MODIFIED MAGNETIC-BASED SOLVENT ASSISTED DISPERSIVE SOLID-PHASE EXTRACTION: APPLICATION TO THE DETERMINATION OF CORTISOL AND CORTISONE IN HUMAN SALIVA

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INTRODUCTION

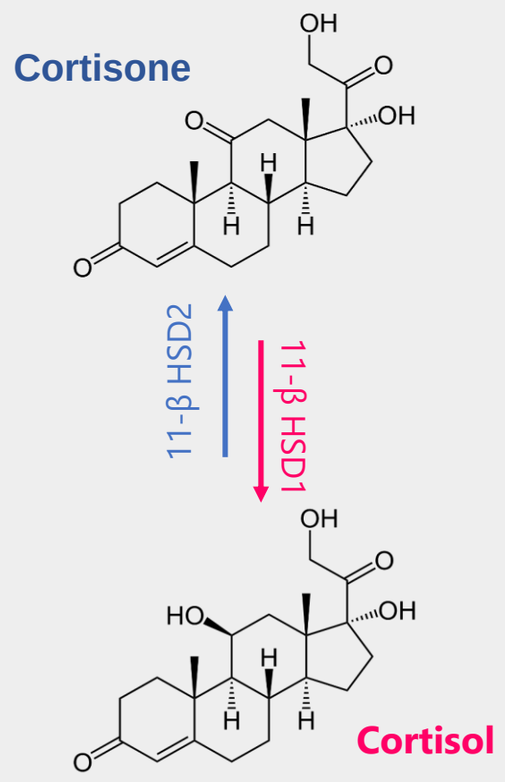
AIM

To develop a method employing a modification of the Magnetic-based Solvent Assisted Dispersive Solid-Phase Extraction (M-SA-DSPE) for the determination of **cortisol** and **cortisone** in saliva

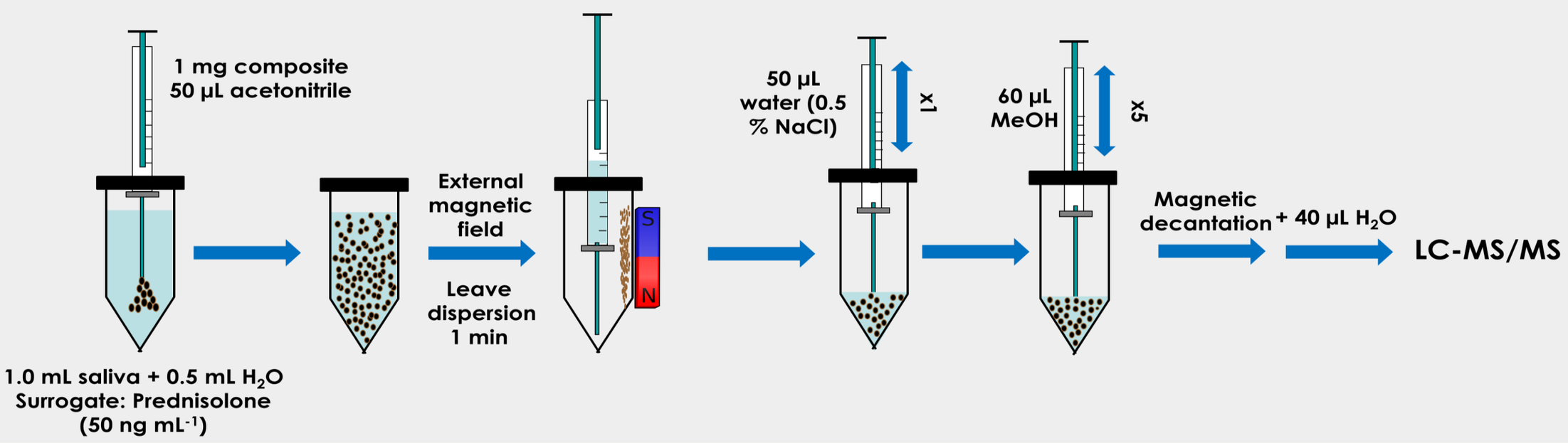
- In this modification, a **magnetic sorbent material** is quickly dispersed in the sample with a **disperser solvent**, analogously to dispersive liquid-liquid microextraction (DLLME)
- **No external agitators** (i.e. vortex, ultrasounds etc.) are needed
- After a **small extraction time**, the magnetic material is easily retrieved by means of an **external magnet**
- Finally, analytes are **desorbed** in a small volume of organic solvent and measured by **LC-MS/MS**

- Analysis of **salivary cortisol** has gained interest in recent years due to its good correlation with **serum cortisol** and the relatively no-invasiveness of saliva sampling
- Abnormal levels of salivary **cortisol** can be an indicator of **Cushing** syndrome, **hypertension** and **sepsis**, among others
- Moreover, simultaneous determination of **cortisol** and **cortisone** can be a good indicator about a malfunction in the parotid gland

- In this work, a **reversed phase polymer** (Strata X^{TM}-RP) embedded **with $CoFe_2O_4$ magnetic nanoparticles** (MNPs) is used as magnetic sorbent



EXPERIMENTAL



RESULTS AND DISCUSSION

Analyte	R^2	MLOD (ng mL^{-1})	MLOQ (ng mL^{-1})	EFs	Repeatability (% RSD)			
					Intra-day		Inter-day	
					1 ng mL^{-1}	10 ng mL^{-1}	1 ng mL^{-1}	10 ng mL^{-1}
Cortisol	0.9990	0.029	0.097	5.2 ± 0.2	4.2	6.1	10.0	6.3
Cortisone	0.9995	0.018	0.060	5.6 ± 0.3	5.0	1.8	9.6	8.7

