DEVELOPMENT OF DISPERSIVE LIQUID-SOLID MICROEXTRACTION: APPLICATION TO THE DETERMINATION OF CORTISONE AND CORTISOL IN HUMAN SALIVA

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INTRODUCTION

AIM

In this work, we introduce a new hybrid microextraction technique that combines dispersive liquid-liquid microextraction (DLLME) and dispersive solid-phase extraction (DSPE) with magnetic nanoparticles (MNPs), termed dispersive liquid-solid microextraction (DLSME)

In this new approach a magnetic material is dispersed into the liquid sample employing a disperser solvent. Later, using an external magnetic field, the sorbent is kept whereas the sample is retired with a syringe. Finally, the analytes are desorbed from the magnetic sorbent with a small volume of organic solvent.

Cortisone and cortisol, widely used as biomarkers of Cushing's syndrome, stress and other diseases, were selected as model compounds for method development and optimization.

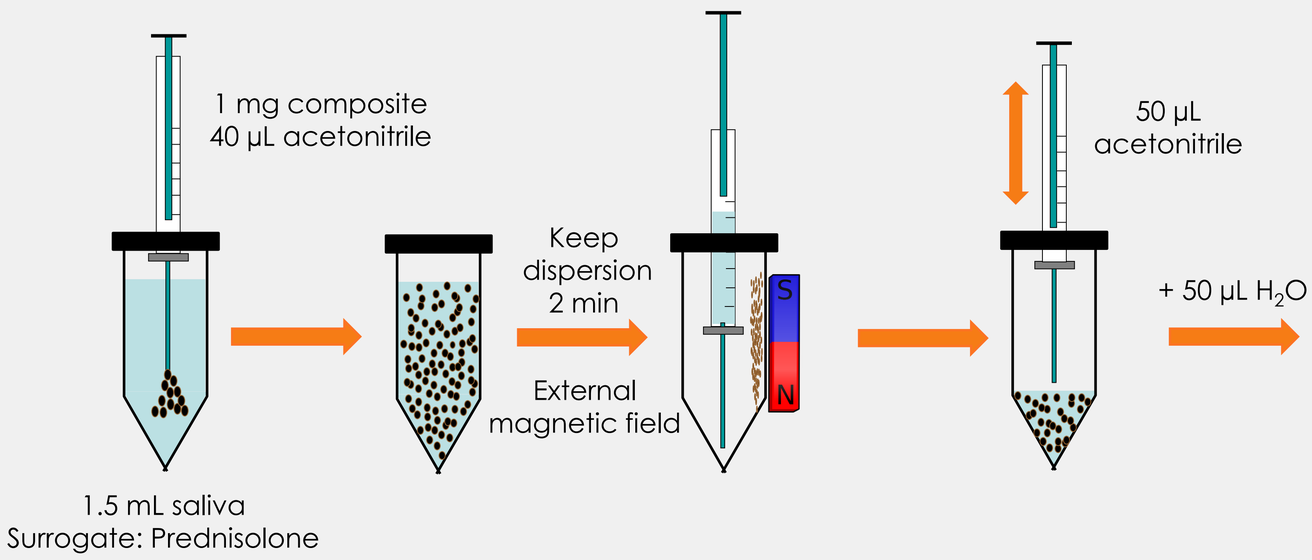
DLLME + DSPE + MNPs → **DLSME Mediated by MNPs**

Cortisone ⇄ **Cortisol**

11-β HSD1

11-β HSD2

EXPERIMENTAL



LC-MS/MS

LC-MS/MS conditions

- **Injection Volume**: 10 µL
- **Column Temperature:** 25 °C
- **Mobile Phase:** MeOH:H2O (50:50%)
- **Flow Rate**: 0.2 mL min^{-1}
- **Column**: Zorbax SB-C18 (1.8 µm, 2.1 x 50 mm)
- **Gas Temperature:** 350 °C
- **Gas Flow:** 11 mL min^{-1}
- **Acquisition Mode**: ESI^+(MRM)

