e-ISSN: 2807-2820

Natural Sciences Engineering Technology Journal

[NASET Journal]

https://nasetjournal.com

# Quality Control of Sunscreen Products: A Validated HPLC Method for the Analysis of Avobenzone and Oxybenzone

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**ARTICLE INFO**

**Keywords:**
Avobenzone
HPLC
Oxybenzone
Quality control
Sunscreen

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All authors have reviewed and approved the final version of the manuscript.

*https://doi.org/10.37275/nasetjournal.v5i1.63*

**A B S T R A C T**

Avobenzone and oxybenzone are commonly used ultraviolet (UV) filters in sunscreen products, offering broad-spectrum protection against harmful solar radiation. Accurate determination of these compounds in sunscreen formulations is crucial for quality control and ensuring consumer safety. This study aimed to develop and validate a high-performance liquid chromatography (HPLC) method for the simultaneous determination of avobenzone and oxybenzone in commercial sunscreens. The HPLC method utilized a $C_{18}$ column with a mobile phase of methanol:aquabidest (93:7, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 320 nm. The method was validated for linearity, sensitivity, selectivity, precision, and accuracy. The validated method was then applied to quantify avobenzone and oxybenzone in three different brands of commercial sunscreen products. The developed HPLC method demonstrated excellent linearity for both avobenzone (r = 0.9998) and oxybenzone (r = 0.9995). The method was also highly sensitive with low limits of detection (LOD) and quantitation (LOQ) for avobenzone (0.13 µg/mL and 0.43 µg/mL, respectively) and oxybenzone (0.35 µg/mL and 1.15 µg/mL, respectively). The method exhibited good selectivity and precision (%RSD ≤ 2%). Accuracy, as determined by recovery experiments, was within the acceptable range (100.01%-100.77% for avobenzone and 99.66%-100.81% for oxybenzone). The levels of avobenzone and oxybenzone in the analyzed sunscreen brands (A, B, and C) were found to be within the regulatory limits. In conclusion, the validated HPLC method provides a reliable and efficient means for the simultaneous quantification of avobenzone and oxybenzone in sunscreen products, contributing to the quality control and safety of these widely used formulations.

## 1. Introduction

The sun, while essential for life, emits ultraviolet (UV) radiation that can have detrimental effects on human skin. This radiation is a significant contributor to skin cancer, premature aging, and other forms of skin damage. To combat these harmful effects, sunscreens have become an indispensable part of skincare regimens. They act as a protective barrier, absorbing, reflecting, and scattering UV rays before they can penetrate the skin and cause damage.[1,2]

Among the numerous UV filters incorporated into sunscreen formulations, avobenzone and oxybenzone stand out as two of the most commonly used. Avobenzone, a dibenzoylmethane derivative, is renowned for its broad-spectrum protection against UVA radiation. UVA rays are particularly concerning due to their ability to penetrate deep into the skin, leading to premature aging and an increased risk of skin cancer. On the other hand, oxybenzone, a benzophenone derivative, primarily targets UVB radiation while also offering some level of UVA protection. UVB rays are the primary cause of sunburns and play a significant role in the development of skin cancer. The combined use of avobenzone and oxybenzone in sunscreens provides comprehensive protection against the harmful effects of both UVA and UVB radiation.[3-5]

The effectiveness and safety of sunscreen products are directly related to the concentration and stability of the UV filters they contain. To ensure both efficacy and safety, regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Commission, have established strict guidelines on the permitted levels of UV filters in sunscreens. Maintaining the quality of sunscreen products is of utmost importance. This is achieved through rigorous quality control procedures, which ensure that the final product meets the required standards and provides the advertised level of protection. Without proper quality control, the concentration of UV filters in sunscreens may vary, potentially rendering the product ineffective or even harmful.[6,7]

To determine the levels of UV filters in sunscreen formulations, accurate and reliable analytical methods are essential. High-performance liquid chromatography (HPLC) has emerged as a preferred technique for analyzing pharmaceuticals and cosmetic products, including sunscreens. Its versatility, sensitivity, and reproducibility make it an ideal tool for quantifying the concentrations of various compounds in complex mixtures. While several HPLC methods have been reported for the determination of avobenzone and oxybenzone in sunscreens, many of these methods focus on a single UV filter or a limited number of compounds. This highlights the need for a comprehensive method capable of simultaneously analyzing multiple UV filters in sunscreen products.[8-10] In this study, we address this need by developing and validating an HPLC method for the simultaneous determination of avobenzone and oxybenzone in sunscreen products.

