

Comparative Analysis of Chemical Additives in Colgate and Oral-B Toothbrush Bristles Using LC-MS

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Abstract

Plastic toothbrushes contribute significantly to plastic pollution, yet we don't know much about the chemical additives in them. Our study aims to provide more information on this. We are going to be doing this by evaluating the differences in chemical additives of toothbrushes from major toothbrush brands. The brands we used were Colgate and Oral B. We took toothbrush bristle samples from these toothbrushes and used liquid chromatography-mass spectrometry (LC-MS) to measure and compare the chemical additives in these bristles. Despite our hypothesis that there would be greater concentrations in the Colgate toothbrushes, we found that there wasn't much difference between the brands. These findings show that there were similar manufacturing standards between the toothbrush brands and we are going to need more research into the environmental effects of toothbrushes and toothbrush bristles.

Introduction

Plastic pollution has become a very important environmental issue due to its widespread use and the lack of ability to decompose. Plastic toothbrushes which are often made of polypropylene or nylon, are a significant contributor to microplastic pollution since they do not decompose. Instead of decomposing, these products break down into smaller particles and get released into the environment as chemical additives (Hopewell et al., 2009). These additives can leach into the environment, causing health risks such as cytotoxicity (Savva et al., 2023).

Since over 70% of the Canadian population brushes their teeth daily, disposable toothbrushes are an overlooked source of environmental contamination (Environment and Climate Change Canada, 2024). However limited research exists on the chemical composition of these products. This study looks into chemical additives in toothbrush bristles from Colgate and Oral B toothbrushes in order to address the lack of research in this field. Our research question was: What are the differences in the types and concentrations of chemical additives found in toothbrush bristles of different brands? Based on prior studies indicating differing cytotoxic profiles between the brands, we hypothesized that Colgate toothbrushes would contain a greater variety and higher concentrations of additives than Oral-B toothbrushes.

Methods

Our study analyzed the chemical composition of toothbrush bristles from three samples: a manual Colgate toothbrush (Sample A), a manual Oral-B toothbrush (Sample B), and an electric Oral-B toothbrush (Sample C). The purpose was to identify and compare the types and relative

intensities of chemical additives across these brands to assess potential environmental and health implications.

For our sample preparation, samples A and C were washed with soap and water and boiled for 10 minutes to remove surface contaminants that may have adhered to the bristles during manufacturing, packaging, or storage. Boiling also helps sterilize the samples, even though this step might lead to partial chemical leaching. Sample B was washed with soap and water but not boiled which could have led to less chemical leaching. We then made three replicates of approximately 0.1 g of white bristles collected from each toothbrush to ensure the reliability of the results. These replicates were stored in Eppendorf tubes to maintain sample integrity before analysis.

Here are the variables we carefully selected: The independent variable was the toothbrush brand since we hypothesized that different manufacturing processes might result in different chemical compositions. The dependent variable was the relative abundance of chemical additives in the bristles, which was identified through LC-MS analysis. The Controlled variables included the color of the bristles, the brand, and the type of bristle, to ensure that the analysis focused on comparable components. On a side note, we chose white bristles as our controlled color instead of a different color since different color dyes may introduce variables in the chemical composition of plastic bristles, and bristle type assuming similar manufacturing procedures and industry standards for the same type of bristle. The Uncontrolled variables included the age of samples, usage time, and manufacture year which created some limitations in our study. Liquid Chromatography-Mass Spectrometry (LC-MS) was chosen as the analytical method due to its high sensitivity in detecting and quantifying chemical additives in complex mixtures and its ability to identify and separate compounds. The sample preparation steps were designed to extract these chemicals efficiently: When we did Acetonitrile Addition, We extracted 1 mL of acetonitrile which a strong solvent capable of dissolving a wide range of organic compounds and was added to each tube. This step allowed for the extraction of chemical additives from the bristles and the separation of polar and non-polar compounds. For Vortexing, The tubes were vortexed for two minutes to enhance the mixing and solubility of the chemicals in the solvent. For Supernatant Transfer, the supernatant was carefully transferred to sample vials to ensure that solid residues remained in the tubes. This step ensured the LC-MS machine analyzed only the dissolved chemical compounds, minimizing interference.

