical methodologies to ensure and confirm the reliability of their results [96].
12. Extraction during analytical procedure: In certain instances, the presence of significant quantities of carotenoids following hydrolysis in the solution, coupled with a low concentration of vitamin A, might necessitate the implementation of multiple extraction procedures [102]. In the context of high-fat samples, the formation of extra soaps during the saponification process has the potential to influence the partition coefficient, thereby favoring the aqueous alcohol phase. Consequently, in such scenarios, it becomes imperative to conduct multiple extractions to ensure the efficient separation of retinol into the solvent [11]. According to Moore et al. [96], the primary cause of variation in retinol analysis in feed among various laboratories is, in fact, the vitamin extraction procedure.

13. Evaporation in the analytical phase: During the process of solvent evaporation, the thermal degradation of retinol solutions can occur, particularly at temperatures exceeding 40 degrees Celsius [11]. Thus, it is vital to control and maintain the temperature below this threshold to prevent the degradation of retinol. Additionally, it is essential to minimize the exposure of the retinol residues to ambient air, as this could potentially compromise the stability of the solution [11].
14. Other factors: During the analytical process, other factors, such as the isomerization of all-trans retinol, quality control protocols, precise equipment calibration, potential human errors, systematic and bias errors, and various other influences, could potentially impact the final analytical results [11].

In summary, analyzing vitamin A in different samples is a complex process that requires careful consideration of various factors. Factors such as sample type, quantity, storage conditions, analytical determinants, and a range of other parameters pose challenges for maintaining accurate measurements of retinol. Understanding and managing these factors are crucial for producing high-quality animal feed and premixes that meet nutritional requirements. Figure 4 illustrates an Ishikawa diagram detailing the factors influencing the determination of vitamin A.

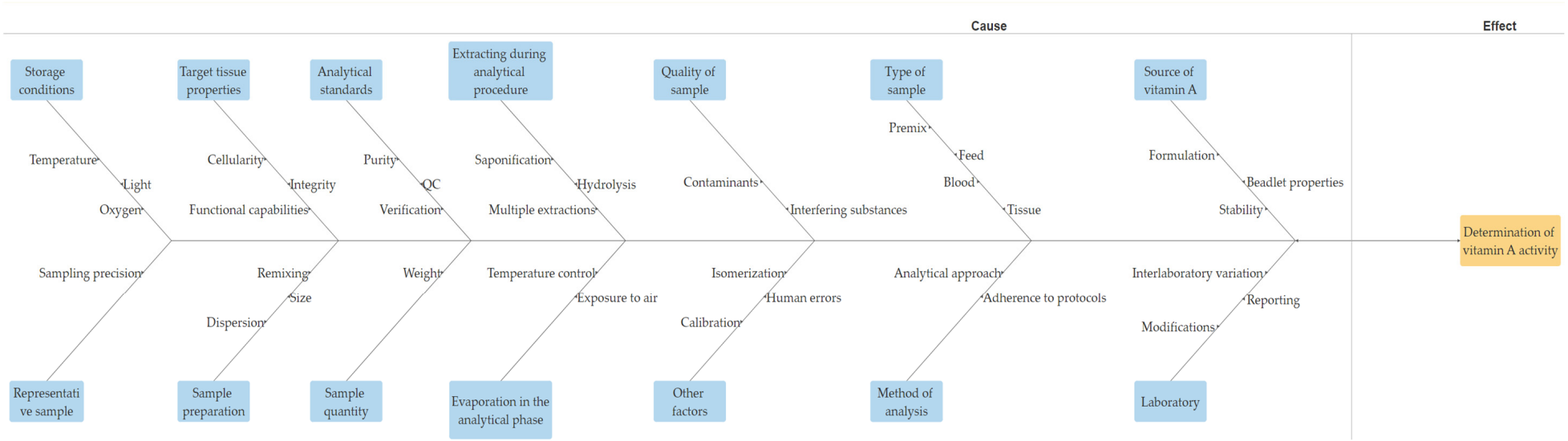

Figure 4. Factors affecting the determination of vitamin A.

