HIGH-THROUGHPUT DETERMINATION OF OXIDATIVE STRESS BIOMARKERS IN SALIVA BY SOLVENT-ASSISTED DISPERSIVE SOLID-PHASE EXTRACTION FOR CLINICAL ANALYSIS

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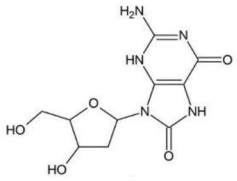
INTRODUCTION

Oxidative stress is produced as a result of a disturbance in the oxidant-antioxidant balance in the organism, when reactive oxygen species are not completely inactivated by cellular antioxidant defenses. This oxidative damage represents a risk of developing atherosclerosis, cancer, heart failure or diabetes, among other pathologies. Oxidative stress induces the oxidation of several biomolecules, including DNA or RNA, forming oxidized nucleotides that are subsequently excreted by different biological fluids. **8-hydroxy-2'-deoxyguanosine (8-oxodG)** and **8-hydroxy-2'-deoxyadenosine (8-oxodA)** formed by oxidative DNA damage are stress oxidative biomarkers associated with diabetes and different types of cancer.

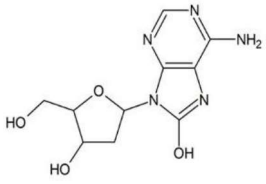
The aim of this work is to develop a high-throughput method for the determination of these **stress oxidative biomarkers** (8-oxodG and 8-oxodA) in **saliva** and apply it to samples from diabetes type II patients to demonstrate its applicability.

The presented method is based on **solvent-assisted dispersive solid-phase extraction (SA-DSPE)** employing a commercial hydrophilic-lipophilic balanced (HLB) polymer (Strata^{TM}-X-RP) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [1].

ANALYTES

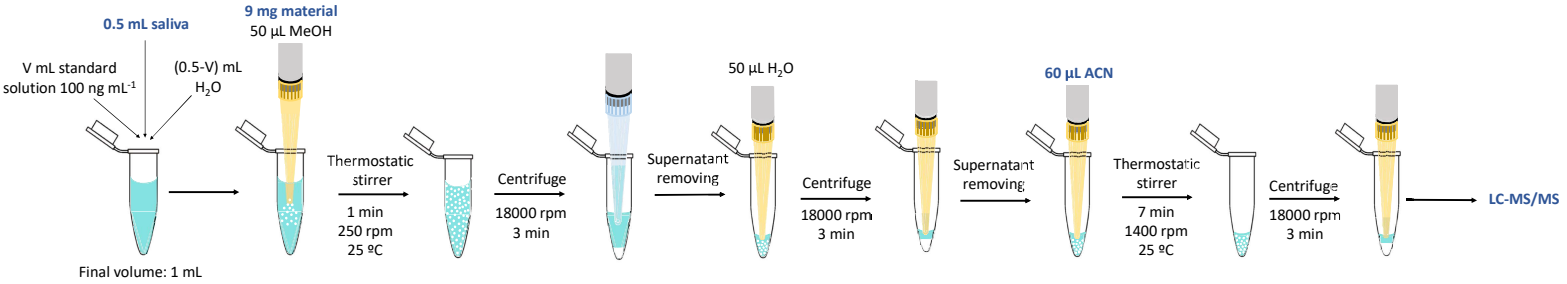


8-hydroxy-2'-deoxyguanosine (8-oxodG)



8-hydroxy-2'-deoxyadenosine (8-oxodA)

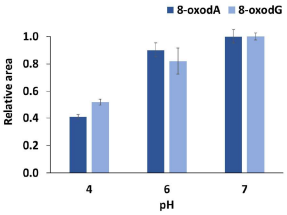
EXPERIMENTAL



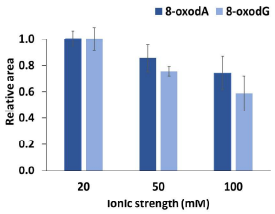
RESULTS AND DISCUSSION

Optimization

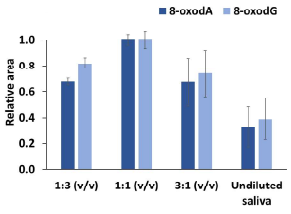
pH



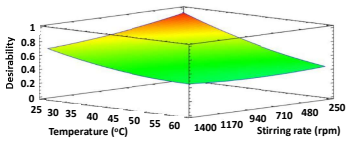
Ionic strength

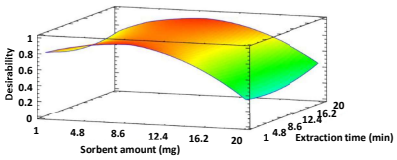


Saliva dilution

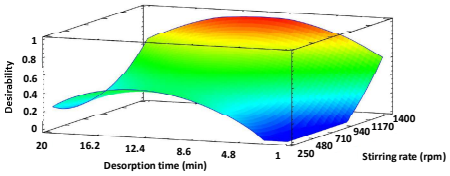


Response Surface Methodology for the extraction procedure (Box-Behnken design)

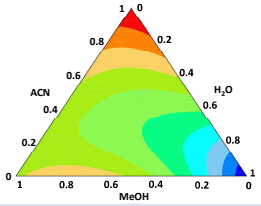




Response Surface Methodology for the desorption procedure (Doehlert design)