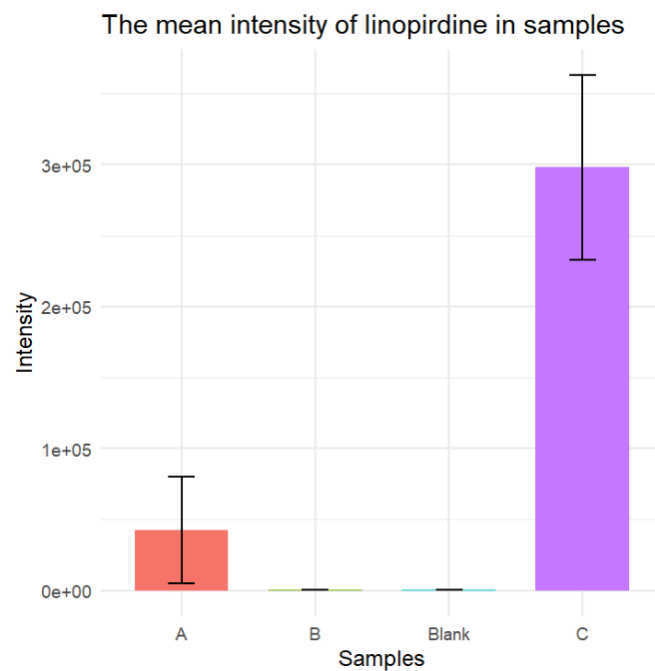
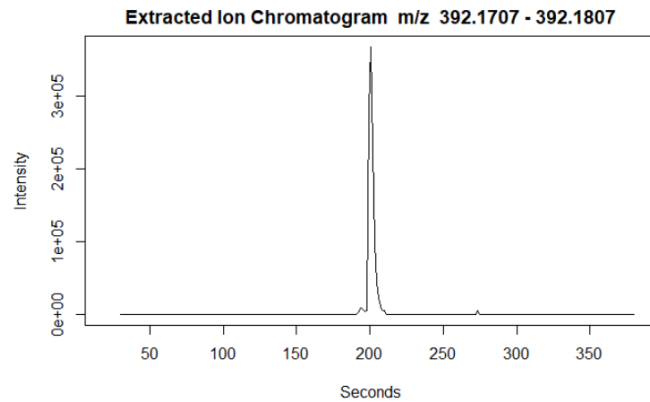
To interpret the data meaningfully, statistical tests were applied: For making Procedure Blanks, we included Blank samples that were composed of acetonitrile only to account for potential contamination or background noise from the solvents or lab equipment. The chemical profiles of blanks were subtracted from sample data to isolate additives specific to the bristles. For the Two-Tailed t-tests, these tests compare the chemical profiles of toothbrush replicates against blanks to identify the significant chemical presence and the chemical profiles between toothbrush brands to detect differences in additive composition.

Each step in our methods was designed to maximize the reliability and relevance of the findings. The use of replicates provided us with statistical robustness, while the controlled variables ensured consistency across samples. LC-MS was chosen for its precision in identifying trace amounts of chemical additives, which is crucial for assessing potential health and environmental risks. By including procedure blanks and performing statistical analyses, the study minimized errors and biases, ensuring that the detected differences were meaningful.

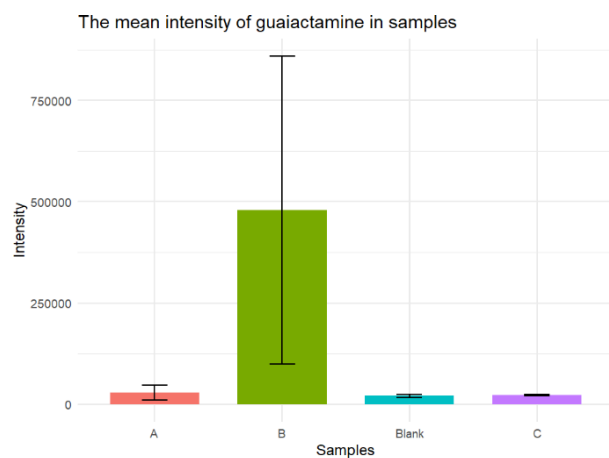
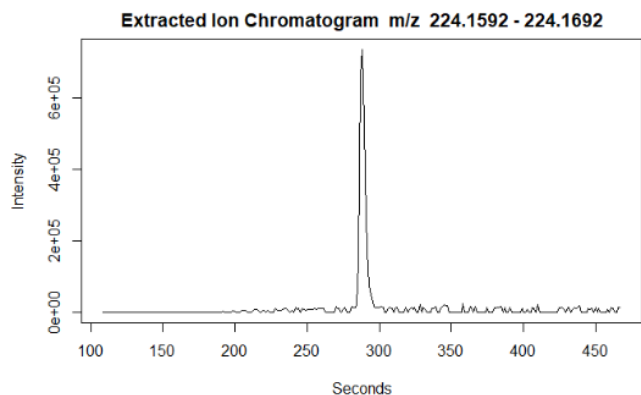
Results

In the LC-MS analysis, four chemicals were identified in varying relative abundances across the toothbrush samples: linopirdine, guaiactamine, troxipide, and anthraquinone. We assessed these chemicals for their potential implications based on their roles and statistical significance.