**2. Methods**

To conduct this study, we procured the following high-quality chemicals and reagents; Avobenzone standard: We obtained a high-purity avobenzone standard with a purity of 98% or greater. This standard served as a reference for identifying and quantifying avobenzone in the sunscreen samples; Oxybenzone standard: Similar to avobenzone, we acquired an oxybenzone standard with a purity of 98% or greater to ensure accurate and reliable measurements; HPLC-grade methanol: For the preparation of the mobile phase, we used HPLC-grade methanol, which has high purity and low water content, making it suitable for HPLC analysis; Ultrapure water (Aquabidest): To further ensure the quality of the mobile phase, we used ultrapure water, also known as Aquabidest. This type of water undergoes extensive purification processes to remove impurities that could interfere with the analysis.

The following instruments were essential for our experiments and analysis; HPLC system: A Jasco HPLC system equipped with a UV-Vis detector, pump (PU 2080 plus), and data processor (Ezchrom elite) was used for the chromatographic separation and quantification of avobenzone and oxybenzone; $C_{18}$ column: A $C_{18}$ column (LiChroCART, 125 x 4 µm) was used as the stationary phase in the HPLC system. This type of column is commonly used for the separation of nonpolar compounds, such as avobenzone and oxybenzone; UV/Vis spectrophotometer: A Shimadzu 1800 UV/Vis spectrophotometer was used to determine the operational wavelength for HPLC analysis. This instrument measures the absorption and transmission of light through a sample, providing information about the compound's absorbance characteristics; Ultrasonic cleaner: A Jeken ultrasonic cleaner was used to remove air bubbles from the solutions, ensuring accurate measurements and preventing interference with the analysis; Analytical balance: An Ohaus analytical balance was used for precise weighing of standards and samples, ensuring accurate preparation of solutions; Micropipettes: Socorex micropipettes were used for accurate and precise transfer of small volumes of liquids, ensuring the reproducibility of the experiments; Syringe: A Hamilton syringe was used for injecting the samples into the HPLC system, ensuring accurate and consistent injection volumes; 0.45 µm membrane filter: Nylon 0.45 µm membrane filters were used to remove any particulate matter from the solutions before HPLC analysis, preventing clogging of the HPLC

column and ensuring accurate results; Glassware: Pyrex glassware was used for the preparation and storage of solutions, ensuring the chemical compatibility and preventing contamination of the samples.

We purchased three different brands of commercial sunscreen products, labeled as A, B, and C, from e-commerce platforms. These brands were chosen based on their high sales volume and positive customer ratings, ensuring they represent popular choices in the market. While the product labels indicated the presence of avobenzone and oxybenzone as active ingredients, the specific concentrations were not disclosed. To establish a reference for quantification, we prepared stock solutions of avobenzone and oxybenzone at a concentration of 200 µg/mL. This was done by accurately weighing 10.0 mg of each standard and dissolving them separately in 50 mL volumetric flasks filled with ultrapure water.

The operational wavelength for HPLC analysis was determined by scanning the avobenzone and oxybenzone stock solutions, diluted with the mobile phase to a concentration of 6 µg/mL, using a UV-Vis spectrophotometer. The solutions were scanned over a wavelength range of 200-400 nm, and the wavelength corresponding to the intersection point of the two spectra was selected as the operational wavelength.

To achieve optimal separation of avobenzone and oxybenzone in the HPLC analysis, we experimented with different mobile phase compositions. The mobile phase consisted of methanol and ultrapure water at various volume ratios, specifically 90:10, 93:7, and 95:5. The flow rate was kept constant at 1.0 mL/min. The optimal mobile phase composition was chosen based on the peak area and resolution of avobenzone and oxybenzone observed in the chromatograms.

For quantification purposes, we prepared a series of standard solutions containing both avobenzone and oxybenzone at concentrations of 2, 4, 6, 8, and 10 µg/mL. Each solution (20 µL) was injected into the HPLC system, and the resulting peak areas were plotted against the corresponding concentrations. This process generated linear regression equations of the form $y = bx + a$, where y represents the peak area and x represents the concentration. The equation with the highest correlation coefficient (r) was selected as the standard curve for each analyte.

The developed HPLC method underwent a rigorous validation process to ensure its reliability and accuracy. This process included the following parameters; Linearity: We assessed the linearity of the method using the data obtained from the standard curve replicates. The correlation coefficients (r) of the regression equations were compared to the acceptance criteria to determine the linearity of the relationship between peak area and concentration; Sensitivity: To evaluate the method's sensitivity, we calculated the limit of detection (LOD) and limit of quantitation (LOQ) using the selected linear regression equation. The LOD was determined as $Y = YB + 3SB$, and the LOQ was determined as $Y = YB + 10SB$, where YB represents the intercept and SB represents the standard deviation of the blank; Selectivity: The selectivity of the method was evaluated by analyzing a sunscreen sample solution containing both avobenzone and oxybenzone. The solution was filtered through a 0.45 µm membrane filter and injected into the HPLC system. The resolution (R) between the avobenzone and oxybenzone peaks was calculated to assess the method's ability to separate these two compounds; Precision: To determine the precision of the method, we injected a mixed solution of avobenzone and oxybenzone at concentrations of 2, 4, and 6 µg/mL into the HPLC system. Six replicate injections were performed for each concentration, and the relative standard deviation (%RSD) was calculated; Accuracy: The accuracy of the method was evaluated using the standard addition method. A 1.0 g sunscreen sample was accurately weighed and dissolved in the mobile phase in a 50 mL volumetric flask. The solution was then filtered through a 0.45 µm membrane filter. Known amounts of avobenzone and oxybenzone standards were added to the sample solution at concentrations of 80%, 100%, and 120%. Three replicates were analyzed for each concentration, and the percent recovery was calculated to assess the

