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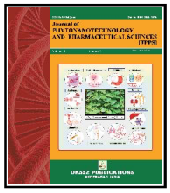

Review Article : Open Access

Analytical methods for determining retinol in skincare formulations: A comprehensive review

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Abstract

Retinol, a derivative of vitamin A, is widely used in various formulations for the treatment of specific skin ailments such as acne, photoaging, and psoriasis. Accurate determination of retinol in these formulations is crucial to ensure their safety, efficacy, and quality. Several analytical techniques have been developed and used to determine retinol in these formulations, including high-performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS), UV-Vis spectroscopy, and fluorescence spectroscopy. HPLC is the most widely used technique, involving the separation of retinol from other compounds using a stationary phase and a mobile phase. LC-MS combines the separation power of liquid chromatography with the detection and identification capabilities of mass spectrometry, providing high sensitivity and selectivity. UV-vis spectroscopy measures the absorption of light by retinol in the UV or visible region to determine its concentration, while fluorescence spectroscopy measures the fluorescence emitted by retinol when excited by a specific wavelength of light. Factors that affect the accuracy of retinol measurement include the stability of retinol, interference from other compounds in the sample, and matrix effects on sample preparation, calibration, and validation of analytical methods, sensitivity of the analytical method, and sample handling and storage. Retinol analysis has various applications, including quality control of retinol-containing skincare products, pharmacokinetic studies in human skin, and safety evaluation in cosmetic/pharmaceutical products. Future research should focus on developing more sensitive and specific analytical methods for retinol measurement, particularly for trace analysis in complex matrices, and exploring alternative techniques for rapid and non-destructive analysis of retinol in skin tissue.

1. Introduction

Retinol is a type of vitamin A, which is a fat-soluble vitamin that is essential for many bodily functions, including maintaining healthy skin (Malbos *et al.,* 2021). Retinol is a type of retinoid, which is a chemical compound that is derived from vitamin A. It is found in many skincare products, such as serums, creams, and lotions, due to its ability to help improve the appearance of fine lines, wrinkles, and uneven skin tone (Stevens, 2021). It works by increasing cell turnover, which helps unclog pores, reduce the appearance of fine lines and wrinkles, and even out the tone of the skin. It also helps stimulate collagen production, which is a protein that helps to keep the skin firm and elastic (Dattola *et al.,* 2020). Furthermore, retinol has been shown to have antioxidant properties, which can help protect the skin from damage caused by free radicals (Zhou *et al.,*2023; Grunebaum and Baumann, 2014). Although, retinol can be highly beneficial to the skin, it can also cause irritation and sensitivity, especially for those with sensitive skin. It is important to start with a low retinol concentration and gradually increase over time to minimize the risk of irritation (Sinbad *et al.,*2019). Vitamin A deficiency is treated with retinol. It is listed as one of the essential medications (Patil *et al.,* 2023) by the World Health Organization (Hodge and Taylor, 2023). Both over-the-counter and generic versions of retinol are readily available. As a result of its potential to contribute to the creation of new products and services, nanotechnology has been identified by the European Commission as a key enabler technology. When applied topically, using nanoscale versions of chemicals or nanocarriers can sometimes result in deeper skin penetration, longer-lasting effects, or even more effective ultraviolet (UV) protection (Hougeir and Kircik, 2012). In targeted therapies, the topical approach is also more efficient since it increases the bioavailability in the target region. Additionally, its application reduces the dangers of systemic adverse effects. Furthermore, retinol should always be used in conjunction with sunscreen, as it can make the skin more sensitive to harmful UV rays (Table 1).

Retinol can be a beneficial ingredient for a variety of skin concerns, but it is essential to use it correctly and consult with a dermatologist to avoid potential adverse effects. Analytical methods are crucial to determine the appropriate concentration of retinol in various skincare formulations, ensuring optimal efficacy and safety (Draelos *et al.,* 2020).

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Table 1: Effects of retinol in different skin diseases

Skin ailment	Description	References
Acne	Retinol can unclog pores, reduce inflammation, and regulate skin cell turnover, making it an effective treatment for mild to moderate acne.	Ruamrak *et al.,* 2009
Aging skin	Retinol can stimulate collagen production, improve skin texture and tone, and reduce the appearance of fine lines and wrinkles, making it a popular anti-aging ingredient.	Sadick *et al.,*2019
Hyperpigmentation	Retinol can help fade dark spots, age spots, and other forms of hyperpigmentation by inhibiting melanin production and promoting skin cell turnover.	Searle *et al.,* 2020
Psoriasis	Retinol can help reduce inflammation, improve skin texture, and alleviate symptoms of psoriasis, a chronic autoimmune disorder that causes scaling and redness of the skin.	Wang *et al.,* 2020
Eczema	Retinol can help soothe and repair the skin barrier, reduce inflammation, and alleviate the symptoms of eczema, a chronic skin condition that causes dry, itchy, and inflamed skin.	Buchholz, 1976

