Optimization of a method for the determination of fatty acids in dental plaque by GC-MS

Analysis of authentic samples from subjects in opioid replacement therapy

S. Roth[1], K. Henkel[1], M. Altenburger[2], V. Auwärter[1], M. A. Neukamm[1]

[1]*Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Freiburg, Germany*

[2]*Department of Operative Dentistry and Periodontology, Center for Dental Medicine, Medical Center – University of Freiburg, Freiburg, Germany*

UNIVERSITÄTS KLINIKUM FREIBURG

Institute of Forensic Medicine
Forensic Toxicology

Background and Objectives

The analysis of non-mineralized dental biofilm (plaque) has revealed high interindividual variances in drug concentrations but so far incorporation patterns are still under investigation. Dental plaque mainly consists of a community of different bacteria. Characteristic fatty acids (FAs) as a constituent of bacterial membrane lipids can be used to characterize certain bacterial groups (e.g. Gram+ and Gram- bacteria). The composition of the bacterial community could influence the incorporation and retention of drugs in this matrix (**Fig. 1**). Therefore, a simple and fast method facilitating routine use in a toxicological laboratory was adopted and optimized for the analysis of 47 FAs in dental plaque as methyl esters (FAME). The method was applied to plaque samples from 10 subjects in opioid replacement therapy to evaluate possible correlations between FAME profiles and drug concentrations.

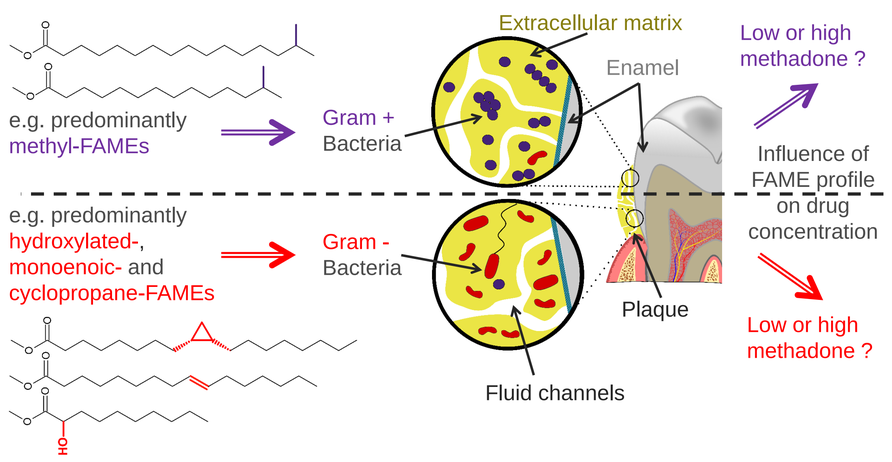


Fig. 1 : Correlation of FAME profiles, bacterial composition and drug results.

Methods

Multivariate optimization of transesterification:

For optimization dried *in-vitro* biofilm was prepared as previously described by *Henkel et al*. [2].

The design illustrated in **Fig. 2** was created in the **optimization software MODDE®**. The 18 experiments were carried out accordingly.

4D contour plots (**Fig. 3**) were used to determine the optimal conditions for best FAME yield.

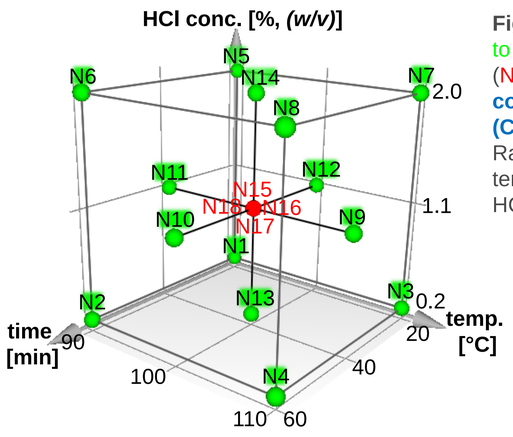
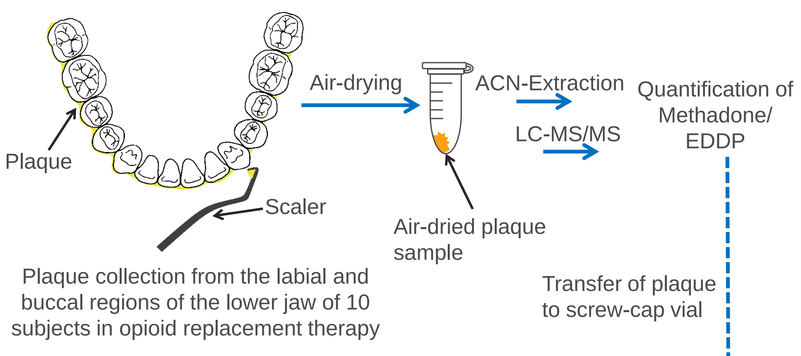