Simplex-Centroid design for the desorption solvent



Validation

Analyte	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Precision (% RSD) (n=5)			
				Intra-day		Inter-day	
				1 ng mL^{-1}	5 ng mL^{-1}	1 ng mL^{-1}	5 ng mL^{-1}
8-oxodG	0.991	0.22	0.72	8.3	10.0	14.0	14.7
8-oxodA	0.992	0.25	0.83	14.8	6.3	5.7	10.0

Working range: 0.5-5 ng mL^{-1}

Analysis of saliva samples

Volunteer	Concentration (ng mL^{-1})	
	8-oxodG	8-oxodA
1	2.4 ± 0.1	1.0 ± 0.1
2	1.1 ± 0.2	<LOD
3	<LOD	1.3 ± 0.3
4	1.3 ± 0.2	2.4 ± 0.4
5	<LOQ	1.9 ± 0.2
6	<LOQ	<LOQ
7	2.8 ± 0.1	6.9 ± 0.1
8	4.4 ± 0.6	5.0 ± 0.5
9	4.0 ± 0.4	3.9 ± 0.5

Expected concentrations:

Healthy person: ≈≤1.5 ng mL^{-1}

Person with a disease: ≥4.0 ng mL

→ Diagnosed with type II diabetes

- As expected, higher concentrations were found for volunteers with **type II diabetes**

CONCLUSIONS

- A **high-throughput** method based on SA-DSPE as clean-up step prior to LC-MS/MS for the determination of **8-oxodG** and **8-oxodA** in **saliva** samples has been developed and validated
- This new approach overcomes the main drawback of long analysis time of the unique previous work where the determination of these oxidative stress biomarkers in saliva was carried out employing microextraction
- The use of thermostatic stirrer allows the extraction of several samples simultaneously, which is beneficial for **routine clinical analysis**
- The method was successfully applied to a total of nine saliva samples (six healthy volunteers and **three volunteers diagnosed with type II diabetes**), obtaining higher concentrations for type II diabetes volunteers, demonstrating its applicability

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ONLINE VERSION

REFERENCES

[1] G. Peris-Pastor, S. Alonso-Rodríguez, J.L. Benedé, A. Chisvert, High-throughput determination of oxidative stress biomarkers in saliva by solvent-assisted dispersive solid-pase extraction, Advances in Sample Preparation. 6 (2023) 100067

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High-throughput determination of oxidative stress biomarkers in saliva by solvent-assisted dispersive solid-phase extraction for clinical analysis

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ABSTRACT

A reliable analytical method for the simultaneous determination of two oxidative stress biomarkers (i.e., 8-hydroxy-2′-deoxyguanosine (8-oxodG) and 8-hydroxy-2′-deoxyadenosine (8-oxodA)) in saliva samples is presented. These biomarkers are produced by an oxidative DNA damage and have gained prominence in the field of medicine as early diagnostic and disease control tools. The method is based on solvent-assisted dispersive solid-phase extraction (SA-DSPE) as a clean-up step, followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). For this purpose, a commercial polymer with a hydrophilic-hydrophobic balance has been used as extraction phase. This balance makes the material suitable for extracting compounds from polar matrices such as saliva. Those variables involved in the extraction were optimized by a Box-Behnken design, whereas those variables affecting the desorption were optimized by a Doehlert design, except the desorption solvent that was optimized by using a Simplex-Centroid design. The method was successfully validated, showing a good linearity at least up to 20 ng mL^{-1}, limits of detection and quantification at the low ng mL^{-1} level, and good precision values (< 15%). Standard addition calibration was employed to correct the observed matrix effects. Finally, this new approach was successfully applied to saliva samples from nine volunteers, three of them with type II diabetes, obtaining notable differences in the concentration values between both groups. The proposed methodology overcomes some of the drawbacks of the only previous work with the same purpose, such as the time-consuming procedure and the consumption of large volumes of organic solvents. To increase the sample throughput and reduce the analysis time, a thermostatic stirrer that allows the extraction of several samples simultaneously was used.

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Other communications presented by GICAPC Research Group at **25th International Symposium on Advances in Extraction Technologies (ExTech 2023):**

YP-50 *Solid-phase immunoextraction followed by liquid chromatography-tandem mass spectrometry for the selective determination of thyroxine in human serum*. V. Vállez-Gomis, J. L. Benedé, A. Combès, A. Chisvert and V. Pichon. **See communication**.

KN-02 *New miniaturized approaches for the analysis of low-availability samples*. A. Chisvert, J. L. Benedé, J. Grau, V. Vállez-Gomis, C. Azorín and G. Peris-Pastor.
July 19th, 10:40h, Auditorium.

YO-02 *Miniaturized magnetic-pipette tip microextraction: A new tool for microsample analysis*. J. Grau, M. Moreno-Guzmán, L. Arruza, M. Á. López, A. Escarpa and A. Chisvert.
July 19th, 12:35h, Auditorium.

YO-28 *Miniaturized stir bar sorptive dispersive microextraction as a high-throughput and feasible approach for low-availability samples*. C. Azorín, J. L. Benedé and A. Chisvert.
July 20th, 17:40h, Atenas room.