2. Retinol formulations for specific skin ailments

Retinol formulations can be precisely tailored to address specific skin problems, and dermatologist guidance recommended ensuring appropriate concentration for individual skin types and conditions (Table 2) (Zasada and Budzisz, 2020). For acne-prone skin, retinol is often combined with salicylic acid or benzoyl peroxide to unclog pores, regulate oil production, and reduce inflammation (Layton, 2009). Ageing skin benefits from higher concentrations ofretinol that stimulate collagen synthesis, improve elasticity, and diminish fine lines (Griffiths *et al.,* 1993). To target hyperpigmentation, retinol synergizes with vitamin C or niacinamide to even skin tone and reduce dark spots (Kang *et al.,* 2001). For inflammatory conditions such as psoriasis and eczema, lower concentrations ofretinol minimize irritation while improving the function of the skin barrier and cell turnover (Weinstein *et al.,* 2003; Knott *et al.,* 2015).

Table 2: Available topical formulation of retinol used in various skin diseases

Skin ailment	Retinol formulation	Concentration	Additional active ingredients	References
Acne	Retinol cream	0.025-0.1%	Benzoyl peroxide, salicylic acid	Layton, 2009
	Retinol gel	0.01-0.1%	Clindamycin, adapalene	Layton, 2009
Aging skin	Retinol serum	0.5-1%	Vitamin C, hyaluronic acid	Griffiths *et al.,* 1993
	Retinol cream	0.5-1%	Peptides, antioxidants	Griffiths *et al.,* 1993
Hyperpigmentation	Retinol brightening cream	0.5-1%	Kojic acid, niacinamide	Kang *et al.,* 2001)
Psoriasis	Retinol ointment	0.025-0.1%	Coal tar, salicylic acid	Weinstein *et al.,* 2003
	Retinol cream	0.1-0.3%	Topical corticosteroids, vitamin D3	Weinstein *et al.,* 2003
Wrinkles	Retinol serum	0.25%	Bisabolol, *Cannabis sativa*, Capric, Caprylic, Ceramides, Ethylhexanoate, Niacinamide, Palmitoyl, Polysorbate 20, Retinol, Squalaneoil, Squalene, Tocopheryl Acetate, Tretinoin, Triglyceride, Vitamin A, Vitamin E	Skin MEDICA

3. Analytical methods to determine the retinol content in skincare products

Analytical methods to determine retinol in various formulations used for specific skin diseases, it is essential to discuss the different techniques used for this purpose.

3.1 High performance liquid chromatography (HPLC)

HPLC is a widely used analytical technique for the measurement of retinol in various formulations used for specific skin ailments (Talwar *et al.,* 1998). Retinol is a vitamin A derivative that is known to have numerous beneficial effects on the skin, including improving skin texture, reducing the appearance of fine lines and wrinkles, and improving overall skin health (Tasioula-Margari and Tsabolatidou, 2015).

HPLC works by separating the individual components in a sample on their chemical properties such as polarity, size, and shape. In the case of retinol analysis, the sample is typically prepared by extracting retinol from the formulation using a suitable solvent. The extracted retinol is then injected into the HPLC system, which consists of a mobile phase (a solvent that flows through the column), a stationary phase (a solid or liquid material that interacts with the sample), a column (a long narrow tube packed with the stationary phase), and a detector (a device that detects the separated components) (Kane *et al.,* 2008; Yang *et al.,* 2015). As the mobile phase flows through the column, the components of the sample interact with the stationary phase and are separated on their chemical properties. Separate components are then detected by the detector, which produces a chromatogram showing the retention times and peak areas of each component (Sabir *et al.,* 2016). In the case of retinol analysis, the

detector used is usually a UV-vis detector, which detects the absorption of light by the retinol molecule at a specific wavelength. The retention time and peak area of the retinol peak in the chromatogram can be used to quantify the amount of retinol in the sample (Craft *et al.,* 2000).

HPLC is a highly sensitive and selective technique that can detect and quantify retinol at low concentrations. However, there are some challenges associated with HPLC analysis of retinol, including the potential for retinol degradation during sample preparation and analysis, the need for specialized equipment and expertise, and the potential for interference from other components in the sample matrix (Chaudhary *et al.,* 2019). To overcome these challenges, careful sample preparation and handling is required and the HPLC method should be carefully optimized for the specific sample matrix and retinol concentration range of interest. Furthermore, the use of internal standards and calibration curves can improve the accuracy and precision of retinol quantification (Tenon *et al.,* 2017).

