Beyond Aligners: Unmasking Hidden Extractables and Leachables in Orthodontic Devices

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Introduction

The Rise of Clear Aligners

Increasingly popular in orthodontics, clear aligners promised to users' improved comfort, compliance and flexibility. It also came with prolonged intraoral exposure. The literature on the potential for leaching components of the polymer used to manufacture the invisible aligners is sparse.

Despite the benefits, user submitted cases in the FDA retrospective analysis report several adverse effect ranging from swelling, hives, itchiness and even difficulty breathing[1].

Previous works examined the potential of leaching in retainers but used conditions far from the in-vivo setting[2].

We used samples closest to user conditions and harnessed the power of Gas Chromatography High Resolution Mass Spectrometry (GC-HRMS) and the power of curated and annotated libraries to find candidate compounds that could be at the origin of the reported adverse effects.

We also used multivariate statistics to establish relationships between the compounds and metadata.

Experimental Design

Control Blanks and Population Selection

Blank selection is key in subtracting endogenous compounds from the samples of interest. We used two blanks as follow:

Synthetic Blank synthetic blank saliva from commercial provider.

Pooled Blank saliva samples collected from 28 individuals who never had orthodontic treatment, dental fillings or root canals with an age distribution like that of population on which the study was conducted.

Population 30 individuals using clear aligners at different ages and stages of treatment

Sample Preparation

All extractions were conducted in glassware to minimize any input of Extractables and Leachables from the plastic pipette tips and centrifuge tubes.

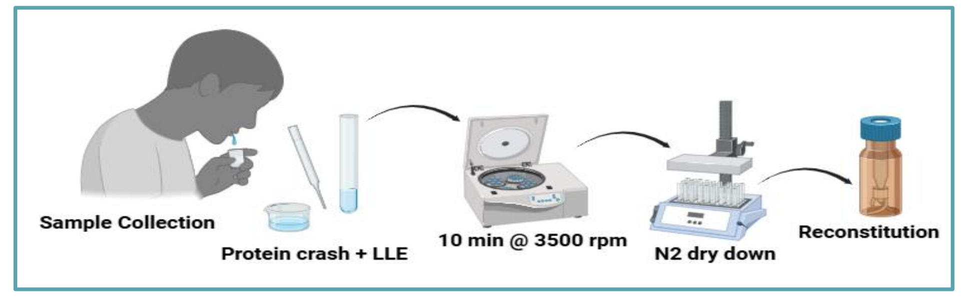

Figure 1. Sample preparation workflow.

Experimental

1 mL saliva was measured in a glass tube and extracted with 3 mL chilled HPLC grade acetonitrile spiked with internal standard (Fentanyl-d5 at 100 ng/mL). The tube was then centrifuged at 3000 rpm for 15 minutes. Then 1 mL of the supernatant was dried down under nitrogen stream. Samples were resuspended in 100 µL solvent of 9:1 methanol and ethyl acetate (Figure 1).

Sample Analysis

Samples were analyzed using the Agilent 7250 accurate mass GC-qTOF equipped with the 8890 GC and a DB-5MS UI column (30m x 0.25 x 0.25). The inlet temperature was 280°C and the transfer line temperature was 325°C. The oven program was as follows: 50°C for 1 min, ramp at 25°C to 170°C, hold for 1 min; then ramp at 15°C to 300°C, hold for 10 min; then ramp at 20 °C to 325°C and hold for 5 min. The solvent delay was 4 min. The data were acquired in electron ionization (EI) mode using mass range 45 to 1000 m/z. The source temperature was 230°C, and the emission current was 5 µA.

A mix of alkanes was run along with the samples and blanks and an Extractables and Leachables (E&L) standard mixture was run as a quality check.

Data Analysis

Retention indices were used in consolidating library matches in both the Agilent HRMS E&L library and the NIST23 library.

Data analysis was conducted on Unknown Analysis software from Agilent that performs compound annotation based on library matching and retention index.

All compounds were individually reviewed based on ExactMass, retention index delta and library match factor (Figure 2).

Canonical Correlation Analysis was performed using the FactoMineR package in R.