5. The Crucial Role of Quality Control: Navigating the Path to Reliable Results

As the field of animal production increasingly emphasizes the need for precise and reliable chemical analytical results, the critical significance of quality control and assurance in safeguarding the integrity of these outcomes is underscored [103]. Within the context of complex laboratory analyses, the looming potential for errors remains a significant concern, as they possess the capability to distort results and undermine the credibility of the entire analytical process [104].

5.1. The Pivotal Role of Quality Control

Quality control plays a pivotal role in upholding the precision and accuracy of vitamin analysis within the field. It serves as a foundational safeguard, ensuring that all analytical procedures adhere to the highest standards of scientific rigor and reproducibility [105]. Through the establishment of a robust system of checks and balances, quality control not only preserves the integrity of analytical outcomes, but also cultivates confidence and trust in the obtained results.

In the context of animal production, where chemical analysis is integral to dietary formulations and achieving optimal nutritional levels in animal feed, the absence of dependable quality control mechanisms could have severe consequences. Such consequences encompass potential harm to animal performance and well-being, challenges in meeting regulatory requirements, and a loss of trust among customers and stakeholders [4,7].

5.2. Implementing Stringent Protocols and Standard Operating Procedures

To maintain analytical rigor, laboratories globally have embraced the adoption of rigorous protocols and standard operating procedures. These protocols are intricately designed to standardize each phase of the analytical process in animal nutrition, from sample collection and preparation to instrumental analysis and data interpretation [106]. The scrupulous crafting of these protocols guarantees that each analyst adheres to a consistent methodology, thus minimizing the introduction of discrepancies and variations that could compromise the accuracy of the final outcomes. Furthermore, these protocols act as a guiding principle, enhancing the path to uniformity and reproducibility, both of which are fundamental to ensuring dependable analytical results [107].

Drawing insights from established practices in the field, it becomes evident that the adoption of stringent protocols is not solely a matter of compliance, but rather, a proactive measure to enhance overall analytical performance and accuracy [108]. The integration of automated systems, advanced instrumentation, and sophisticated data management tools further strengthens the implementation of these protocols, facilitating real-time monitoring and streamlined data analysis [109]. Laboratories that prioritize the adoption of such protocols often position themselves at the forefront of analytical excellence, poised to deliver results that not only meet but surpass the expectations of precision and reliability [110].

5.3. Navigating the Challenges of Consistency and Accuracy

The pursuit of analytical precision in laboratory settings is a complex endeavor that is not without its challenges. Various factors, such as the dynamic nature of analytical processes and the continuous advancements in technology, contribute to a formidable environment that necessitates ongoing vigilance and adaptability [111]. In this fast-paced setting, the importance of regular monitoring and proficiency testing becomes increasingly evident [112].

Laboratories are required to actively conduct regular performance assessments and proficiency testing on animal feed, premixes, and biological samples to ensure the effectiveness of their analytical procedures and the competence of their staff [112–114]. These evaluations act as benchmarks, assessing the efficacy of current protocols and identifying areas that require refinement and adjustment. Insights gained from such assessments not only enable laboratories to proactively address potential shortcomings, but also foster a

culture of continuous improvement, where the pursuit of analytical excellence in vitamin analysis becomes a sustained commitment rather than a fleeting goal [115–117].

Furthermore, the intricate interplay of human factors and the inherent variability of analytical instruments necessitate a comprehensive approach to monitoring and control [118]. Laboratories must invest in comprehensive training programs to cultivate a skilled workforce proficient in adhering to standardized protocols and equipped with the knowledge to recognize and mitigate potential sources of error [1,119]. By nurturing a culture of precision and accountability, laboratories can instill a sense of collective responsibility, where each individual understands their role in maintaining the integrity of analytical data.

Given the evolving regulatory frameworks and the persistent demand for heightened precision, laboratories must remain resolute in their commitment to quality control and assurance [108]. The journey towards reliable results is fraught with challenges and intricacies; however, it is through unwavering dedication to stringent protocols, continuous monitoring, and proficiency testing that laboratories can navigate this path confidently, emerging as bastions of analytical excellence in an ever-evolving landscape of scientific exploration and innovation.