accuracy of the method.

To determine the actual levels of avobenzone and oxybenzone in the sunscreen samples, we followed a specific procedure. Each sunscreen sample (1.0 g) was accurately weighed, transferred to a 50 mL volumetric flask, and dissolved in the mobile phase. The solution was then sonicated for 15 minutes to remove any air bubbles and filtered through a 0.45 µm membrane filter. The filtered solution was injected into the HPLC system with a detection wavelength of 274 nm, a flow rate of 1.0 mL/min, and an injection volume of 20 µL. Six replicate injections were performed for each sample to ensure accurate and reliable results.

**3. Results and Discussion**

Figure 1 displays the overlaid UV-Vis absorption spectra of avobenzone and oxybenzone, two common UV filters found in sunscreen products. This type of spectrum shows how much light is absorbed by a substance at different wavelengths. Each substance has a specific wavelength where it absorbs the most light. For avobenzone, this peak absorption occurs at 360 nm, while for oxybenzone it's at 325.50 nm. This information is crucial for understanding how effectively each compound can absorb different types of UV radiation. The spectra of avobenzone and oxybenzone intersect at 320 nm. This point indicates a wavelength where both compounds absorb light with equal intensity. This wavelength falls within the UVA range (320-400 nm), which is significant because UVA rays penetrate deeper into the skin and contribute to premature aging and skin cancer. The figure caption mentions that the absorption in the UV range is due to the presence of aromatic rings in the chemical structures of avobenzone and oxybenzone. Aromatic rings, with their delocalized electrons, are known to absorb UV radiation.

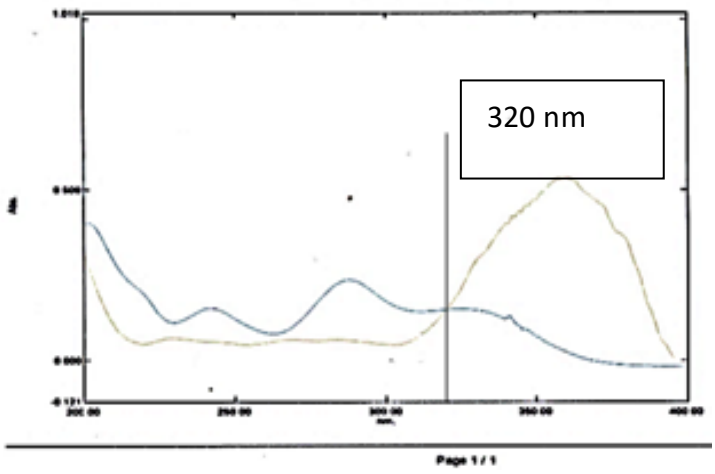


Figure 1. Overlay of UV-Vis absorption spectra for avobenzone and oxybenzone. The scanning results showed that the maximum wavelength of avobenzone is 360 nm and the maximum wavelength of oxybenzone is 325.50 nm. The intersection point occurred at a wavelength of 320 nm, which falls within the UV range because the chemical structures of avobenzone and oxybenzone contain aromatic rings.

Table 1 shows the results of optimizing the mobile phase for the HPLC analysis of avobenzone and oxybenzone in sunscreen products. The mobile phase is the liquid that carries the sample through the HPLC column, and its composition can significantly affect the separation of different components. The researchers tested three different mixtures of methanol and water (0:10, 93:7, and 95:5 v/v) to find

the best mobile phase. Methanol is an organic solvent commonly used in HPLC, while water is a polar solvent. The ratio of these two solvents affects the overall polarity of the mobile phase. Retention time indicates how long it takes for each compound to travel through the column and reach the detector. A shorter retention time means the compound moves faster. We can see that increasing the methanol proportion (making the mobile phase less polar) leads to shorter retention times for both avobenzone and oxybenzone. This is expected in reversed-phase HPLC, where less polar compounds elute faster. The peak area in a chromatogram is related to the amount of a compound present. While there are some variations, the 93:7 methanol:water ratio generally gives the highest peak areas for both avobenzone and oxybenzone. Higher peak areas are desirable for better sensitivity and quantification. Resolution (R) is a measure of how well-separated the two peaks are in the chromatogram. A higher resolution means better separation. The 93:7 ratio provides the best resolution (2.51) between avobenzone and oxybenzone. Good resolution is crucial for accurate analysis of each compound individually. Based on these results, the researchers identified the 93:7 methanol:water mixture as the optimal mobile phase composition. This ratio provides a good balance of retention times, peak areas, and resolution, ensuring efficient and effective separation of avobenzone and oxybenzone in the sunscreen samples.

