SHS-GC-MS Optimization for Volatile Organic Compounds Analysis in Oral Fluid and Urine

Poster #3c

Bruno Ruiz Brandão da Costa[1], Bruno Toledo Freitas[2], Delia Rita Tapia Blácido[2], Bruno Spinosa De Martinis[2].

[1]*School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo. Ribeirão Preto, São Paulo, Brazil.*
[2]*Chemistry Department of Faculty of Philosophy, Sciences and Languages of Ribeirão Preto – University of São Paulo. Ribeirão Preto, São Paulo, Brazil.*

INTRODUCTION

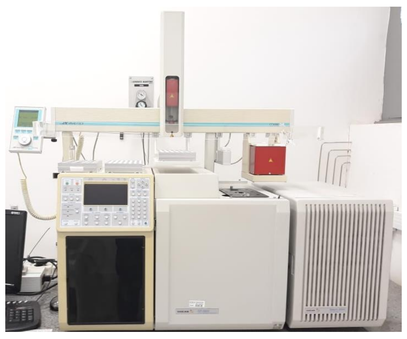
- Physiological processes naturally produce Volatile Organic Compounds (VOCs). In a pathological condition, different processes occur, causing the generation of VOCs which are not usually produced and/or changing their concentration [1].
- VOCs can be analyzed in biological samples (for example, oral fluid and urine) to identify potential disease biomarkers, notably cancer [2].
- Static Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS). depends on numerous factors, such as incubation time (t_{inc}), temperature (T), salt addition (NaCl), agitation (A), sample volume (SV) and pH [3].
- Factorial experimental designs can be used in order to maximize the process and reduce the number, duration, and cost of experiments [4].

METHODS

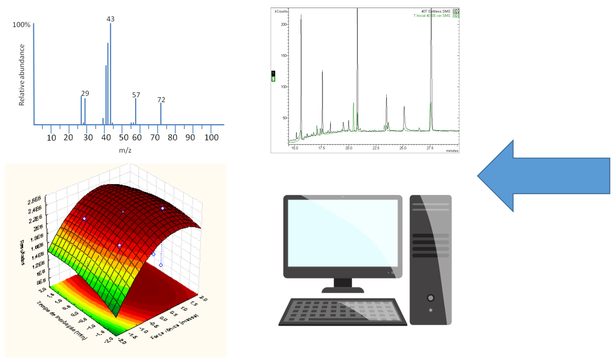
- Plackett-Burman (PB) factorial design was used to evaluate how the variables affected the total response area. The significant ones were optimized by central composite design (CCD) and Response Surface Methodology.

Sample collection

Sample preparation



GC-MS



Statical analysis in Statistica 7.0

GC-MS: Gas Chromatography CP-3800 coupled to Ion Trap Mass Spectrometry Saturn 2000 (Varian)

Fig 1. Experimental design and analysis.

Oral fluid optimization parameters:

Table 1. PB design for oral fluid

Plackett-Burman			
Variables	**Levels**		
	-1	**0**	**1**
t_{inc} (min)	5	12.5	20
T (°C)	60	77.5	95
NaCl (g)	0	0.3	0.6
A (rpm)	250	475	700
SV (mL)	0.5	1	1.5
pH	Ac	Neu	Bas

Table 2. CCD values for oral fluid

CCD 2^3					
Variables	**Levels**				
	-1.68	**-1**	**0**	**1**	**1.68**
t_{inc} (min)	5	8	12.5	17	20
T (°C)	60	66	77.5	89	95
NaCl (g)	0.2	0.36	0.6	0.84	1

Urine optimization parameters*:

Table 3. PB design for urine

Plackett-Burman			
Variables	**Levels**		
	-1	**0**	**1**
t_{inc} (min)	5	10	15
NaCl (g)	0.6	0.8	1.2
A (rpm)	250	475	700
SV (mL)	2	3	4
pH	Ac	Neu	Bas

Table 4. CCD values for urine

CCD 2^3					
Variables	**Levels**				
	-1.68	**-1**	**0**	**1**	**1.68**
t_{inc} (min)	10	16	20	26	30
A (rpm)	400	490	550	640	700
NaCl (g)	0.8	0.96	1.2	1.44	1.6

T was kept at 95 **°C**

RESULTS

Oral fluid:

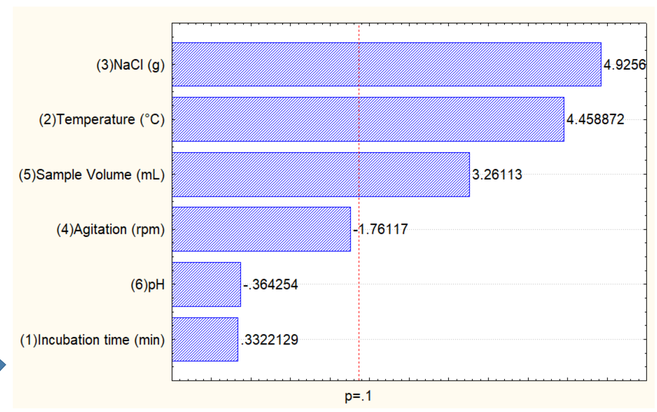
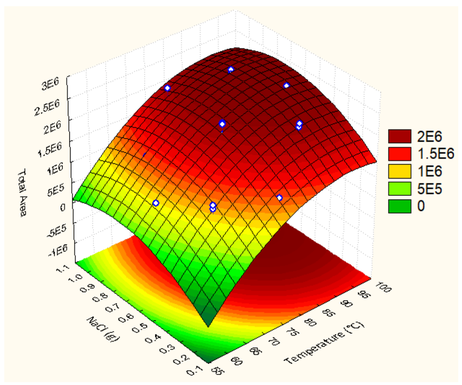


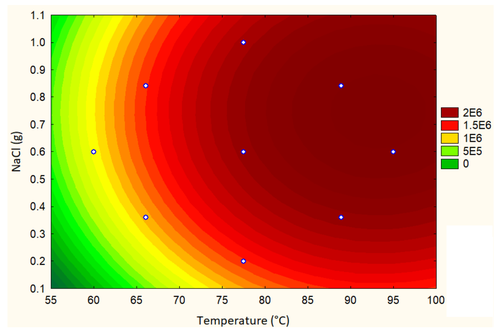
Fig 2. Pareto Chart – PB Oral Fluid

- SV was fixed at 1 mL;
- Agitation at 250 rpm;
- Neutral pH;
- **CCD: T_{inc}, T** and **NaCl** were optmized.



R^2 = 0.964

Equation: 2093172 + 486142 X_2 – 170231 X_2^2 + 168263X_3 - 153601X_3^2



Optimized conditions for oral fluid: T_{inc} = 5 min, T = 95°C; NaCl = 0.8 g

Fig 3. Oral fluid CCD 2^3 results

Urine:

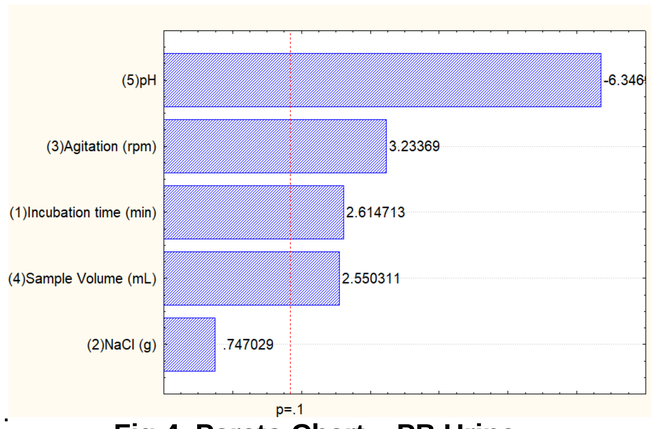
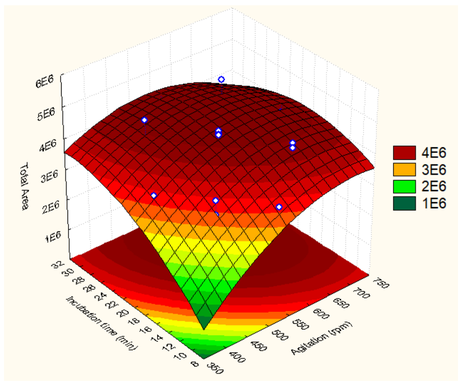


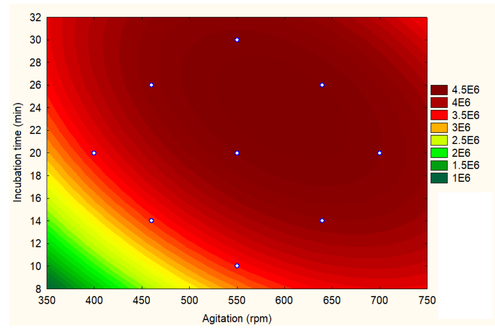
Fig 4. Pareto Chart – PB Urine

- SV was fixed at 4 mL;
- Acidic pH;
- **CCD 2^3: T_{inc}, A** and **NaCl** were analyzed.



R^2 = 0.932

Equation: 4411519 + 623648 X_1 – 349830 X_1^2 + 552270 X_2 - 335023 X_2^2 + 422662 X^3 – 156801 X_3^2 -305891 X_1X_2 + 272366 X_1X_3 – 139797 X_2X_3



Optmized agitation: 550 rpm. t_{inc} and NaCl need a CCD 2^2 optmization.

Fig 5. Urine CCD 2^3 results

CONCLUSION AND PERSPECTIVE

- Factorial design allowed the optmization of six variables related to VOCs analysis in OF and Urine. Once these matrices have different characteristics and VOC profiles, the optimized values also differ.
- These parameters will be used to analyze the OF and urine of individuals with Head and Neck Cancers, to determine possible biomarkers of this disease.

REFERENCES

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ACKNOWLEDGMENT

CAPES

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