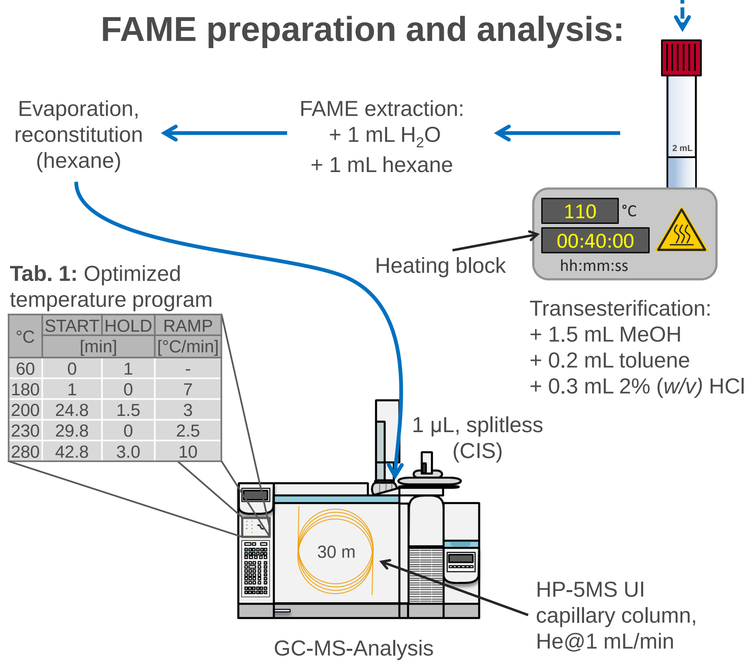
Fig. 2: 14 experiments (N1 to N14) and 4 center-points (N15 to N18) in a **central-composite face-centered (CCF) design** space. Ranges: time, 20 to 60 min; temperature, 90 to 110 °C; HCl conc., 0.2 to 2.0% (*w/v*)

Plaque collection and drug analysis [3]:



Plaque collection from the labial and buccal regions of the lower jaw of 10 subjects in opioid replacement therapy

FAME preparation and analysis:



Tab. 1: Optimized temperature program

°C	START [min]	HOLD [min]	RAMP [°C/min]
60	0	1	-
180	1	0	7
200	24.8	1.5	3
230	29.8	0	2.5
280	42.8	3.0	10

Results and Discussion

Optimization of transesterification:

Optimal conditions for the transesterification reaction were as follows: incubation for **40 min** at **110 °C** with **2% (*w/v*) HCl** concentration.

The results displayed in **Fig. 3** are representative for the 29 FAMEs in the *in-vitro* biofilm samples.

The summary of fit plot showed **good model statistics** (high values for model fit (R2), predictive capability (Q2), absence of lack of fit (Va) and reproducibility (Re)).

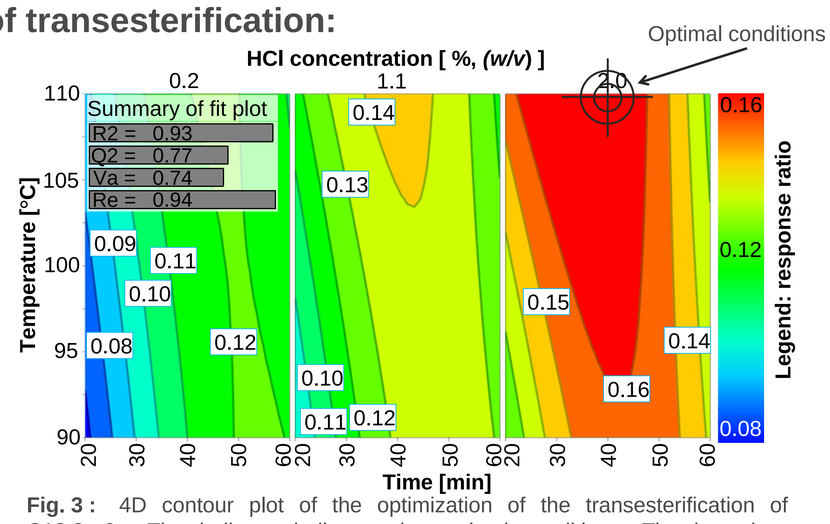


Fig. 3 : 4D contour plot of the optimization of the transesterification of C18:2n-6c. The bullseye indicates the optimal conditions. The bar chart inscribed in the upper left corner shows good model statistics.

Optimization of GC temperature program

The temperature program was optimized for best possible **separation of 47 FAMEs**. Slight alterations of pre-existing methods in the literature [4,5] led to the program displayed in **Tab. 1**. **Baseline separation** was achieved for all FAMEs in single ion monitoring mode.