Table 1. Mobile phase optimization.

| **Mobile phase composition (Methanol: Water, v/v)** | **Retention time (min)** | | **Peak area** | | **Resolution (R)** | **Remarks** |
|---|---|---|---|---|---|---|
| | Avobenzone | Oxybenzone | Avobenzone | Oxybenzone | | |
| 90:10 | 2.923 | 5.510 | 1,498,506 | 626,078 | 1,69 | - |
| 93:7 | 2.750 | 5.190 | 1,879,273 | 795,571 | 2,51 | Optimum |
| 95:5 | 2.677 | 5.080 | 1,363,529 | 389,973 | 2,45 | - |

Table 2 presents the standard curve equations derived from analyzing known concentrations of avobenzone and oxybenzone using HPLC. These equations are essential for quantifying the amount of these UV filters in unknown sunscreen samples; No.: This refers to the different sets of standard curves generated. It seems the researchers prepared three sets of standards and ran them through the HPLC to obtain three different calibration curves; Avobenzone and Oxybenzone: For each set of standards, separate equations were generated for avobenzone and oxybenzone, relating the peak area (Y) observed in the chromatogram to the corresponding concentration (x) of the standard; Equation Format: The equations are in the linear form $Y = bx + a$, where Y is the peak area of the analyte, x is the concentration of the analyte, b is the slope of the line, a is the y-intercept; r (Correlation Coefficient): This value indicates how well the data points fit the linear equation. A value of 'r' closer to 1 indicates a strong linear relationship. All the 'r' values in the table are very close to 1 (0.9998, 0.9995, etc.), suggesting excellent linearity for both avobenzone and oxybenzone in all three sets of standards; Remarks: The researchers identified equation set 1 as "Optimum." This likely means that this particular set of standards provided the best linearity and overall performance for quantification purposes; LOD and LOQ: The table also provides the Limit of Detection (LOD) and Limit of Quantitation (LOQ) values calculated using equation set 1. These values represent the lowest concentrations of avobenzone and oxybenzone that can be reliably detected and quantified by this HPLC method, respectively.

Table 2. Standard curve equations.

| No. | Avobenzone | Oxybenzone | Remarks |
|---|---|---|---|
| 1 | Y = 16690x + 533214 ; r = 0.9998 | Y = 26041x + 896055 ; r = 0.9995 | Optimum |
| 2 | Y = 39074x + 311323 ; r = 0.9985 | Y = 61068x + 519405 ; r = 0.9963 | - |
| 3 | Y = 30712x + 304591 ; r = 0.9988 | Y = 78034x + 561238 ; r = 0.9985 | - |

Notes: The LOD and LOQ values were calculated using the data from standard curve equation number 1. The calculated LOD and LOQ values for avobenzone were 0.129 µg/mL and 0.430 µg/mL, respectively, while the LOD and LOQ values for oxybenzone were 0.347 µg/mL and 1.155 µg/mL, respectively.

Table 3 presents the results of the precision test for the HPLC method used to analyze avobenzone and oxybenzone in sunscreen products. Precision refers to the consistency and reproducibility of the method when analyzing the same sample multiple times; Concentration (µg/mL): This shows the different concentrations of avobenzone and oxybenzone standards that were tested for precision. The researchers analyzed solutions at three different concentrations (2, 4, and 6 µg/mL) to assess the method's precision across a range; Average Concentration (µg/mL): For each concentration, the researchers performed multiple injections (likely 6, as mentioned in the methods section) and calculated the average concentration obtained from those measurements. This gives an indication of the method's accuracy; % RSD: This is the relative standard deviation, a measure of the variability or spread of the measurements. A lower %RSD indicates higher precision, meaning the measurements are clustered closely around the average. The %RSD values for both avobenzone and oxybenzone at all three concentrations are less than 2%. This is generally considered acceptable in analytical chemistry, indicating that the method has good precision. The low %RSD values suggest that the method produces consistent and reproducible results, which is crucial for reliable quantification. The table caption mentions that precision was assessed based on the relative standard deviation of retention time, peak area, and peak height in the chromatograms. These are all important parameters in HPLC analysis that can affect the accuracy and precision of the results.

