# Analysis of parabens in over-the-counter cosmetics by gas chromatography-mass spectrometry and their binding affinity to bovine serum albumin by fluorescence spectroscopy

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# Analysis of parabens in over-the-counter cosmetics by gas chromatography-mass spectrometry and their binding affinity to bovine serum albumin by fluorescence spectroscopy

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**Abstract**:

Cosmetics are an integral part of the beauty world and by extension, most of what is incorporated in our social lives, therefore, the ingredient contents of cosmetic formulas can influence overall health and well-being. Preservatives like parabens, are required within their formulas, however, reports show a correlation between cancers, such as breast cancer, and paraben exposure leading to its restricted use and banning of various forms of paraben within cosmetics. Once parabens pass the dermal barrier, they move within the body toward various organs and can bind to estrogenic receptors and affect their function. Foundations from four common drug store cosmetics were analyzed via gas chromatography-mass spectrometry (GC-MS) to determine the presence and identify of the parabens contained within. All four formulas contained the preservative methyl paraben, however, each foundation varied with having either propyl, butyl, or ethyl paraben as the additional preservative due to varying cosmetic formulas. Lastly, the binding affinity of each paraben to the major transport protein bovine serum albumin (BSA), was quantified via fluorescence quenching to model the travelling efficiency of the different parabens within the body.

**Introduction**:

Preservatives are added to cosmetics and other personal-care product (PCP) formulas to inhibit microbial growth, thus increasing their overall shelf life and protect consumers from bacterial growth in multiple use products. Benzoic acid and esters of *p*-hydroxybenzoic acid, such as parabens, are often used as preservatives in various household products [2, 7, 12]. Methyl paraben, ethyl paraben, propyl paraben, and butyl paraben are the most common esters used since they have antibacterial properties as they inhibit substrate uptake in bacteria [12]. However, there has been some controversy regarding the relationship between parabens and the inhibition of estrogenic activity, as well as the presence of parabens in human breast tumors [9, 10]. As a result, the European union established strict regulations regarding the concentration of parabens permitted in all household products. The total concentration of parabens must be below 0.8% of the total product mass with a maximum concentration of 0.4% product mass for a single paraben [8]. The United States on the other hand, has no FDA regulations on the concentration of parabens in their products [6].

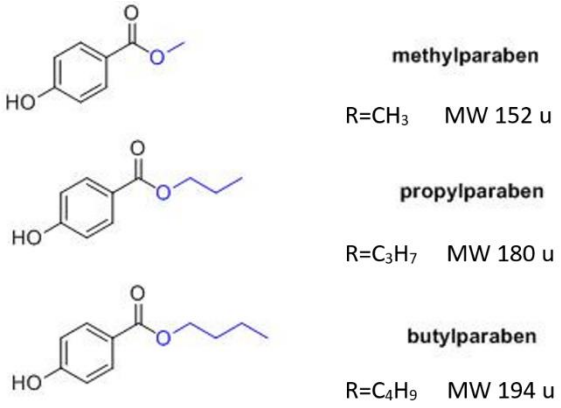


**Figure 1.** The chemical structure of the four most common parabens used in cosmetics [15].

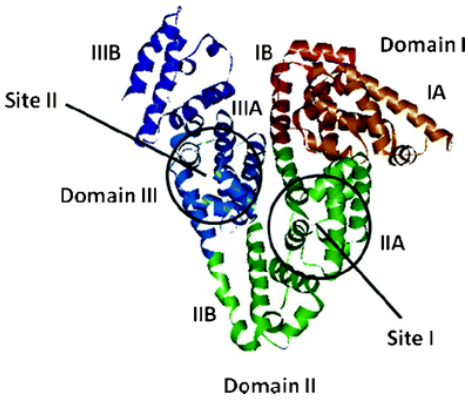


**Figure 2.** The molecular structure of the receptor protein Bovine Serum Albumin (BSA)[16].



Multiple analytical methods have been adapted to identify and quantify these preservatives in various cosmetic formulations such as foundations and lotions. Gas chromatography-mass spectrometry (GC-MS) is quick and efficient way to identify various molecules. GC-MS requires a small amount of sample to be vaporized, separated in a capillary

column, and analyzed through an attached mass-spectrometer. GC-MS was used as the first separation and detection tool for the analysis of these preservatives within various over-the-counter cosmetic foundations [1, 5, 11, 13, 14]. This study uses this method to determine the various amounts of parabens present within the samples.