RESULTS AND DISCUSSION

Figures of merit of the proposed method

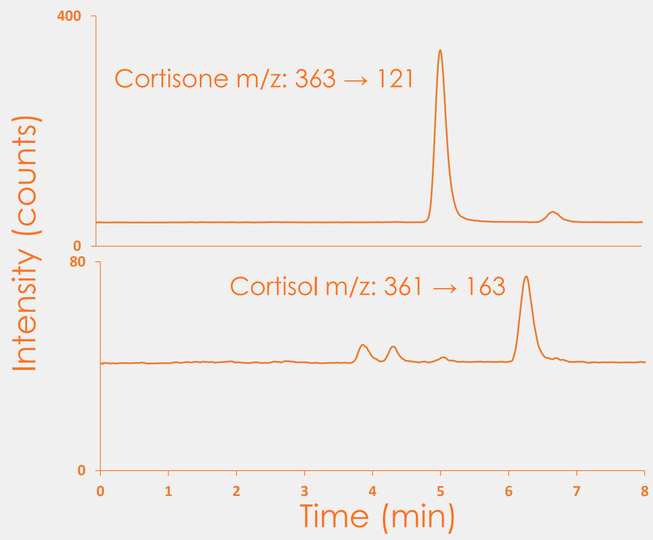
- High levels of **linearity**, that reached at least 20 ng mL^{-1}, were obtained for both compounds
- Low **method limits of detection** (MLODs) and good values of **precision** (repeatability) and **enrichment factors** were achieved
- **Recoveries** were studied employing an artificial saliva sample: results did not show significant matrix effect

	MLOD (ng L^{-1})	EF	R^2	Repeatability (% RSD)				Recoveries %	
				Intra-day		Inter-day			
				1 ng mL^{-1}	10 ng mL^{-1}	1 ng mL^{-1}	10 ng mL^{-1}	1 ng mL^{-1}	10 ng mL^{-1}
Cortisone	19.3	9.5 ± 0.5	0.9995	5.0	1.8	9.6	8.7	97 ± 9	105 ± 7
Cortisol	33.2	8.6 ± 0.5	0.9990	4.2	6.1	10.6	6.3	98 ± 8	105 ± 5

Analysis of real samples

- Samples from 4 volunteers were studied
- Different amounts of cortisol (between 0.48-2.20 ng mL^{-1})and cortisone (3.8-10.0 ng mL^{-1}) were found

	Volunteer 1 (ng mL^{-1})	Volunteer 2 (ng mL^{-1})	Volunteer 3 (ng mL^{-1})	Volunteer 4 (ng mL^{-1})
Cortisone	3.8 ± 0.2	4.2 ± 0.3	7.5 ± 0.4	10.0 ± 0.2
Cortisol	0.54 ± 0.03	0.48 ± 0.04	1.81 ± 0.11	2.20 ± 0.07



Chromatogram obtained by applying DLSME to a real saliva sample

CONCLUSIONS

- This new approach allows faster determination employing small amounts of sample, organic solvents and composite with the minimum sample treatment. Any supporting equipment (vortex, centrifuge, ultrasounds…) is not necessary neither
- The proposed method provides good analytical features and allows the determination of cortisone and cortisol in saliva samples

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Consulta otras comunicaciones presentadas por el GICAPC en la **XXII Reunión de la Sociedad Española de Química Analítica:**

Determination of nitro musks in environmental waters by stir bar sorptive dispersive microextraction followed by thermal desorption-gas chromatography-mass spectrometry. J.L. Benedé, A. Chisvert, A. Salvador. Flash Communication. Ver comunicación.

Stir bar sorptive-dispersive microextraction mediated by a magnetic nanoparticles-metal organic framework composite for the determination of n-nitrosamines in cosmetic products. P. Miralles, I. Van Gemert, A. Chisvert, A. Salvador. Flash Communication. Ver comunicación.

Development of an analytical method for the determination of acrylamide in cosmetic products based on dispersive liquid-liquid microextraction. L. Schettino, J.L. Benedé, A. Chisvert, A. Salvador. Flash Communication. Ver comunicación.

Determination of hydroxylated ingredients with preservative activity in cosmetic products by gas chromatography-mass spectrometry. C. Azorín, J.L. Benedé, A. Chisvert, A. Salvador. Ver comunicación.

A green analytical method for the determination of hydroxyethoxyphenyl butanone in cosmetic products. P. Miralles, J.L. Benedé, A. Mata-Martín, A. Chisvert, A. Salvador. Ver comunicación.

Determination of polycyclic aromatic hydrocarbons in cosmetics by stir bar sorptive dispersive microextraction and gas chromatography-mass spectrometry. Vállez-Gomis, J. Grau, J.L. Benedé, A. Chisvert, A. Salvador. Ver comunicación.

Reversed-phase dispersive liquid-liquid microextraction prior to liquid chromatography-tandem mass spectrometry for the determination of acrylamide in cosmetic products. L. Fernández, J.L. Benedé, A. Chisvert, A. Salvador. Ver comunicación.

Development of dispersive liquid-solid microextraction: application to the determination of cortisone and cortisol in human saliva. J. Grau, J.L. Benedé, A. Chisvert, A. Salvador. Ver comunicación.