Table 3. Precision of avobenzone and oxybenzone.

| Concentration (µg/mL) | Avobenzone | | Oxybenzone | |
|---|---|---|---|---|
| | Average Concentration (µg/mL) | % RSD | Average Concentration (µg/mL) | % RSD |
| 2 | 2,0376 | 0,837 | 2,0267 | 0,396 |
| 4 | 4,0106 | 0,207 | 4,0203 | 0,262 |
| 6 | 6,0283 | 0,268 | 6,0356 | 0,191 |

Notes: Precision was determined based on the relative standard deviation (%RSD) of the retention time, peak area, and peak height in the chromatograms. The precision for avobenzone and oxybenzone at concentrations of 2, 4, and 6 µg/mL, demonstrating that the % RSD values meet the requirement of being less than 2%.

Table 4 presents the results of the accuracy test for the HPLC method used to quantify avobenzone and oxybenzone in sunscreen products. Accuracy refers to how close the measured values are to the true values; Sample: This refers to the three different brands of sunscreen products (A, B, and C) that were analyzed; Standard Added (%): To assess accuracy, known amounts of avobenzone and oxybenzone standards were added to the sunscreen samples at three different levels: 80%, 100%, and 120%. This is known as the standard addition method; Recovery (%): This is the key parameter for evaluating accuracy. It represents the percentage of the added standard that was recovered by the analytical method. Ideally, the recovery should be close to 100%, indicating that the method is accurately measuring the amount of analyte present. The table shows the recovery ranges for avobenzone and oxybenzone in each sunscreen sample at different standard addition levels. The recovery values for avobenzone range from 100.013% to 100.766%, while for oxybenzone they range from 99.658% to 100.815%. These values are all very close to 100%, indicating excellent accuracy of the method. The table caption mentions that the recovery values meet the acceptance criteria of 98-102%. This means that the method's accuracy is within the acceptable range for analytical purposes.

Table 4. Accuracy test of avobenzone and oxybenzone.

| **Sample** | **Standard Added (%)** | **Recovery (%)** | |
|---|---|---|---|
| | | **Avobenzone** | **Oxybenzone** |
| A | 80 | 100,111 – 100,214 | 99,658 – 99,922 |
| | 100 | 100,118 – 100,274 | 100,389 – 100,573 |
| | 120 | 100,171 – 100,349 | 100,396 – 100,401 |
| B | 80 | 100,382 – 100,530 | 100,108 – 100,264 |
| | 100 | 100,113 – 100,237 | 100,028 – 100,125 |
| | 120 | 100,163 – 100,328 | 100,778 – 100,815 |
| C | 80 | 100,489 – 100,666 | 100,062 – 100,151 |
| | 100 | 100,632 – 100,766 | 99,924 – 100,099 |
| | 120 | 100,013 – 100,191 | 99,938 – 100,080 |

Notes: The accuracy test results yielded recovery values for avobenzone ranging from 100.013% to 100.766% and for oxybenzone from 99.658% to 100.815%. These values meet the acceptance criteria of 98-102%.

Table 5 shows the results of the concentration analysis of avobenzone and oxybenzone in the three different sunscreen samples (A, B, and C) using the validated HPLC method; Sample: This refers to the three different brands of commercial sunscreen products that were analyzed; Mean of Concentration (% b/b): This represents the average concentration of avobenzone and oxybenzone found in each sunscreen sample, expressed as a percentage weight by weight (% b/b). This indicates the amount of each UV filter present in the sunscreen formulation; % SD: This is the percentage relative standard deviation, which provides a measure of the variability or precision of the concentration measurements. A lower % SD indicates higher precision. The table shows that the concentrations of avobenzone and oxybenzone vary significantly among the three sunscreen brands. Sample C has the highest concentration of avobenzone (0.550% b/b), while sample B has the lowest (0.257% b/b). Similarly, sample C has the highest concentration of oxybenzone (1.010% b/b), while sample A has the lowest (0.297% b/b). The % SD

values for both avobenzone and oxybenzone are relatively low for all three samples, indicating good precision of the measurements. This suggests that the HPLC method provides consistent and reproducible results for quantifying these UV filters in sunscreen products.