The first one we analysed was linopirdine. Below the first figure shows where linopirdine was detected in the samples and the second figure compares the mean intensity of linopirdine detected across samples.

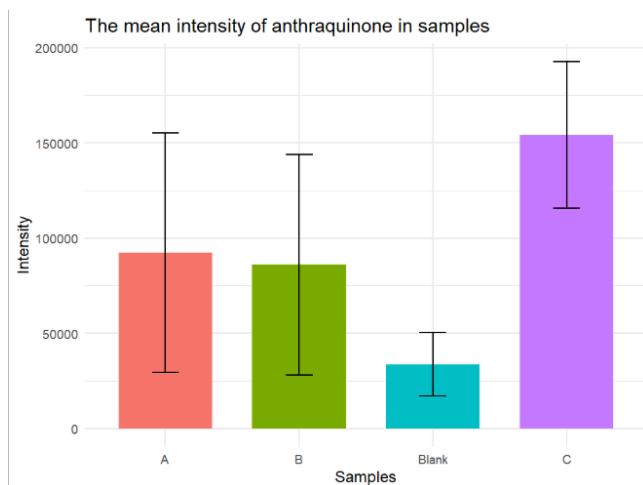
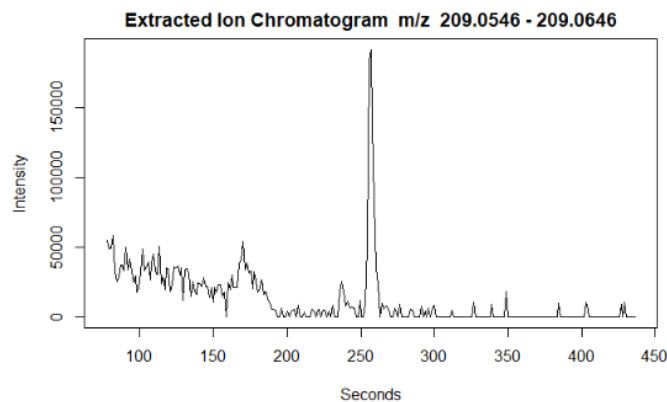


As shown in the figure above, linopirdine, a neuroprotective agent, was detected in all three samples, with no statistically significant differences between the brands ($p > 0.05$) even though it was highest in sample C. Its presence was unexpected since it is not a commonly known additive for toothbrush bristles. It is possible that the detection resulted from contamination during the manufacturing process or unintentional exposure during handling and packaging. Similarly, guaiactamine, a compound known to affect the nervous system was also analysed and produced the same figures where the first figure shows where guaiactamine was detected in the samples and the second figure compares the mean intensity of guaiactamine detected across samples.



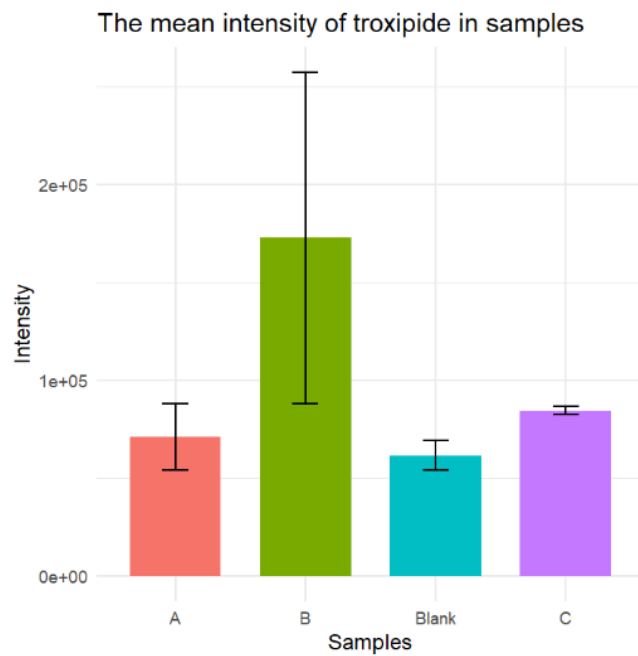
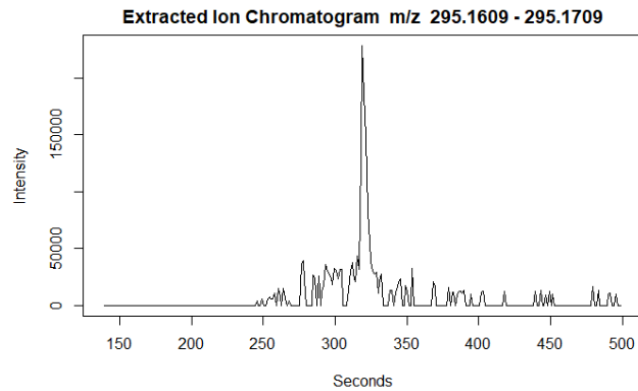
This compound showed no significant differences across the samples ($p > 0.05$). This chemical's consistent presence in all toothbrush samples suggests potential contamination or residual chemicals from industrial processes, though its role in toothbrush manufacturing remains unclear.