3.2 Liquid chromatography-mass spectrometry (LC-MS)

LC-MS is a powerful analytical technique that combines the separation power of liquid chromatography with the detection and identification capabilities of mass spectrometry (Tyanova *et al.,* 2015). LC-MS has become an essential tool in many areas of research, including pharmaceuticals, environmental analysis, forensic analysis, and metabolomics (Segerand Salzmann, 2020). The LC part of the technique involves the separation of components in a sample using a liquid mobile phase that flows through a column packed with a stationary phase. The stationary phase can be a solid support or a liquid coating, and it interacts with the components in the sample on their chemical properties such as polarity, hydrophobicity, and size. As the mobile phase flows through the column, the components in the sample are separated on their interactions with the stationary phase. The MS part of the technique involves the detection and identification of the separated components in the sample on their mass-to-charge ratio (m/z). MS works by ionizing the components in the sample, which generates ions that are then separated on their m/z using an electric or magnetic field. The separated ions are then detected by a detector, which produces a mass spectrum that can be used to identify the components in the sample (McCormick and Napoli, 1982).

LC-MS can be used in various modes, including a positive ion mode, a negative ion mode, and a multiple reaction monitoring (MRM) mode. In positive ion mode, the components in the sample are ionized by adding protons, which generates positively charged ions (la Marca *et al.,* 2012). In negative-ion mode, the components in the sample are ionized by removal of electrons, which generates negatively charged ions. The MRM mode is a highly selective and sensitive mode that involves the detection of specific precursor ions and their corresponding product ions, which are generated by the fragmentation of the precursor ions (Albanes *et al.,* 2016). One of the main advantages of LC-MS is its high sensitivity, which allows for the detection and quantification of components in a sample at low concentrations. LC-MS is also highly selective, which means that it can distinguish between different components in a sample based on their chemical properties and mass spectra (Al-Khelaifi *et al.,* 2018). Additionally, LC-MS is a versatile technique that can be used to analyze a wide range of samples, including small molecules, peptides, proteins, and lipids. Despite its many advantages.

LC-MS also has some limitations. One of the main limitations is the complexity of the technique, which requires specialized equipment, expertise, and software. Another limitation is the potential for matrix effects, which can interfere with the separation and detection of components in a sample. Matrix effects can arise from the presence of coeluting compounds, sample matrix components, or contaminants that can affect the ionization and detection of components in the sample (Gika *et al.,* 2014).

3.3 UV-vis spectroscopy

UV-vis spectroscopy is a non-destructive analytical techniqueused for retinol measurement. It involves measuring the absorption of light by retinol in the UV or visible region to determine its concentration (Picollo *et al.,* 2018). UV-vis spectroscopy is a widely used analytical technique for the qualitative and quantitative analysis of compounds based on their absorption of light in the ultraviolet and visible regions of the electromagnetic spectrum (Liu *et al.,* 2011). Retinol, a form of vitamin A, can be measured using UV-vis spectroscopy due to its characteristic absorption at a specific wavelength (Khan *et al.,* 2010; Khan *et al.,* 2010).

The sample is exposed to light in the ultraviolet or visible region of the spectrum, and the intensity of the transmitted or absorbed light is measured. The absorption spectrum of a compound is unique and can be used to identify and quantify the compound in a sample (Wingerath *et al.,* 1999). For measurement, the sample is first prepared by extracting retinol from the sample matrix using a suitable method such as liquid-liquid extraction with organic solvents. The sample was then dissolved in a suitable solvent such as ethanol or hexane. A UV-vis spectrophotometer is used to measure the absorbance of the retinol solution at a specific wavelength, typically around 325 nm, which is the wavelength of maximum absorbance for retinol. A calibration curve is prepared using standard solutions of retinol in the concentration range of interest, and the concentration of retinol in the sample is calculated by comparing its absorbance with the calibration curve (Schäffer *et al.,* 2010). Moreover, the accuracy and precision of the method can be affected by interferences from other compounds in the sample matrix. Therefore, proper sample preparation and calibration are crucial for accurate and reliable results (Betz *et al.,* 2011).

3.4 Fluorescence spectroscopy

Fluorescence spectroscopy is a highly sensitive technique that is used for retinol measurement. It involves measuring the fluorescence emitted by retinol when excited by a specific wavelength of light to determine its concentration (Hink, 2015). Fluorescence spectroscopy is a powerful analytical technique that is used for the qualitative and quantitative analysis of compounds on their fluorescence properties. Retinol, a form of vitamin A, exhibits fluorescence properties that can be used for its measurement by fluorescence spectroscopy (Kahan, 1971; Lakowicz *et al.,* 1999).