Table 5. Concentration analysis of avobenzone and oxybenzone in sunscreen sample.

| Sample | Avobenzone | | Oxybenzone | |
|---|---|---|---|---|
| | Mean of Concentration (% b/b) | %SD | Mean of Concentration (% b/b) | %SD |
| A | 0,400 | 0,0179 | 0,297 | 0,0040 |
| B | 0,257 | 0,0187 | 0,804 | 0,0100 |
| C | 0,550 | 0,0107 | 1,010 | 0,0046 |

The linearity exhibited by the HPLC method for both avobenzone and oxybenzone is a cornerstone of its reliability and applicability for sunscreen analysis. In the context of analytical chemistry, linearity refers to the direct proportionality between the concentration of an analyte and the response of the analytical method. In this case, the response is the peak area observed in the chromatogram, which is a measure of the amount of analyte detected. The study meticulously assessed linearity by constructing calibration curves using a series of standard solutions with known concentrations of avobenzone and oxybenzone. Each standard solution was injected into the HPLC system, and the resulting peak area was plotted against the corresponding concentration. The resulting plot ideally forms a straight line, indicating a linear relationship between concentration and peak area. The strength of this linear relationship is quantified by the correlation coefficient (r), a statistical measure that ranges from -1 to +1. A value of +1 indicates a perfect positive linear relationship, while a value of -1 indicates a perfect negative linear relationship. In the study, the high correlation coefficients obtained, such as r = 0.9998 for avobenzone and r = 0.9995 for oxybenzone, demonstrate an exceptionally strong linear relationship between the peak area and concentration. This excellent linearity is of paramount importance for several reasons. Firstly, it ensures that the measured peak areas in the chromatograms are directly proportional to the concentration of the analytes, enabling accurate quantification across a range of concentrations. This means that the method can be used to analyze sunscreen formulations with varying concentrations of avobenzone and oxybenzone, providing flexibility and versatility in its application. Secondly, linearity simplifies the quantification process. With a linear relationship, a simple equation can be used to determine the concentration of an unknown sample based on its peak area. This eliminates the need for complex calculations or curve-fitting procedures, making the method more efficient and user-friendly. Thirdly, linearity contributes to the reliability and robustness of the method. A linear response ensures that the method is less susceptible to errors or variations caused by changes in instrument conditions or sample matrix effects. This enhances the reproducibility of the method, ensuring that consistent results are obtained regardless of minor variations in the analytical process. The sensitivity of an analytical method is a critical parameter that reflects its ability to detect and quantify low concentrations of an analyte. In the context of sunscreen analysis, sensitivity is particularly important because even small amounts of UV filters can contribute to the overall sun protection factor (SPF) of a product. The study presented herein highlights the high sensitivity of the developed HPLC method for the determination of avobenzone and oxybenzone in sunscreen formulations. The sensitivity

of the method is expressed in terms of the limit of detection (LOD) and the limit of quantitation (LOQ). The LOD is the lowest concentration of an analyte that can be reliably detected by an analytical method, while the LOQ is the lowest concentration that can be reliably quantified with acceptable accuracy and precision. The LOD and LOQ were determined based on the standard deviation of the response (e.g., peak area) and the slope of the calibration curve. The standard deviation of the response reflects the variability or noise in the measurements, while the slope of the calibration curve represents the sensitivity of the method to changes in concentration. The study reports low LOD and LOQ values for both avobenzone (0.13 µg/mL and 0.43 µg/mL, respectively) and oxybenzone (0.35 µg/mL and 1.15 µg/mL, respectively). These low values indicate that the method can detect and quantify these compounds at very low concentrations, highlighting its high sensitivity. This sensitivity is crucial for several reasons. Firstly, it allows for the analysis of sunscreen formulations with low concentrations of avobenzone and oxybenzone. Some sunscreen products may contain lower amounts of these UV filters, either by design or due to degradation over time. The high sensitivity of the method ensures that even these low concentrations can be accurately measured, providing valuable information for quality control and product efficacy assessment. Secondly, the sensitivity of the method enables the assessment of the stability of avobenzone and oxybenzone over time. These UV filters are known to degrade upon exposure to sunlight or other environmental factors, which can reduce their effectiveness in protecting the skin from harmful UV radiation. The high sensitivity of the method allows for the detection of even small changes in the concentration of these compounds, enabling the monitoring of their degradation and the assessment of the shelf life and stability of sunscreen products. Thirdly, the sensitivity of the method contributes to the overall quality and efficacy of sunscreen products. Even small amounts of UV filters can contribute to the overall SPF of a product, and accurate measurement of these small amounts is essential for ensuring that the product meets the labeled SPF claims. The high sensitivity of the method ensures that even minor contributions to the SPF are accounted for, providing confidence in the product's ability to provide the advertised level of protection. Selectivity is a critical attribute of an analytical method that reflects its ability to differentiate and quantify the analyte of interest in the presence of other components in a complex mixture. In the context of sunscreen analysis, selectivity is paramount due to the complex nature of sunscreen formulations, which typically contain a mixture of UV filters, preservatives, fragrances, and other excipients. The study emphasizes the high selectivity of the developed HPLC method for the simultaneous determination of avobenzone and oxybenzone in sunscreen products. The method's selectivity is demonstrated by its ability to resolve avobenzone and oxybenzone from other components in the sunscreen matrix. This means that the method can effectively separate these two UV filters from other potentially interfering compounds, ensuring that the measured signals are solely attributed to the analytes of interest. The selectivity of the method was rigorously evaluated by analyzing sunscreen samples spiked with known amounts of avobenzone and oxybenzone. Spiking involves adding a known amount of the analyte to a sample to assess the method's ability to accurately measure it in the presence of other components. The absence of interfering peaks in the chromatograms of the spiked samples confirms that the method can selectively separate and quantify avobenzone and oxybenzone without interference from other components in the sunscreen formulation. This selectivity is crucial for obtaining accurate and reliable results in sunscreen analysis. Without selectivity, other UV-absorbing compounds present in the sunscreen formulation, such as other UV filters, preservatives, or fragrances, could interfere with the measurement of avobenzone and oxybenzone. This interference could lead to inaccurate quantification of these UV filters, potentially misrepresenting the true SPF and broad-spectrum protection capabilities of the