The next one we analysed was anthraquinone. Below the first figure shows where anthraquinone was detected in the samples and the second figure compares the mean intensity of anthraquinone detected across samples.



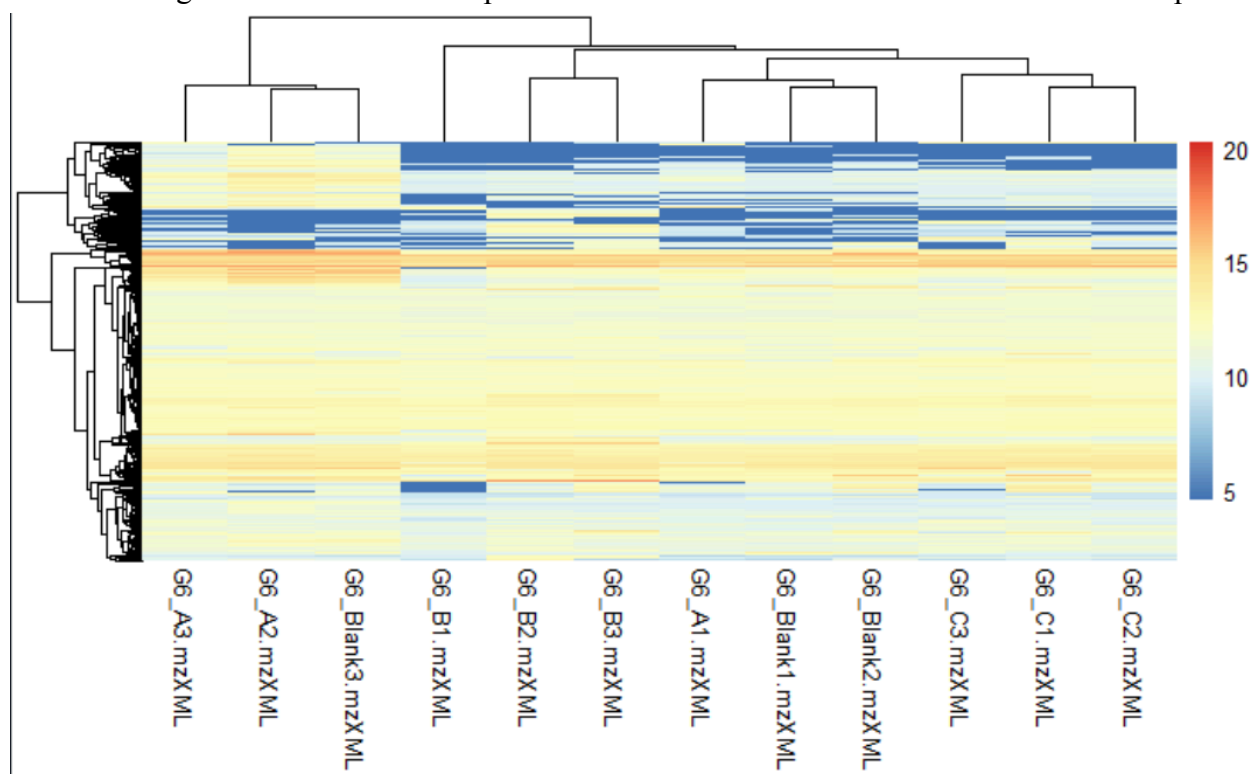
As shown in the figures above and the data we collected, anthraquinone, a compound commonly used as an antimicrobial and antifungal agent, exhibited the highest significance in Sample C (electric Oral-B) compared to the blanks ($p = 0.044$). While its presence may indicate the intentional use of antimicrobial coatings, its classification as a carcinogen by the National Toxicology Program raises health concerns. This finding underscores the need for manufacturers to disclose additive compositions to ensure consumer safety.