The sample is excited with light of a specific wavelength, and the fluorescence emission of the sample is measured at a longer wavelength. The intensity of fluorescence emission is directly proportional to the concentration of the analyte in the sample (Karoui and Blecker, 2011). To measure retinol using fluorescence spectroscopy, the sample is first prepared by extracting retinol from the sample matrix using a suitable method such as liquid-liquid extraction with organic solvents (Gurgel *et al.,* 2018; Erhardt *et al.,*

2002). The sample is then dissolved in a suitable solvent such as ethanol or hexane and at a specific wavelength, typically around 485 nm, which is the wavelength of maximum emission for retinol (Blayo *et al.,* 2014; Kahan, 1971). The sample is excited with light of a specific wavelength, typically around 330 nm, which is the wavelength of maximum excitation for retinol. A calibration curve is prepared using standard solutions of retinol in the concentration range of interest, and the concentration of retinol in the sample is calculated by comparing its fluorescence emission with the calibration curve. Additionally, the method is less affected by interferences from other compounds in the sample matrix (Liu *et al.,* 2018). However, fluorescence spectroscopy can be affected by factors such as quenching, photobleaching, and the inner filter effect, which can affect the accuracy and precision of the method. Proper sample preparation, calibration, and optimization of experimental conditions are therefore crucial for accurate and reliable results (Ishikawa-Ankerhold *et al.,* 2012).

4. Factors affecting the accuracy of retinol measurement

Retinol measurement accuracy can be affected by various factors, including:

Stability of retinol: Retinol can degrade with time or due to exposure to light and air. Therefore, it is essential to ensure that retinol remains stable throughout the sample preparation and analysis process (Tolleson *et al.,* 2005).

Interference of other compounds in the sample: Interference from other compounds present in the sample can affect the accuracy of the retinol measurement. For example, other vitamins, lipids, and pigments can absorb or fluoresce at similar to those of retinol and interfere with its measurement (Greaves *et al.,* 2014).

Matrix effects on sample preparation: The sample matrix can affect the accuracy of the retinol measurement. Matrix effects can arise from differences in the physical and chemical properties of the sample matrix, such as the presence of interfering compounds, pH, ionic strength, and viscosity. These factors can affect the extraction efficiency, stability, and purity of retinol in the sample (Silvestro *et al.,* 2013).

Calibration and validation of analytical methods: The accuracy of the retinol measurement depends on the calibration and validation of the analytical methods used. Calibration is the process of establishing a relationship between the instrument response and the concentration of retinol, while validation is the process of evaluating the accuracy, precision, and robustness of the method. Calibration and validation should be performed using suitable standards and quality control materials and the results should be within acceptable limits of error (Boqué *et al.,* 2002).

Sensitivity of the analytical method: The sensitivity of the analytical method used for the measurement ofretinol can affect the accuracy of the results. Methods with lower detection and quantification are preferred for accurate and reliable retinol measurement (Arnold *et al.,* 2012).

Sample handling and storage: The accuracy of retinol measurement can be affected by how samples are handled and stored before and during analysis. Factors such as temperature, light exposure, and contamination can affect the stability and purity of retinol in the sample (Czuba *et al.,* 2020).

5. Applications of retinol analysis

Retinol analysis has various applications, some of which are:

Quality control of retinol-containing skincare products: Retinol's prominence in antiageing and skin rejuvenation creams, lotions, and serums necessitates rigorous quality control. Analysis is critical to verify label claims regarding retinol concentration, ensure product safety by detecting impurities or contaminants, and conform to overall product quality and consistency (Kong *et al.,* 2016; Jun *et al.,* 2021; Sheftel *et al.,* 2019).

Pharmacokinetic studiesin human skin: Understanding the absorption, distribution, metabolism, and elimination (pharmacokinetics) of topically applied retinol is vital. As a fat-soluble vitamin capable of skin absorption and systemic accumulation, the pharmacokinetic profile informs the development of topical formulations designed for optimal skin penetration and efficacy (Caspers *et al.,* 2019; Croce *et al.,* 2020; Lim and Chen, 2021; Gannon and Tanumihardjo, 2015; Tycękiewicz *et al.,* 2018).

Safety evaluation in cosmetics and pharmaceutical products: While retinol is generally safe at appropriate concentrations, excessive levels can cause adverse effects such as skin irritation, redness, and peeling. Retinol analysis underpins safety assessments by quantifying concentrations to ensure they remain within acceptable limits for cosmetic and pharmaceutical applications. This analysis supports toxicological studies that evaluate the safety profile of retinol and its derivatives (Racz *et al.,* 2020; Marles *et al.,* 2017; Li *et al.,* 2021; Morris *et al.,* 2012).