Most paraben-containing cosmetics are applied topically, which allows parabens to absorb into the skin and into the bloodstream. Parabens have been shown to bind to bovine serum albumin (BSA) which is a prevalent protein found in the blood plasma of many species [10]. This binding affinity between BSA and parabens could be an indication of how quickly the anti-microbial drug can travel through the body after it is absorbed through the skin. Parabens each have a specific binding constant which can be calculated experimentally. A method developed for analyzing the relationship between BSA and a paraben substrate is fluorescence quenching. Fluorescence quenching is the reduction of fluorescence that results from the formation of a protein-substrate complex. The method fluorescence quenching is method for the analysis of protein-substrate interaction and provides accurate results within a timely manner and demonstrates sensitivity [10]. The degree of fluorescence quenching can indicate the strength of binding between the protein and substrate. Measuring the change of fluorescence quantifies the amount of quenching that occurs within the protein-substrate complex.



**Experimental**:

*Reagents*

Methyl *p*-hydroxybenzoate (MP), propyl *p*-hydroxybenzoate (PP), and butyl *p*-hydroxybenzoate (BP), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. The stock paraben standards for the GC-MS samples were 1 mg/mL and the cosmetic samples were purchased from Sephora and CVS (Table 1) [13]. Methanol was used as a solvent for the

GC-MS samples. A 45 μM stock solution of BSA was prepared in 0.1 M potassium phosphate buffer with a pH of 7.4 to match physiological pH. 50 μM stock solutions of methyl paraben, propyl paraben, and butyl paraben were also prepared in the 0.1 M potassium phosphate buffer with a pH of 7.4.

**Table 1.** The list of cosmetics tested, the country of origin, and the parabens present in the cosmetics according to the ingredients list.

| COUNTRY OF ORIGIN | COSMETIC | TYPE OF PARABENS PRESENT |
|---|---|---|
| N/A | Neutrogena Healthy Skin Enhancer | Methyl, ethyl, and propyl |
| USA | Maybelline Fit Me Matte+Poreless | Methyl and butyl |
| USA | Covergirl tru blend | Methyl and propyl |
| USA | L'oreal True match Lumi | Methyl, ethyl, and butyl |
| France | Make up Forever Mat Velvet | Methyl and propyl |
| USA | Laura Mercier tinted moisturizer | Methyl and propyl |



*Gas-Chromatography Mass Spectroscopy*

The cosmetic samples were prepared with a concentration of 0.1 g of cream per 1 mL of methanol. The parabens were dissolved in the methanol and the remaining ingredients of the cosmetics were separated via centrifugation for 10 minutes at 13,000 rpm. The supernatant was extracted and the remaining water was dried via anhydrous magnesium sulfate. The remaining supernatant was isolated after centrifuging and filtered via syringe with a 45 μM membrane

adapter. In order to avoid cross contamination between samples, the column was baked for 10 minutes at 280 °C after each run. The starting temperature for the column oven was 50 °C and rose at a rate of 25 °C/min to a final temperature of 150 °C and was held for 5 minutes. Then, the temperature increased from 150 °C to 170 °C at 3 °C/min and then 170 °C to 280 °C at a rate of 25 °C/min and was held for 10 minutes with a total running time of 30 minutes [13]. The samples were run in triplicates to ensure reproducibility.

*Fluorescence Spectroscopy*

1.5 μM Methyl paraben, propyl paraben, and butyl paraben solutions were each mixed with 1.5 μM BSA in a potassium phosphate buffer solution. Blanks and samples were measured at an excitation at 280 nm and emission from a range of 300 nm to 450 nm with the slits at 2 nm. The blanks contained only 1.5 μM of the paraben of interest in buffer [10]. They were then equilibrated at 298 K for 15 minutes before measuring fluorescence. Each sample containing BSA yielded a 1:1 ratio of enzyme and substrate and were equilibrated in a water bath at 298 K for 15 minutes before the initial measurement. After the first measurement, the samples were

scanned every 10 minutes for a total of 60 minutes. Four scans total were taken and averaged per measurement.