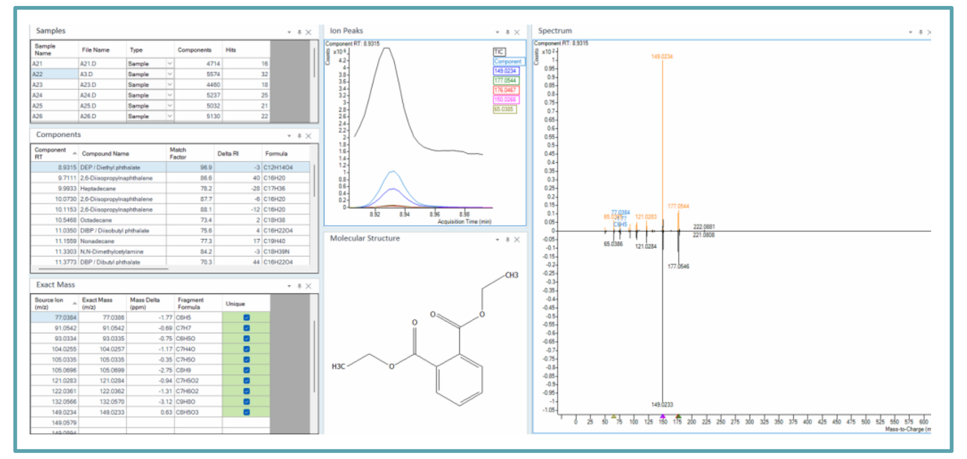

Figure 2. Data review in the Unknown Analysis using multiple features.

Results and Discussion

Compound Annotation

A total of 885 unique compounds were detected across the population of 30 individuals. Endogenous compounds were filtered using the blank subtraction feature, and only compounds present in over 80% of the population were kept (except for metabolites of Caprolactam). Hence, 55 compounds (Figure 3) were identified as potential E&Ls from the retainers. This include known plasticizers such as caprolactam, amides and phthalates. Notable as well that we found a variety of amides as well as a candidate metabolite of caprolactam (n-Caproic acid) in some individuals which could be a result of metabolism.

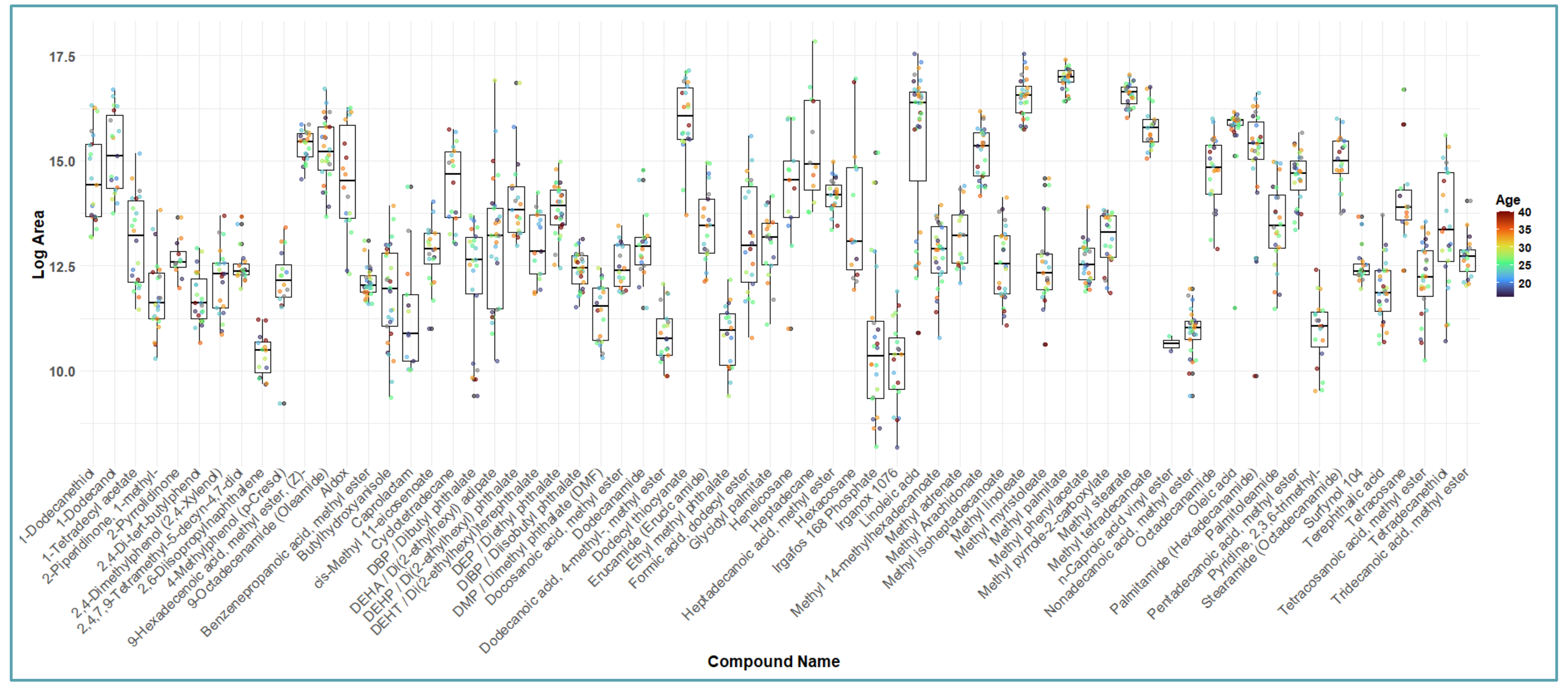

Figure 3. Compounds identified across the 30 individuals in the population using clear aligners.