6. Conclusion

This article provides an overview of the analytical methods used to determine retinol in various formulations for the treatment of specific skin diseases. Retinol, a vitamin A derivative, is widely used in skincare products for its beneficial effects on skin health. Accurate determination of retinol concentration is crucial for ensuring product safety, efficacy, and quality. The most commonly used analytical techniques include HPLC, LC-MS, UV-vis spectroscopy, and fluorescence spectroscopy. Each method has its advantages and limitations, and factors such as retinol stability, sample matrix effects, calibration, and validation can affect the precision of retinol measurement. Retinol analysis has various applications, including quality control of skincare products, pharmacokinetic studies on human skin, and safety evaluation in cosmetic and pharmaceutical products.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Albanes, D.; Panagiotou, O.; Weinstein, S.; Moore, S. and Mondul, A. (2016). Metabolomic profile of serum retinol concentrations in the ATBC Study Cohort.FASEB J., **30**:34-37.

Al-Khelaifi, F.; Diboun, I.; Donati, F.; Botrè, F.; Alsayrafi, M.; Georgakopoulos, C.; Yousri, N. A.; Suhre, K. and Elrayess, M. A. (2018). Metabolomics profiling of xenobiotics in elite athletes: relevance to supplement consumption. J. Int. Soc. Sports Nutr., **15**(1):48.

Arnold, S. L.; Amory, J. K.; Walsh, T. J. and Isoherranen, N. (2012). A sensitive and specific method for measurement of multiple retinoids in human serum with UHPLC-MS/MS. J. Lipid Res., **53**(3):587-598.

Betz, J. M.; Brown, P. N. and Roman, M. C. (2011). Accuracy, precision, and reliability of chemical measurements in natural products research. Fitoterapia, **82**(1):44-52.

Boqué, R.; Maroto, A.; Riu, J. and Rius, F. X. (2002). Validation of analytical methods. Grasas Aceites, **53**(1):128-143.

Buchholz, V. W. (1976). Studies on the influence of vitamin A on the DNBC-eczema of the guinea pig. Dermatol. Monatsschr., **162**(4):312-316.

Caspers, P. J.; Nico, C.; Bakker Schut, T. C.; de Sterke, J.; Pudney, P. D.; Curto, P. R.; Illand, A. and Puppels, G. J. (2019). Method to quantify the *in vivo* skin penetration of topically applied materials based on confocal Raman spectroscopy. Transl. Biophotonics, **1**(1-2):e201900004.

Chaudhary-Webb, M.; Schleicher, R. L.; Erhardt, J. G.; Pendergrast, E. C. and Pfeiffer, C. M. (2019). An HPLC ultraviolet method using low sample volume and protein precipitation for the measurement of retinol in human serum suitable for laboratories in low- and middle-income countries. J. Appl. Lab. Med., **4**(1):101-107.

Craft, N. E.; Haitema, T.; Brindle, L. K.; Yamini, S.; Humphrey, J. H. and West, K. P. (2000). Retinol analysis in dried blood spots by HPLC. J. Nutr., **130**(4):882-885.

Croce, A. C.; Ferrigno, A.; Berardo, C.; Bottiroli, G.; Vairetti, M. and Di Pasqua, L. G. (2020). Spectrofluorometric analysis of autofluorescing components of crude serum from a rat liver model of ischemia and reperfusion. Molecules, **25**(6):1327.

Czuba, L. C.; Zhong, G.; Yabut, K. C. and Isoherranen, N. (2020). Analysis of vitamin A and retinoids in biological matrices. In: Methods Enzymol., **637**:309-340.

Dattola, A.; Silvestri, M.; Bennardo, L.; Passante, M.; Scali, E.; Patruno, C. and Nisticò, S. P. (2020). Role of vitamins in skin health: A systematic review. Curr. Nutr. Rep., **9**:226-235.

Draelos, Z. D.; Gunt, H.; Zeichner, J. and Levy, S. (2020). Clinical evaluation of a nature-based bakuchiol anti-ageing moisturizer for sensitive skin. J. Drugs Dermatol., **19**(12):1181-1183.

Erhardt, J. G.; Craft, N. E.; Heinrich, F. and Biesalski, H. K. (2002). Rapid and simple measurement of retinol in human dried whole blood spots. J. Nutr., **132**(2):318-321.

Gannon, B. M. and Tanumihardjo, S. A. (2015). Comparisons among equations used for retinol isotope dilution in the assessment of total body stores and total liver reserves. J. Nutr., **145**(5):847-854.