**Results**:

Samples were taken from 0.2 g/mL cosmetic solutions. Originally 0.1 g/mL were used, however, a higher concentration was required for more prominent paraben peaks to appear in the GC-MS. Only Covergirl and Make up Forever did not yield peaks prominent enough for the spectrum search to identify the parabens within the concentrated cosmetic solutions.

**Table 2.** The retention times reported from the gas chromatography-mass spectrometry for the parabens in the cosmetic samples and 1 mg/mL paraben stock concentrations which were identified via mass spectral analysis.

| **Cosmetic** | *Neutrogena* | | | *Maybelline* | | *Covergirl* | |
|---|---|---|---|---|---|---|---|
| **Type of Paraben** | Methyl | Ethyl | Propyl | Methyl | Butyl | Methyl | Propyl |
| **Avg. Retention Time (min)** | 12.708 | 13.777 | 16.162 | 13.536 | 17.756 | N/A | N/A |
| **Cosmetic** | *L'Oréal* | | | *Make up Forever* | | | |
| **Type of Paraben** | Methyl | Ethyl | Butyl | Methyl | Propyl | | |
| **Avg. Retention Time (min)** | 12.847 | 13.901 | 15.935 | N/A | N/A | | |
| **Cosmetic** | *Laura Mercier* | | *Stock* | | | | |
| **Type of Paraben** | Methyl | Propyl | Methyl | Propyl | Butyl | | |
| **Avg. Retention Time (min)** | N/A | N/A | 13.592 | 16.723 | 17.988 | | |



The paraben peaks were integrated, however, Covergirl, Laura Mercier, and Make up Forever did not yield large enough peaks for integration. Although L'Oréal could yield clean enough peaks to identify methyl, ethyl, and butyl paraben, only the methyl paraben peak was large enough to have an integrated area. The stock solutions yielded larger integrated areas compared to the cosmetic samples between a factor of 1 to 3 indicating that the concentration of

the parabens in make up is less than the standard concentration of 1mg/ml by at least that factor assuming quantitative recovery by the sample prep protocol.

**Table 3.** The integrated areas of paraben peaks in the various cosmetic samples and 1 mg/mL paraben stock solutions which were identified via mass spectral analysis and areas were acquired via GC-MS.

| **Cosmetic** | *Neutrogena* | | | *Maybelline* | | *Covergirl* | |
|---|---|---|---|---|---|---|---|
| **Type of Paraben** | Methyl | Ethyl | Propyl | Methyl | Butyl | Methyl | Propyl |
| **Area** | 2.29E+04 | 9.89E+03 | 7.43E+03 | 1.60E+04 | 5.76E+03 | N/A | N/A |
| **Cosmetic** | *L'Oréal* | | | *Make up Forever* | | | |
| **Type of Paraben** | Methyl | Ethyl | Butyl | Methyl | Propyl | | |
| **Area** | 1.04E+04 | N/A | N/A | N/A | N/A | | |
| **Cosmetic** | *Laura Mercier* | | *Stock* | | | | |
| **Type of Paraben** | Methyl | Propyl | Methyl | Propyl | Butyl | | |
| **Area** | N/A | N/A | 5.29E+06 | 1.26E+04 | 1.83E+05 | | |