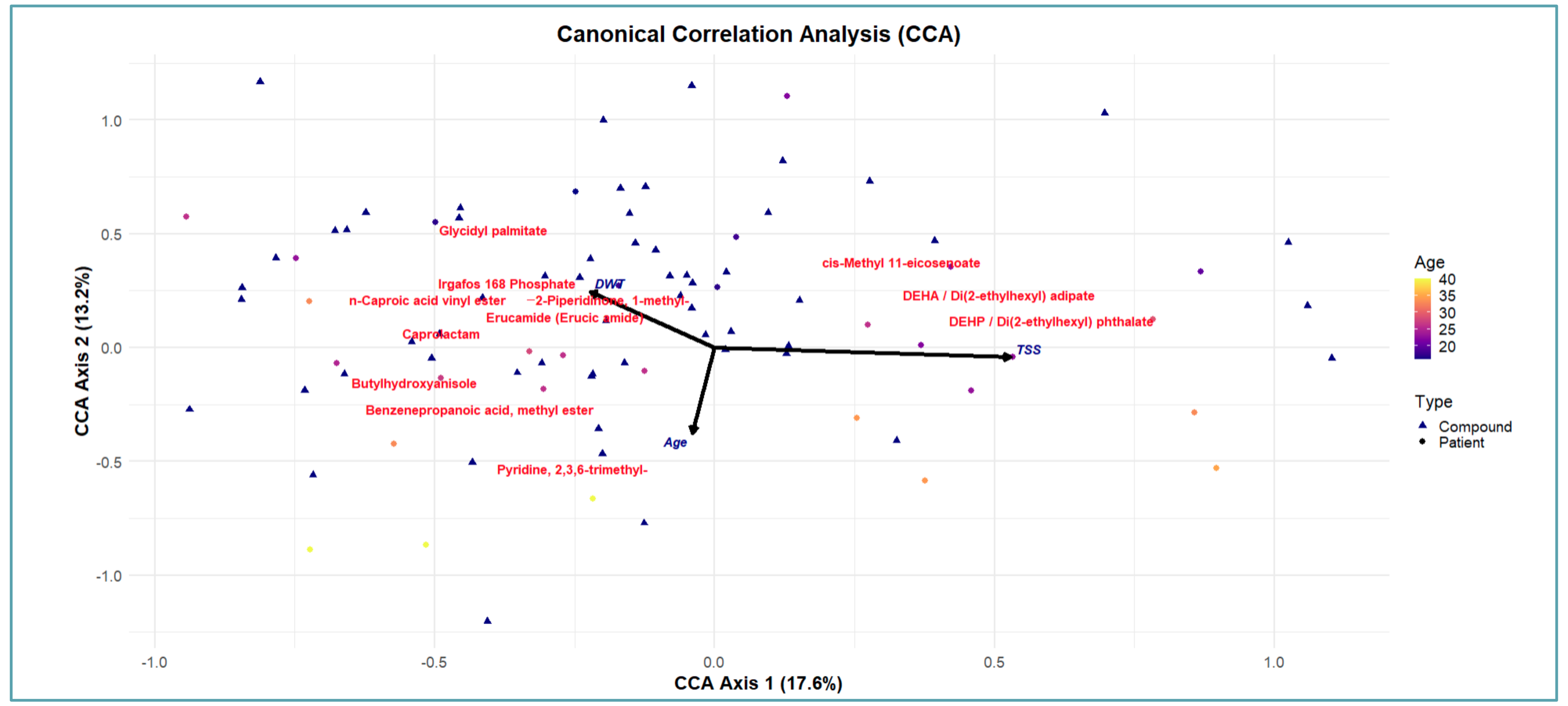

Figure 4. Correlating population metadata (Age, DWT and TSS) to the normalized compound intensity.

Results and Discussion

Establishing Relationships between Compounds and Population Metadata

Although the approach was not quantitative, the relative abundance of compounds could be used in the multivariate space to establish relationships between a matrix of predictors: Age, Daily Wear Time (DWT) and Time Since the Start of treatment (TSS), and a matrix of explanatory variables: the exogenous compounds unique to the population and suspected to be coming from the clear aligners (Figure 4). Canonical Correlation Analysis is adequate to address this dataset. All compound abundances were z-score normalized. Data scaling was set to allow inferences about relationships between predictor and explanatory variables. Only the 12 compounds contributing most to the model were plotted for ease of visualization.