Gika, H. G.; Wilson, I. D. and Theodoridis, G. A. (2014). LC-MS-based holistic metabolic profiling.Problems, limitations, advantages, and future perspectives. J. Chromatogr. B, **966**:1-6.

Greaves, R. F.; Woollard, G. A.; Hoad, K. E.; Walmsley, T. A.; Johnson, L. A.; Briscoe, S.; Koetsier, S.; Harrower, T. and Gill, J. P. (2014). Laboratory medicine best practice guideline: Vitamins A, E and the carotenoids in blood. Clin. Biochem. Rev., **35**(2):81.

Griffiths, C. E.; Finkel, L. J.; Tkanfaglia, M. G.; Hamilton, T. A. and Voorhees, J. J. (1993). An *in vivo* experimental model for effects of topical retinoic acid in human skin. Br. J. Dermatol., **129**(4):389-394.

Grunebaum, L. D. and Baumann, L. S. (2014). Nonprescription topical treatments for skin rejuvenation. Facial Plast. Surg., **30**(1):3-11.

Gurgel, C. S.; Grilo, E. C.; Lira, L. Q.; Assunção, D. G.; Oliveira, P. G.; de Melo, L. R.; de Medeiros, S. V.; Pessanha, L. C.; Dimenstein, R. and Lyra, C. O. (2018). Vitamin A nutritional status in high and low income postpartum women and its effect on colostrum and the requirements of the term newborn. J. Pediatr. (Porto Alegre), **94**(2):207-215.

Hink, M. A. (2015). Fluorescence correlation spectroscopy. In: Adv. Fluoresc. Microsc., pp:135-150.

Hodge, C. and Taylor, C. (2023). Vitamin A deficiency. In: Stat Pearls [Internet]. Stat Pearls Publishing.

Hougeir, F. G. and Kircik, L. (2012). A review of delivery systems in cosmetics.Dermatol.Ther., **25**(3):234-237.

Ishikawa-Ankerhold, H. C.; Ankerhold, R. and Drummen, G. P. (2012). Advanced fluorescence microscopy techniques: FRAP, FLIP, FLAP, FRET and FLIM. Molecules, **17**(4):4047-4132.

Jun, S. H.; Kim, H.; Lee, H.; Song, J. E.; Park, S. G. and Kang, N. G. (2021). Synthesis of retinol-loaded lipid nanocarrier *via* vacuum emulsification to improve topical skin delivery. Polymers, **13**(5):826.

Kahan, J. (1971). The fluorescence properties of vitamin A. In: Methods Enzymol., **18**:574-591.

Kane, M. A.; Folias, A. E. and Napoli, J. L. (2008). HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues. Anal. Biochem., **378**(1):71-79.

Kang, S.; Leyden, J. J.; Lowe, N. J.; Ortonne, J. P.; Phillips, T. J.; Weinstein, G. D.; Bhawan, J.; Lew-Kaya, D. A.; Matsumoto, R. M.; Sefton, J. and Walker, P. S. (2001). Tazarotene cream for the treatment of facial photodamage: A multicenter, investigator-masked, randomized, vehicle-controlled, parallel comparison of 0.01%, 0.025%, 0.05%, and 0.1% tazarotene creams with 0.05% tretinoin emollient cream applied once daily for 24 weeks. Arch. Dermatol., **137**(12):1597-1604.

Karoui, R. and Blecker, C. (2011). Fluorescence spectroscopy measurement for quality assessment of food systems: A review. Food Bioprocess Technol., **4**:364-386.

Khan, A.; Khan, M. I.; Iqbal, Z.; Shah, Y.; Ahmad, L. and Watson, D. G. (2010). An optimized and validated RP-HPLC/UV detection method for simultaneous determination of all-trans-Retinol (vitamin A) and α-Tocopherol (vitamin E) in human serum: Comparison of different particulate reversed-phase HPLC columns. J. Chromatogr. B, **878**(25):2339-2347.

Khan, M. I.; Khan, A.; Iqbal, Z.; Ahmad, L. and Shah, Y. (2010). Optimization and validation of RP-LC/UV–VIS detection method for simultaneous determination of fat-soluble anti-oxidant vitamins, all-trans-retinol and á-tocopherol in human serum: Effect of experimental parameters. Chromatographia, 71:577-586.

Knott, A.; Achterberg, V.; Smuda, C.; Mielke, H.; Sperling, G.; Dunckelmann, K.; Vogelsang, A.; Krüger, A.; Schwengler, H.; Behtash, M. and Kristof, S. (2015). Topical treatment with coenzyme Q10 containing formulas improves skin's Q10 level and provides antioxidative effects. Biofactors, **41**(6):383-390.