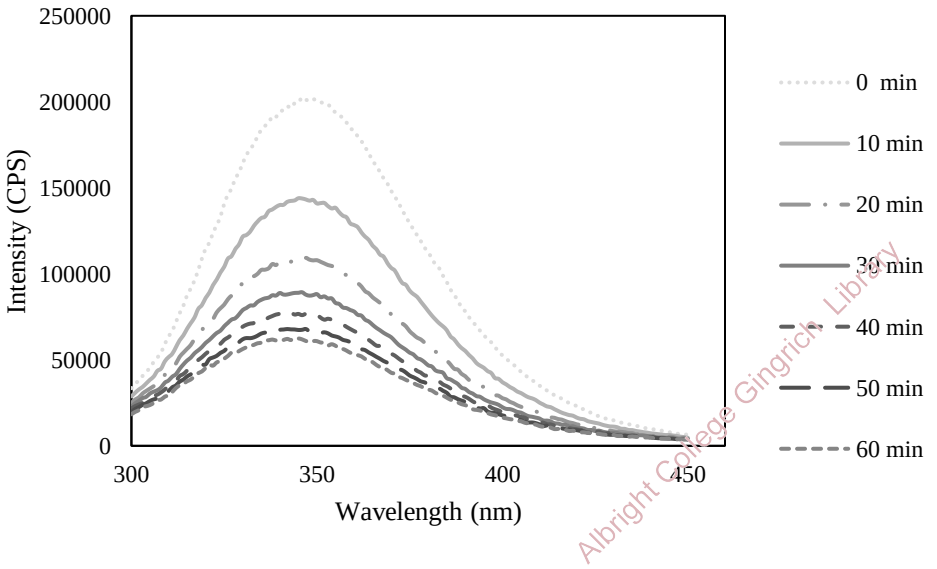



**Figure 3.** The change in intensity of fluorescence within the 1:1 ratio of 1.5 μM bovine serum albumin (BSA) and methyl paraben substrate complex over time.

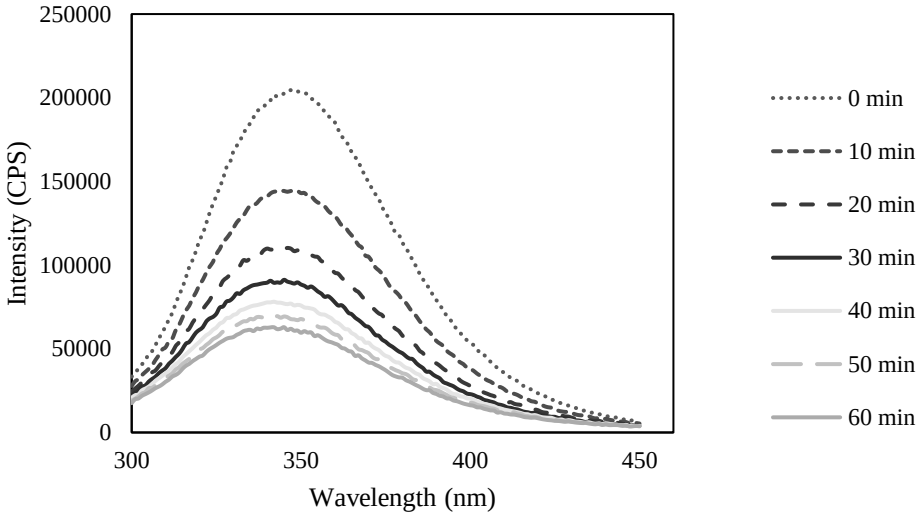


**Figure 4.** The change in intensity of fluorescence within the 1.5 μM bovine serum albumin (BSA) and propyl paraben substrate complex over time.

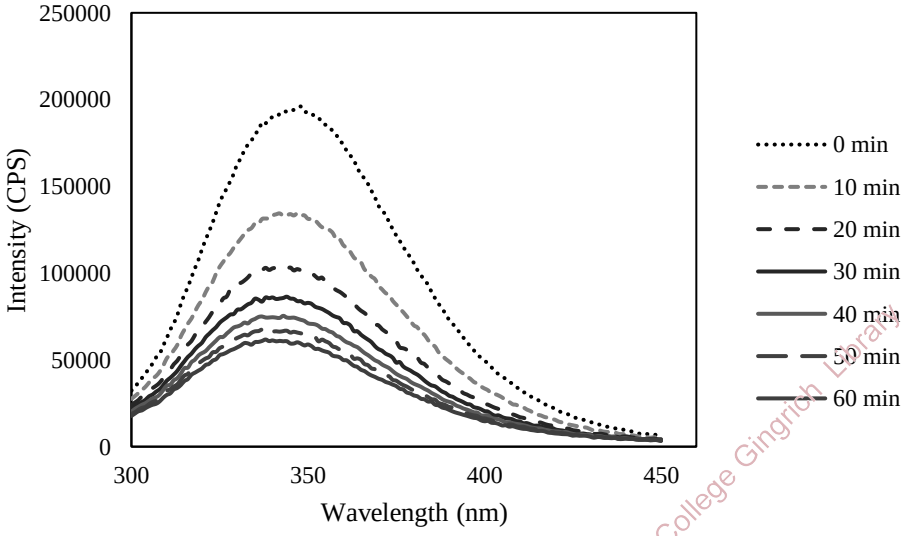




**Figure 6.** The change in intensity of fluorescence within the 1.5 μM bovine serum albumin (BSA) and butyl paraben substrate complex over time.