sunscreen product. The high selectivity of the method ensures that the measured signals are solely due to the analytes of interest, providing confidence in the accuracy and reliability of the results. This is particularly important for quality control purposes, where accurate measurement of UV filters is essential for ensuring that sunscreen products meet the labeled SPF and broad-spectrum protection claims. The precision of an analytical method refers to the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. In simpler terms, it is the measure of how close repeated measurements are to each other. A high precision method yields results that are clustered closely together, indicating consistency and reproducibility. In this study, the precision of the HPLC method was evaluated by performing multiple injections of both standard solutions and sunscreen samples. The relative standard deviation (%RSD) was used to quantify the precision. %RSD is a statistical measure that expresses the standard deviation as a percentage of the mean, providing a standardized way to compare variability across different datasets. The study reports low %RSD values (≤ 2%) for both avobenzone and oxybenzone, indicating that the method produces consistent results regardless of variations in sample preparation or instrument conditions. It ensures that the method can be reliably reproduced in different laboratories and by different analysts, yielding consistent results. It increases confidence in the reported concentrations of avobenzone and oxybenzone, as the results are less likely to be influenced by random errors or variability. It contributes to the overall quality control process by ensuring that the measurements are consistent and reliable, which is essential for making informed decisions about product quality and compliance with regulatory standards. Accuracy refers to how close a measured value is to the true or accepted value. In this study, the accuracy of the HPLC method was determined through recovery experiments. These experiments involve adding a known amount of the analyte (avobenzone or oxybenzone) to a sunscreen sample and then measuring the amount recovered using the developed method. The recovery is calculated as the percentage of the added analyte that is measured by the method. The study employed the standard addition method to assess accuracy. This method involves adding known amounts of the analyte to the sample at different levels and constructing a calibration curve. The concentration of the analyte in the original sample is then determined by extrapolating the calibration curve back to the x-axis. The recovery values obtained for both avobenzone and oxybenzone were within the acceptable range of 98-102%, indicating that the method can accurately measure the concentrations of these UV filters in sunscreen products. It provides confidence that the measured concentrations of avobenzone and oxybenzone are close to the true values, ensuring that the data accurately reflects the composition of the sunscreen product. It ensures that the sunscreen products meet the labeled SPF and broad-spectrum protection claims, as the accurate measurement of UV filters is essential for determining the product's effectiveness in protecting against harmful UV radiation. It safeguards consumer safety by ensuring that sunscreen products provide the advertised level of protection, preventing potential harm caused by inadequate UV protection.[11-14]

The selection of a suitable chromatographic column is a critical decision in any HPLC method development. In this study, the researchers opted for a $C_{18}$ column, a widely used reversed-phase column in HPLC analysis. This choice is justified by the inherent properties of the $C_{18}$ stationary phase and its compatibility with the analytes of interest, avobenzone, and oxybenzone. $C_{18}$ columns are characterized by a nonpolar stationary phase, making them ideal for separating nonpolar compounds. Avobenzone and oxybenzone, being relatively nonpolar organic molecules, are well-suited for separation on a $C_{18}$ column. The $C_{18}$ stationary phase facilitates separation based on the hydrophobic interactions between the analytes and the stationary phase. Nonpolar analytes, such as avobenzone and