6. Reflection and Future Prospects: Charting the Course for Enhanced Analytical Precision

One of the key findings highlighted in this overview emphasizes the crucial importance of recognizing the complex interplay between the sampling of test materials, sample preparation, and the selected analytical approach in the analysis of retinol. Variations in sample matrices, including diverse feeds like total mixed ration and complimentary feed, as well as various biological samples, consistently underscore the need for tailored methodologies. It is apparent that a standardized approach often falls short in capturing the intricacies within these matrices [120–122]. Furthermore, the need for robust preservation protocols to account for the stability and potential degradation of vitamin A during storage and analysis has been highlighted in recent research [94].

Delving deeper into the range of influencing factors, the role of method validation has emerged as a crucial factor in ensuring the reliability and accuracy of vitamin A determination [123,124]. A meticulous validation process, including considerations for specificity, linearity, and precision, serves as a safeguard against erroneous results [122]. Moreover, the recognition that calibration strategies must be adapted to the characteristics of the analytical technique employed underscores the necessity for a nuanced and adaptable approach [125].

A significant aspect gleaned from the review of historical progress in vitamin A analysis techniques is the continual evolution of analytical technologies. Traditional HPLC has evolved into more advanced methodologies such as chromatography on-line coupled to mass spectrometry, offering greater precision and sensitivity [59,76,78,126]. The integration of novel separation techniques, alongside advanced detection methodologies, shows promise in mitigating the challenges posed by complex matrices and low concentrations [127,128]. Embracing these cutting-edge technologies is crucial to achieving enhanced analytical precision.

Envisioning the future, interdisciplinary collaboration and technological advancements will serve as the foundation for achieving unparalleled precision in vitamin A determination [109,129,130]. Recognizing the multifaceted nature of the challenges at hand, interdisciplinary collaboration, including experts from nutrition science, analytical chemistry, and bioinformatics, presents an opportunity to develop a holistic approach to understanding vitamin A analysis [131–133]. By creating a collaborative ecosystem that facilitates the exchange of knowledge and methodologies, comprehensive frameworks for accurate determination can be synthesized.

Simultaneously, the rapid advancement of miniaturized and portable analytical devices offers the potential for on-site analysis and real-time monitoring, minimizing the risk of sample degradation and allowing for immediate intervention [48,134]. Additionally, the

integration of data science and analytical chemistry can leverage advanced algorithms and machine learning techniques to interpret complex datasets, unravel intricate patterns, and enhance the predictive capabilities of retinol determination [135,136].

Moving forward, it is crucial to prioritize research and development initiatives that bring together the strengths of various disciplines and harness the potential of cutting-edge technologies. By fostering an ecosystem that promotes collaborative innovation and embraces technological advancements, the goal of achieving unparalleled analytical precision in vitamin A determination can be realized. Pursuing this vision not only promises to revolutionize the field of nutritional analysis, but also has the potential to drive advancements in analytical methodologies across various domains, paving the way for a future characterized by unprecedented precision and reliability.

7. Conclusions

Based on the information provided in this scientific review paper, the following conclusions can be drawn:

1. The accurate determination of vitamin A is crucial for animal health and product quality.
2. Historical advancements in analysis techniques have evolved from basic methods to sophisticated chromatographic and spectroscopic approaches, improving precision and sensitivity.
3. Various factors, including sample quality, the method of analysis, and storage conditions, significantly impact analytical precision in retinol determination, necessitating a comprehensive understanding and careful consideration.
4. Emphasizing the critical role of quality control through stringent protocols and regular proficiency testing is essential for ensuring consistent and reliable results.
5. Future progress in analytical precision lies in the integration of advanced technologies, such as miniaturized devices and data-driven approaches, promising to overcome current challenges and enhance accuracy in vitamin A analysis.

In light of these insights, a concerted focus on addressing the complexities of vitamin A analysis through continued advancements in technology and stringent quality control measures is imperative to ensuring precise and reliable determination, thereby bolstering animal welfare and feed safety standards.

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