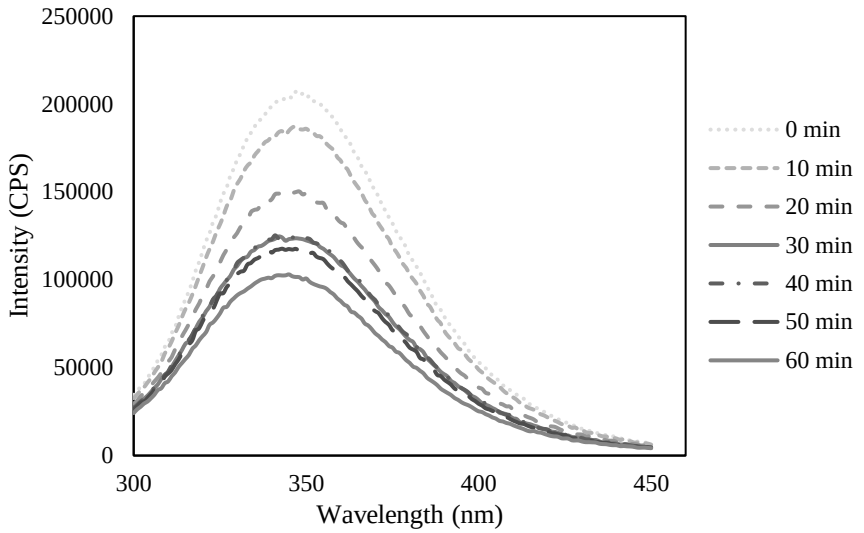


**Figure 6.** The change in intensity of fluorescence within the 1.5 μM bovine serum albumin (BSA) without added parabens over time.

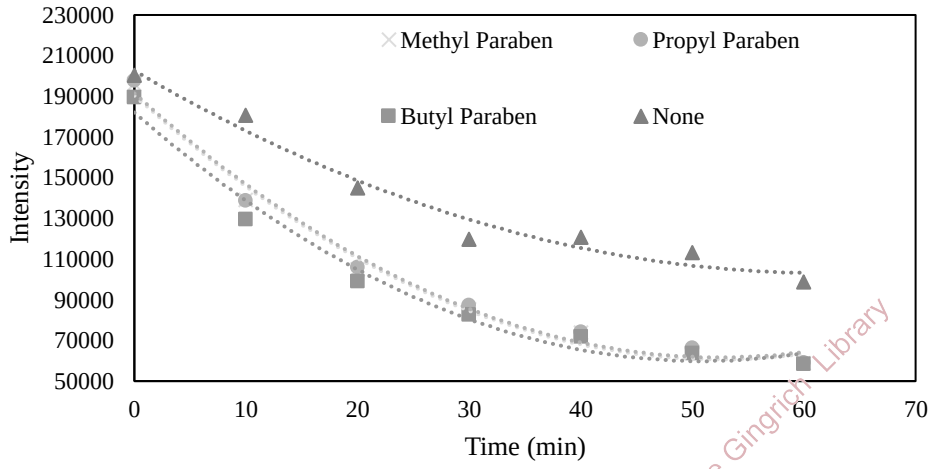




**Figure 7.** The maximum intensity of 1:1 ratio of 1.5 μM BSA-paraben substrate complex during a period of 60 minutes. Blanks and samples were measured at an excitation at 280 nm

and emission from a range of 300 nm to 450 nm with the slits at 2 nm. These values are from the maximum intensity at 350 nm.

**Table 4.** The trendline equations and $R^2$ for each of the 1:1 ratio of 1.5 μM BSA-paraben substrate complexes during a period of 60 minutes.

| Substrate | $R^2$ | Equation |
|---|---|---|
| Methyl Paraben | 0.988 | $y = 47.009x^2 - 4937.3x + 190387$ |
| Propyl Paraben | 0.986 | $y = 47.104x^2 - 4945.2x + 191475$ |
| Butly Paraben | 0.980 | $y = 47.188x^2 - 4803.1x + 182058$ |
| None | 0.969 | $y = 26.083x^2 - 3222.6x + 202670$ |