Kong, R.; Cui, Y.; Fisher, G. J.; Wang, X.; Chen, Y.; Schneider, L. M. and Majmudar, G. (2016). A comparative study of the effects of retinol and retinoic acid on histological, molecular, and clinical properties of human skin.J. Cosmet. Dermatol., **15**(1):49-57.

la Marca, G.; Villanelli, F.; Malvagia, S.; Ombrone, D.; Funghini, S.; De Gaudio, M.; Fallani, S.; Cassetta, M. I.; Novelli, A.; Chiappini, E. and de Martino, M. (2012). Rapid and sensitive LC-MS/MS method for the analysis of antibiotic linezolid on dried blood spot. J. Pharm. Biomed. Anal., **67**:86-91.

Lakowicz, J. R. (2006). Principles of Fluorescence Spectroscopy. Springer US.

Layton, A. (2009). The use of isotretinoin in acne. Dermatoendo-crinol., **1**(3):162-169.

Li, C.; Gu, X. and Xiong, D. (2021). Tolerance and effectiveness of a gel cream containing oat and feverfew on capsaicin sting positive dry sensitive skin. J. Am. Acad. Dermatol., **85**(3):AB166.

Lim, Z. W. and Chen, W. L. (2021). Association between micronutrient concentrations and human epididymis protein 4. J. Inflamm. Res., **14**:4945-4954.

Liu, J.; Zamora, A.; Castillo, M. and Saldo, J. (2018). Using front-face fluorescence spectroscopy for prediction of retinol loss in milk during thermal processing. LWT, **87**:151-157.

Liu, Z.; Lee, H. J.; Garofalo, F.; Jenkins, D. J. and El-Sohemy, A. (2011). Simultaneous measurement of three tocopherols, all-trans-retinol, and eight carotenoids in human plasma by isocratic liquid chromatography. J. Chromatogr. Sci., **49**(3):221-227.

Malbos, D.; Buxeraud, J.; Faure, S. and Desport, J. C. (2021). La vitamine A. Actual. Pharm., **60**(608):23-26.

Marles, R. J.; Roe, A. L. and Oketch-Rabah, H. A. (2017). US pharmacopeial convention safety evaluation of menaquinone-7, a form of vitamin K. Nutr. Rev., **75**(7):553-578.

McCormick, A. M. and Napoli, J. L. (1982). Identification of 5,6-epoxyretinoic acid as an endogenous retinol metabolite. J. Biol. Chem., **257**(4):1730-1735.

Morris, P. J.; Salt, C.; Raila, J.; Brenten, T.; Kohn, B.; Schweigert, F. J. and Zentek, J. (2012). Safety evaluation of vitamin A in growing dogs. Br. J. Nutr., **108**(10):1800-1809.

Patil, S.; Zamwar, U. M. and Mudey, A. (2023). Etiology, epidemiology, pathophysiology, signs and symptoms, evaluation, and treatment of vitamin A (retinol) deficiency. Cureus, **15**(11):e48778.

Picollo, M.; Aceto, M. and Vitorino, T. (2019). UV-Vis spectroscopy. Phys. Sci. Rev., 4:20180008.

Racz, B.; Varadi, A.; Pearson, P. G. and Petrukhin, K. (2020). Comparative pharmacokinetics and pharmacodynamics of the advanced Retinol-Binding Protein 4 antagonist in dog and cynomolgus monkey. PLoS One, **15**(1):e0228291.

Ruamrak, C.; Lourith, N. and Natakankitkul, S. (2009). Comparison of clinical efficacies of sodium ascorbyl phosphate, retinol and their combination in acne treatment. Int. J. Cosmet. Sci., **31**(1):41-46.

Sabir, A. M.; Moloy, M. and Bhasin, P. S. (2013). HPLC method development and validation: A review. Int. Res. J. Pharm., **4**(4):39-46.

Sadick, N.; Edison, B. L.; John, G.; Bohnert, K. L. and Green, B. (2019). An advanced, physician-strength retinol peel improves signs of aging and acne across a range of skin types including melasma and skin of color. J. Drugs Dermatol., **18**(9):918-923.

Schäffer, M. W.; Roy, S. S.; Mukherjee, S.; Nohr, D.; Wolter, M.; Biesalski, H. K.; Ong, D. E. and Das, S. K. (2010). Qualitative and quantitative analysis of retinol, retinyl esters, tocopherols and selected carotenoids out of various internal organs from different species by HPLC. Anal. Methods, **2**(9):1320-1332.

Searle, T.; Al-Niaimi, F. and Ali, F. R. (2020). The top 10 cosmeceuticals for facial hyperpigmentation. Dermatol. Ther., **33**(6):e14095.

Seger, C. and Salzmann, L. (2020). After another decade: LC–MS/MS became routine in clinical diagnostics. Clin. Biochem., **82**:2-11.