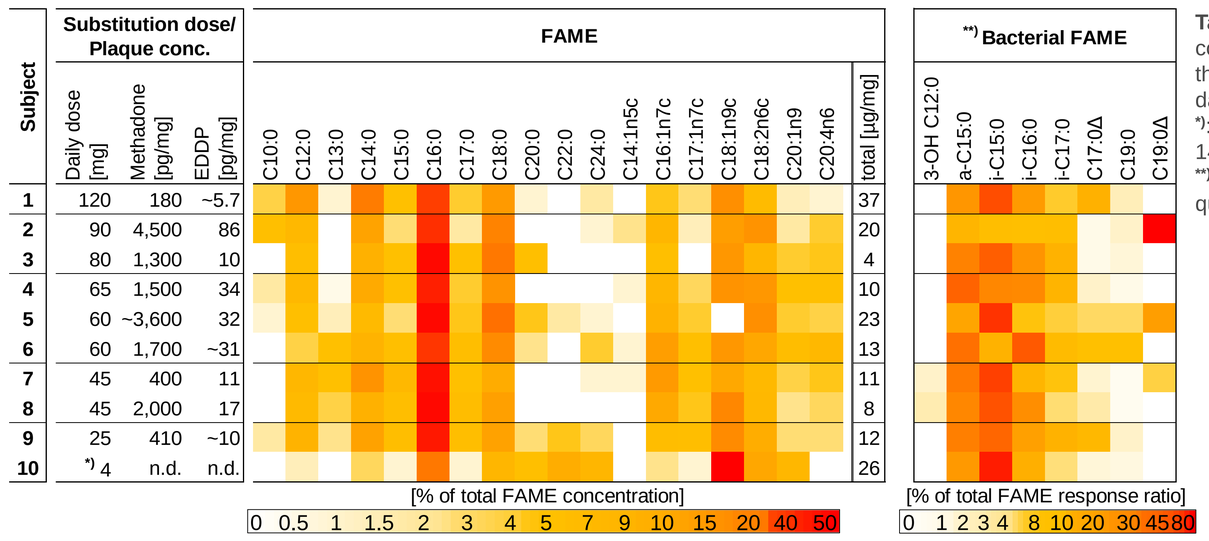
Validation of the analysis method

47 FAMEs were covered by the analysis method, 34 of them were determined quantitatively. Linearity was proven within **concentration ranges** from **5.0 to 4,000 ng/mL**. Accuracy and precision were within a 20% margin. The **LOQ** ranged from **5.0 to 50 ng/mL**.

Drug concentrations and FAME profiles of 10 subjects

The total amount of FAMEs detected in the authentic plaque samples varied from 4.0 to 37 µg/mg with C16:0 (1.9 to 13 µg/mg) and C18:1n-9c (0.5 to 13 µg/mg) as the most prevalent FAMEs. Among the 13 FAMEs of exclusively bacterial origin the highest responses were obtained for iso-C15:0 and cyclopropane-C19:0.

The amount of **methadone and EDDP showed high variations** even in the plaque of subjects that received the same daily dose (e.g. subjects 4 to 6, **Tab. 2**). Variations were also visible in the FAME profiles. Especially the profiles of **bacterial FAMEs showed high variations** between the subjects. These variations are an indication for differences in the bacterial composition of the plaque. This **could explain the variations in drug concentrations** even if no exact match between a specific FAME profile and low or high methadone/EDDP concentrations was found in the 10 subjects that were investigated.



Subject	Daily dose [mg]	Methadone [pg/mg]	EDDP [pg/mg]	total [µg/mg]
1	120	180	~5.7	37
2	90	4,500	86	20
3	80	1,300	10	4
4	65	1,500	34	10
5	60	~3,600	32	23
6	60	1,700	~31	13
7	45	400	11	11
8	45	2,000	17	8
9	25	410	~10	12
10	*) 4	n.d.	n.d.	26

Tab. 2: Methadone and EDDP concentrations and FAME profiles in the plaque of 10 subjects in order of daily dose.
*): subject received buprenorphine; 140 pg/mg were found in the plaque.
**): Bacterial FAME were determined qualitatively.

Conclusion

The presented method is **well suited for routine laboratories** and offers a tool to indirectly characterize the bacterial composition of dental plaque via FA profiles. Thus, the method enables to **investigate correlations** between **drug concentrations** and the **bacterial composition of dental plaque.** This may help to better interpret drug results and to establish plaque as novel alternative matrix in forensic toxicology.

Acknowledgement

Kerstin Henkel acknowledges financial support from the State of Baden-Württemberg (Promotionsstipendium nach dem Landesgraduiertenförderungsgesetz, LGFG).

References

[1] Roth SR, *et al;* 2018 *Bioanalysis* (accepted).
[2] Henkel K, *et al*; 2018 *Talanta.*
[3] Henkel K; 2018 Oral Presentation at the 56th TIAFT Annual Meeting, Ghent (Belgium).
[4] Reich M, *et al*; 2012 *J. Lipid Res.*
[5] Thurnhofer S, *et al*; *2005 J. Agric. Food Chem.*

Contact

Kerstin Henkel
Institute of Forensic Medicine
Albertstraße 9
79104 Freiburg, Germany
kerstin.henkel@uniklinik-freiburg.de