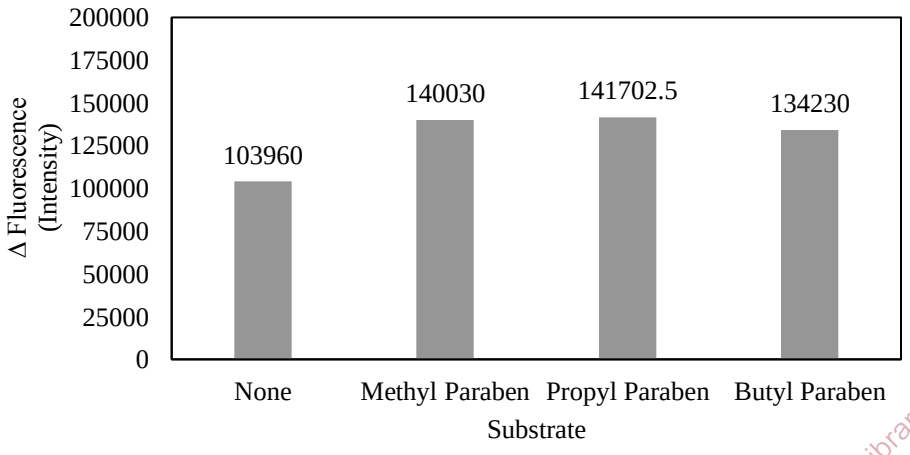




**Figure 8.** The change of fluorescence of each 1.5 μM BSA-paraben substrate complex and BSA by itself. Blanks and samples were measured at an excitation at 280 nm and emission from a range of 300 nm to 450 nm with the slits at 2 nm. The maximum wavelength for the measurements were at 350 nm.

The intensity of fluorescence was measured for a 1:1 ratio of bovine serum albumin (BSA) and methyl paraben at a concentration of 1.5 μM in 0.1 M potassium phosphate buffer with a measured pH of 7.4. There is a decrease in the maximum intensity in the BSA-methyl paraben substrate complex over time with no shift in wavelength (Figure 3). The maximum

intensity of each interval was graphed and there was a second power polynomial decrease over time due to the binding affinity between the protein and substrate (Figure 7). This trend is indicative of the change in conformation with binding that results in changes in fluorescence [3]. The same trends were observed in the intensities of BSA-propyl paraben substrate complex, BSA-butyl paraben complex, and BSA by itself over the same length of time and at the same concentrations of 1.5 μM with a stoichiometric ratio of 1:1(Figure 4-7). However, BSA by itself exhibited a small plateau for intensity between 30 to 50 minutes (Figure 6). This acted as the baseline for the Δ in fluorescence. The Δ in fluorescence of each BSA-paraben substrate complex and BSA alone were graphed for comparison.

**Discussion**:

The gas chromatography-mass spectrometry results were primarily qualitative rather than quantitative. The measurements of the integrated areas for the peaks for the parabens were the closest approximation of quantitative information. The 1 mg/mL stock solutions of methyl, propyl, and butyl paraben yielded the largest areas with the respective values of $5.29 \times 10^6$, $1.26 \times 10^4$, and $1.83 \times 10^5$ (Table 3). For Neutrogena, the methyl, ethyl, and propyl paraben peaks yielded areas with the respective values of $2.29 \times 10^4$, $9.89 \times 10^3$, and $7.43 \times 10^3$ (Table 3). When comparing the methyl paraben stock area to the methyl paraben area for the Neutrogena extract of parabens, the stock solution yielded a larger integrated area by a factor of 2. This difference in area indicates that there is significantly less than 1 mg/mL of methyl paraben in the solution provided the extraction process used for the cosmetics was quantitative.



The same concept can be applied to the areas of propyl paraben stock and the propyl paraben in the Neutrogena sample. The stock solution yields an area that is larger than the sample by an estimated factor of 2. For Maybelline, the methyl and butyl parabens yielded areas

1.60 x $10^4$ and 5.76 x $10^3$ which were respectively lower than the stock solutions by a factor of 2 (Table 3). Only the methyl paraben peak in L'Oréal could be integrated and yielded an area of 1.04 x $10^4$ (Table 3). When comparing this area value to the methyl stock solution it was smaller by a factor of 2. The Covergirl, Laura Mercier, and Make up Forever samples did not yield large enough or distinct enough peaks to be integrated. This inability to integrate these peaks could result from only a trace amount of parabens being present in these cosmetic formulas. Most cosmetics contain 0.01% - 0.3% of their total mass product-worth of parabens for efficiency.