The analysis revealed that the model explained about a third of the variance seen in the population of clear aligners users (sum of CCA axes), i.e. over 30% of the variance observed in these compounds is attributed to Age, DWT and TSS. Suggesting that the release and metabolism of these compounds is variable in function of the three reported metrics.

Notably, caprolactam, n-caproic acid, glycidyl palmitate and piperidinone are most associated with DWT, adipate and phthalate with TSS and trimethyl pyridine along with the BHT and benzene propionate (benzene ring compounds) were most associated with age.

It's worth mentioning that caprolactam is most likely a signature compound of the aligners used in this study since previous data collected in our lab showed its presence in aligners with minimal sample processing (using GC-Thermal Desorption HRMS). As shown by the contribution plot (Figure 5), caprolactam is the strongest contributor to variance in the population further suggesting its differential metabolism.

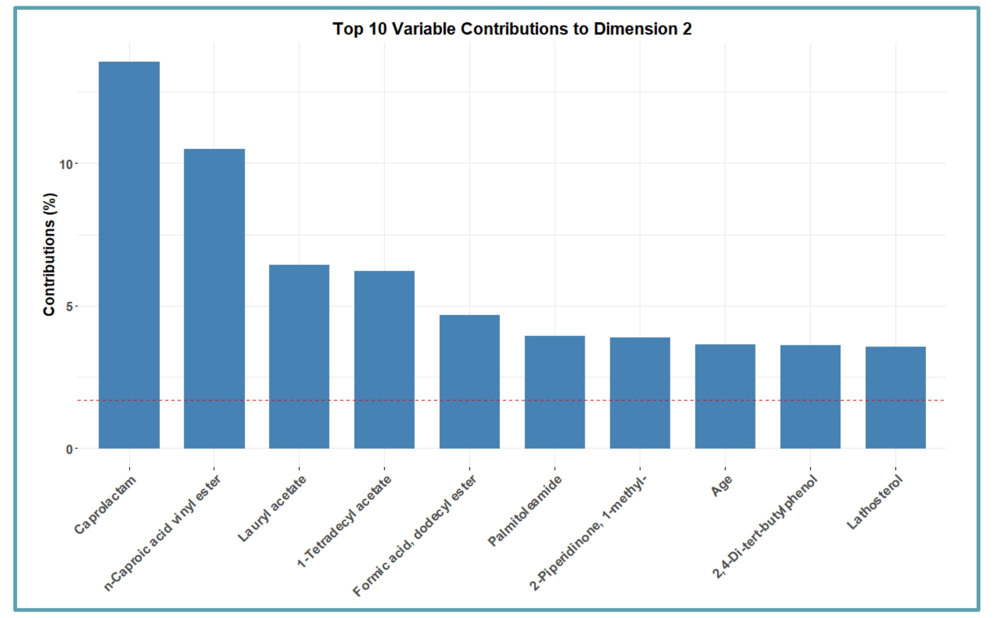

Figure 5. top 10 contributors to the CCA model.

Applications and Toxicological Profile

Some of the identified compounds are tightly linked to clear plastic manufacturing with a toxicological profile matching reported cases of adverse effects[1] (Table 1). Additional compounds that are not listed in the table share a similar toxicological profile.

Table 1. Toxicological profile of some of the chemicals identified in our analysis.

Compound	Function	Toxicological Profile
Caprolactam	Plasticizer	Skin, eye, mucus and nervous toxicity
Oleamide	Lubricant (PET processing)	Skin and eye irritation, allergen
Butylhydroxy-anisole	Added to plastics to prevent degradation from heat and light	Cancer causing in California
Phthalates identified in this study	Plasticizer in plastics and resins	Reproductive toxicity; potential endocrine disruptor

Conclusions

The aligners used in the current study were approved in the late 90s when E&Ls methods were not as prominent or sensitive as they are today. This study stands out with:

- The first report of numerous E&L compounds with known potential adverse effects in a population of clear aligner users
- Providing an insight of the relationships between E&Ls and user's metadata such as age, DWT and TSS
- Providing the necessary libraries to find parent compounds and their potential metabolites

References

[1] R. Nalliah, M. Lee, S. Rampa, and V. Allareddy, "Adverse clinical events reported during Invisalign treatment: Analysis of the MAUDE database," American Journal of Orthodontics and Dentofacial Orthopedics, vol. 152, no. 5, pp. 706–710, 2017, doi: 10.1016/j.ajodo.2017.06.014.

[2] A. Alhendi, R. Khounganian, A. Almudhi, and S. Ahamad, "Leaching of different clear aligner systems: An in vitro study," Dentistry Journal, vol. 10, no. 2, 2022, doi: 10.3390/dj10020027.