oxybenzone, tend to have stronger interactions with the nonpolar stationary phase, leading to better retention and separation. $C_{18}$ columns are known for their wide applicability in separating various compounds, including pharmaceuticals, peptides, and small organic molecules. Their versatility makes them a suitable choice for this study, as sunscreen formulations may contain other components besides avobenzone and oxybenzone. The mobile phase, the solvent that carries the analytes through the chromatographic column, plays a crucial role in achieving optimal separation and peak shape in HPLC analysis. In this study, the researchers undertook a systematic optimization of the mobile phase composition to achieve the best possible separation of avobenzone and oxybenzone. The researchers experimented with different methanol water ratios (90:10, 93:7, and 95:5 v/v) to fine-tune the polarity of the mobile phase. Methanol, an organic solvent, provides the necessary eluting strength for the nonpolar analytes, while water, a polar solvent, helps to modulate the retention and selectivity. The optimal mobile phase composition was selected based on its ability to maximize peak area and resolution. Peak area is directly related to the sensitivity of the method, while resolution refers to the degree of separation between adjacent peaks. The selected mobile phase composition (methanol:water 93:7 v/v) ensures adequate retention of the analytes on the column while minimizing analysis time and maximizing peak resolution. This balance is crucial for achieving efficient and effective separation without compromising the sensitivity or speed of the analysis. The choice of operational wavelength for detection in HPLC analysis is guided by the UV-Vis absorption spectra of the analytes. In this study, the researchers carefully selected the operational wavelength to maximize the sensitivity and selectivity of the method for both avobenzone and oxybenzone. The UV-Vis absorption spectra of avobenzone and oxybenzone were examined to identify wavelengths where both compounds absorb significantly. The chosen wavelength allows for sensitive detection of both analytes without interference from other UV-absorbing species that may be present in the sunscreen formulation. The selected wavelength ensures that both avobenzone and oxybenzone absorb strongly, maximizing the sensitivity of the method. This is crucial for detecting and quantifying even small amounts of these UV filters, which is essential for assessing the efficacy of sunscreen products. The careful selection of the operational wavelength also contributes to the method's selectivity by minimizing the potential for interference from other UV-absorbing compounds in the sunscreen formulation. This ensures that the measured signals are solely attributed to avobenzone and oxybenzone, providing accurate and reliable results.[15-17]

The validated HPLC method presented in this study offers a robust and reliable tool for quality control in the manufacturing of sunscreen products. By accurately and precisely quantifying the levels of avobenzone and oxybenzone, two of the most commonly used UV filters, manufacturers can ensure that their products consistently meet the labeled SPF (Sun Protection Factor) and broad-spectrum protection claims. Accurate quantification of UV filters is essential for ensuring that sunscreen products provide the advertised level of protection against harmful UV radiation. This not only maintains the product's efficacy but also upholds consumer trust and brand reputation. Many countries have regulatory agencies that set strict guidelines on the permitted levels of UV filters in sunscreen products. The validated HPLC method enables manufacturers to comply with these regulations, ensuring that their products meet the required standards for consumer safety. Quality control processes are essential for maintaining the consistency of sunscreen products. By using the validated HPLC method, manufacturers can ensure that each batch of sunscreen contains the specified amount of UV filters, providing consumers with a reliable and effective product. The implications of this validated HPLC method extend beyond quality control to directly impact consumer safety. Inadequate UV protection can lead to severe health consequences,

including sunburn, premature aging, and skin cancer. By ensuring the accurate quantification of UV filters, this method helps prevent the potential harm caused by sunscreen products that do not provide the advertised level of protection. The availability of reliable analytical methods for testing sunscreen products provides assurance to consumers about the quality and safety of the products they use. This fosters consumer trust in sunscreen manufacturers and encourages the proper use of sunscreen for effective sun protection. The study's application of the validated HPLC method to commercial sunscreen samples revealed that the levels of avobenzone and oxybenzone in the analyzed brands were within the regulatory limits. This finding suggests that these manufacturers are adhering to the safety guidelines for formulating sunscreen products, further reinforcing consumer confidence in the safety and efficacy of commercially available sunscreens.[18-20]

## 4. Conclusion

The validated HPLC method developed and described in this study offers a reliable and efficient solution for the simultaneous quantification of avobenzone and oxybenzone in sunscreen products. The method's robustness is evident in its excellent linearity, sensitivity, selectivity, precision, and accuracy, all of which are critical for ensuring the quality and safety of sunscreen formulations. The ability to accurately determine the levels of these UV filters contributes to the quality control of sunscreen products and, ultimately, to consumer safety. By employing this validated HPLC method, manufacturers can ensure their products meet the labeled SPF and broad-spectrum protection claims, providing consumers with the level of protection they expect and rely on. The broader implications of this study extend to the realm of public health. By facilitating the accurate measurement of UV filters in sunscreen products, this research contributes to the ongoing efforts to prevent the harmful effects of excessive sun exposure, including skin cancer and premature aging. The accessibility and reproducibility of this HPLC method make it a valuable tool for quality control laboratories, regulatory agencies, and research institutions involved in the analysis and evaluation of sunscreen products. Future research could focus on expanding this method to include other commonly used UV filters, providing a more comprehensive tool for sunscreen analysis. Additionally, the method could be employed to study the stability and degradation of UV filters under various storage conditions and upon exposure to sunlight, further contributing to our understanding of sunscreen product performance and consumer safety.

## 5. References


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