Benzoic acid was often found in the cosmetics and was the more prominent peak compared to butyl paraben which had a similar retention time (Table 2). When comparing the GC retention times, all samples containing methyl paraben were a 1-minute range between 12.708 minutes to 13.592 minutes. For propyl paraben, the range was smaller with a window of 16.162 minutes to 16.723 minutes. The GC retention times for butyl paraben had the largest range from 15.935 minutes to 17.998 minutes (Table 2). This larger window could be attributed to impurities which could affect the boiling point of the samples, therefore, adjusting the elution times within the GC-MS.



The results from the fluorescence spectrometry show a common trend where the maximum intensity of the BSA-paraben substrate complex decreases over time. Figure 3 shows the fluorescence spectra of the 1.5 μM BSA-methyl paraben substrate complex at the various time intervals, ranging from 0 minutes of waiting up to 60 minutes of time lapsing. There is a decrease in the peak intensity as time increases. This decrease in intensity is shown to have a parabolic relationship with a $R^2$ value of 0.988 (Table 4). The decrease in intensity can be attributed to fluorescence quenching as methyl paraben is affecting the conformation of the active site of bovine serum albumin (BSA), therefore, affecting the emission and excitation

properties of the protein depending how much the fluorophores within the protein are affected. The presence of fluorescence quenching supports the increasing binding affinity of the BSA-methyl paraben substrate complex. The quenching effect levels out as it reaches an equilibrium interaction.

In Figure 4, the maximum intensity of the 1.5 μM BSA-propyl paraben substrate complex decreases over time. There is a decrease in the peak intensity between intervals and this trend depicts a parabolic relationship with a $R^2$ values of 0.986 (Figure 4 and Table 4). The maximum intensity of the 1.5 μM BSA-butyl paraben substrate complex decreases over time just like the other BSA-paraben substrate complexes (Figure 5). There is a confirmed parabolic trend regarding the decrease of maximum intensity of the BSA-butyl substrate complex between intervals with a $R^2$ value of 0.980 (Figure 7 and Table 4). When 1.5 μM BSA is scanned via fluorescence spectroscopy, the common trend of a decrease in maximum intensity is observed (Figure 6). However, between intervals of 30-50 minutes there is a slight plateau in maximum intensity which could represent the preferred conformation of BSA within the buffer. There is still a slight parabolic relationship in the decrease of maximum intensity of 1.5 μM BSA overtime with a $R^2$ value of 0.969 (Figure 7 and Table 4).



In Figure 8, the change in fluorescence of each BSA-paraben substrate complex was compared. BSA without any paraben yielded the lowest change in fluorescence which supports that fluorescence quenching occurs within the BSA-paraben substrate complexes. Propyl paraben shows the most prominent effects of fluorescence quenching, methyl paraben shows moderate quenching, and butyl paraben displays the least amount of fluorescence quenching (Figure 8). These results support the idea that propyl paraben demonstrates the highest binding affinity for BSA compared to methyl paraben and butyl paraben. Whereas, butyl paraben demonstrates the

lowest binding affinity for BSA. The more quantitative measurement of binding affinity is the value of binding constants for each substrate. However, further study is needed to calculate these constants.

**Conclusion**:

Overall, the GC-MS results confirmed the presence of the parabens listed in the ingredient lists. The paraben peaks within the cosmetic samples and stock solutions were integrated and compared. The parabens in the Neutrogena sample yielded areas smaller by a factor of 2 when compared to the stock solution areas. The same thing occurs for the methyl peak in the Maybelline. However, the remaining parabens in the Maybelline sample, the Covergirl sample, Laura Mercier sample, and Make up Forever sample did not yield large enough peaks to be integrated. Indicating that only trace amounts of paraben were found in the formula. Fluorescence spectroscopy was used to confirm the presence of fluorescence quenching within the BSA-paraben substrate complex. Propyl paraben yielded the most quenching, which was followed by methyl paraben, and then Butyl paraben. The presence and binding affinity of parabens can affect the conformation of the protein and can indicated by the change in overall fluorescence.



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