Sheftel, J.; Sowa, M.; Mourao, L.; Zoué, L. T.; Davis, C. R.; Simon, P. W. and Tanumihardjo, S. A. (2019). Total adipose retinol concentrations are correlated with total liver retinol concentrations in male Mongolian gerbils, but only partially explained by chylomicron deposition assessed with total α-retinol. Curr. Dev. Nutr., **3**(2):096.

Silvestro, L.; Tarcomnicu, I. and Savu, S. R. (2013). Matrix effects in mass spectrometry combined with separation methods. Comparison HPLC, GC and discussion on methods to control these effects. In: Tandem Mass Spectrom. Mol. Charact. Intech. Open.

Sinbad, O. O.; Folorunsho, A. A.; Olabisi, O. L.; Ayoola, O. A. and Temitope, E. J. (2019). Vitamins as antioxidants. J. Food Sci. Nutr. Res., **2**(3):214-235.

Stevens, S. L. (2021). Fat-soluble vitamins. Nurs. Clin. North Am., **56**(1):33-45.

Talwar, D.; Ha, T. K.; Cooney, J.; Brownlee, C. and St O'Reilly, D. J. (1998). A routine method for the simultaneous measurement of retinol, α-tocopherol and five carotenoids in human plasma by reverse phase HPLC. Clin. Chim. Acta, **270**(2):85-100.

Tasioula-Margari, M. and Tsabolatidou, E. (2015). Extraction, separation, and identification of phenolic compounds in virgin olive oil by HPLC-DAD and HPLC-MS. Antioxidants, **4**(3):548-562.

Tenon, M.; Feuillère, N.; Roller, M. and Birtiæ, S. (2017). Rapid, cost-effective and accurate quantification of Yucca schidigera Roezl. steroidalsaponins using HPLC-ELSD method. Food Chem., **221**: 1245-1252.

Tolleson, W. H.; Cherng, S. H.; Xia, Q.; Boudreau, M.; Yin, J. J.; Wamer, W. G.; Howard, P. C.; Yu, H. and Fu, P. P. (2005). Photodecomposition and phototoxicity of natural retinoids. Int. J. Environ. Res. Public Health, **2**(1):147-155.

Tyanova, S.; Temu, T.; Carlson, A.; Sinitcyn, P.; Mann, M. and Cox, J. (2015). Visualization of LC-MS/MS proteomics data in MaxQuant. Proteomics, **15**(8):1453-1456.

Tyœkiewicz, K.; Gieysztor, R.; Maziarczyk, I.; Hodurek, P.; Rój, E. and Skalicka-WoŸniak, K. (2018). Supercritical fluid chromatography with photodiode array detection in the determination of fat-soluble vitamins in hemp seed oil and waste fish oil. Molecules, **23**(5):1131.

Wang, H. M.; Wu, C.; Jiang, Y. Y.; Wang, W. M. and Jin, H. Z. (2020). Retinol and vitamin A metabolites accumulate through RBP4 and STRA6 changes in a psoriasis murine model. Nutr. Metab.,**17**:1-15.

Weinstein, G. D.; Koo, J. Y.; Krueger, G. G.; Lebwohl, M. G.; Lowe, N. J.; Menter, M. A.; Lew-Kaya, D. A.; Sefton, J.; Gibson, J. R.; Walker, P. S. and The Tazarotene Cream Study Group. (2003). Tazarotene cream in the treatment of psoriasis: two multicenter, double-blind, randomized, vehicle-controlled studies of the safety and efficacy of tazarotene creams 0.05% and 0.1% applied once daily for 12 weeks. J. Am. Acad. Dermatol., **48**(5):760-767.

Wingerath, T.; Kirsch, D.; Spengler, B. and Stahl, W. (1999). Analysis of cyclic and acyclic analogs of retinol, retinoic acid, and retinal by laser desorption ionization-, matrix-assisted laser desorption ionization–mass spectrometry, and UV/Vis spectroscopy. Anal. Biochem., **272**(2):232-242.

Yang, Y.; Lu, D.; Zhang, J.; Li, Y.; Zheng, B. and Sun, C. (2015). Simultaneous HPLC–DAD determination of retinol and eight vitamin E isomers in human serum. Chromatographia, **78**:1359-1366.

Zasada, M. and Budzisz, E. (2020). Randomized parallel control trial checking the efficacy and impact of two concentrations of retinol in the original formula on the ageing skin condition: pilot study. J. Cosmet. Dermatol., **19**(2):437-443.

Zhou, S.; Chen, J. and Cao, R. (2023). Association between retinol intake and periodontal health in US adults. BMC Oral Health, **23**(1):61.

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