Another one we analysed was troxipide. Below the first figure shows where troxipide was detected in the samples and the second figure compares the mean intensity of troxipide detected across samples



Troxipide, a gastroprotectant drug, was also detected across the samples but with no significant differences between the brands ($p > 0.05$). Its presence is likely incidental, possibly originating from contamination during the manufacturing process or residuals from shared industrial environments.

The next thing we did was a visual representation of the LC-MS data in the form of heatmaps.



This heatmap shows uniform distributions of chemical additives across the samples. While the heatmaps provided an overview of the chemical profiles, they also highlighted minimal variability between the brands, which suggests that similar manufacturing techniques and materials may be used across both Colgate and Oral-B toothbrushes.

Discussion

Our study's findings showed no substantial differences in chemical additives between the two leading toothbrush brands. This challenges our initial hypothesis that Colgate toothbrushes would contain a greater variety and concentration of chemical additives compared to Oral-B toothbrushes. Despite this lack of differentiation, detection of certain additives, such as anthraquinone, raise important environmental and health concerns.

The presence of anthraquinone, especially in the electric Oral-B toothbrush (Sample C), suggests that certain products may include antimicrobial coatings. While this feature could be beneficial in reducing bacterial growth, the health risks associated with this chemical should not be overlooked. Anthraquinone is a suspected carcinogen and its use in consumer products means stricter regulatory measures and a call for action is needed.

The lack of significant differences in linopirdine, guaiactamine, and troxipide across samples shows that these chemicals might not be intentional additives but contaminants introduced

during manufacturing. Also, shared supply chains or processing facilities could explain the similar chemical profiles observed between the brands. This raises an important question about the transparency of manufacturing processes and the need for stricter quality control measures.

Several limitations may have influenced the results. For example, boiling samples to sterilize them likely led to partial chemical leaching, reducing the detectable levels of certain additives. Additionally, the focus on white bristles excluded potential variability introduced by dyes, which might have contributed to additional chemicals. These limitations underscore the importance of refining sample preparation methods in future studies to ensure the integrity of the chemical profiles.

From an environmental perspective, the findings emphasize the need for reducing reliance on non-biodegradable toothbrush materials. Millions of plastic toothbrushes are discarded annually, which contributes to microplastic pollution and the release of potentially harmful additives into ecosystems. Biodegradable alternatives, such as bamboo toothbrushes, could be a viable solution. However, further research would be needed to assess the chemical composition and environmental impact of these alternatives compared to traditional plastic toothbrushes.

Future research should aim to expand the analysis of this issue. This should include examining the chemical composition of toothbrush handles, which are often made from different types of plastics, and comparing new versus used toothbrushes to evaluate changes in additive concentrations over time. Also, studies could investigate whether these findings align with disclosed industry standards and regulatory compliance. Finally, comparing chemical profiles between plastic and biodegradable materials would also provide valuable insights into the sustainability of alternative toothbrush designs.

In summary, this study provides a preliminary understanding of the chemical additives present in toothbrush bristles from major brands. While no significant differences were observed between Colgate and Oral-B toothbrushes, the detection of potentially hazardous chemicals highlights the need for enhanced transparency in manufacturing practices and the development of safer, more sustainable products.

Conclusion

Our study provides very important and valuable insights into the chemical additives in toothbrushes. Despite our hypothesis, we made that Colgate toothbrushes would contain more additives at higher concentrations, our results showed that there were minimal differences between the two brands. This shows that similar materials and manufacturing processes were used across the industry. However, we found chemical additives such as anthraquinone, which is known to have carcinogenic properties. This shows that we need increased regulatory oversight and transparency in producing toothbrushes and other consumer products.

There are several opportunities for future research to build on our findings. One way we can do this is by expanding the scope of analysis to include toothbrush handles and dyed bristles would provide a more comprehensive understanding of chemical additives in these products. Another thing we could do is investigate the effects of usage and aging on additive concentrations. This could also offer insights into long-term environmental and health impacts. We could also compare chemical profiles across different materials, such as bamboo or bioplastics which could identify more sustainable alternatives to traditional plastic toothbrushes.

Overall, our study highlights the need for greater accountability and innovation within the consumer goods industry. By prioritizing safer materials and sustainable practices, manufacturers can reduce the environmental footprint of everyday products while protecting public health. Collaboration between industry stakeholders, researchers, and policymakers will be crucial in achieving these goals.

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