- **High levels of linearity**, at least 20 ng mL^{-1}, were obtained for both compounds

Relative recoveries form spiked samples

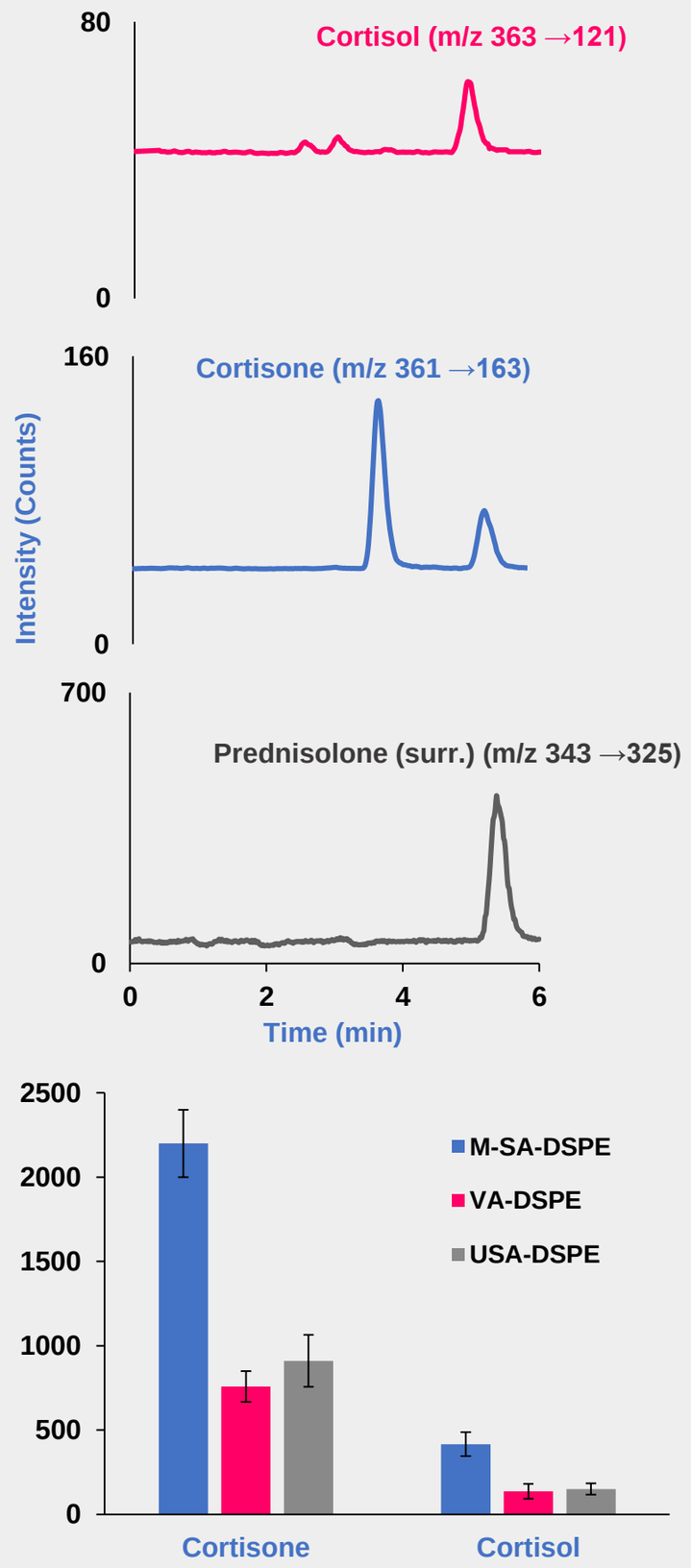
Sample	Amount spiked (ng mL^{-1})	Amount found (ng mL^{-1})		Relative recovery (%)	
		Cortisol	Cortisone	Cortisol	Cortisone
1	0	2.0 ± 0.2	8.1 ± 0.7	-	-
	1	2.86 ± 0.03	9.04 ± 0.05	87 ± 3	94 ± 5
	5	6.41 ± 0.05	12.9 ± 0.6	88 ± 5	96 ± 12
	10	11.7 ± 0.3	17.6 ± 0.3	97 ± 3	95 ± 3
2	0	1.03 ± 0.01	6.6 ± 0.5	-	-
	1	1.88 ± 0.06	7.61 ± 0.09	86 ± 6	96 ± 9
	5	5.5 ± 0.2	12.0 ± 0.7	89 ± 5	108 ± 13
	10	10.7 ± 0.7	17.7 ± 0.5	97 ± 3	111 ± 5
3	0	1.55 ± 0.01	4.3 ± 0.3	-	-
	1	2.44 ± 0.07	5.32 ± 0.09	89 ± 7	99 ± 9
	5	6.0 ± 0.4	9.6 ± 0.5	89 ± 9	107 ± 11
	10	12.1 ± 0.7	14.9 ± 0.2	106 ± 7	106 ± 2

- 3 samples form 3 different volunteers were spiked at 3 levels of concentration in order to study the **relative recoveries** (%)
- Recoveries were obtained between 86 and 111 % for **cortisol** and **cortisone** showing **no significant matrix effect**

M-SA-DSPE vs.VA-DSPE and USA-DSPE

- M-SA-DSPE was compared with other dispersive strategies (i.e. Vortex assisted-DSPE (VA-DSPE) and ultrasounds assisted-DSPE (USA-DSPE)) showing higher **extraction performance** in saliva samples

Chromatogram from a saliva sample



CONCLUSIONS

- A **modified M-SA-DESPE** method has been developed and validated for the determination of **cortisol** and **cortisone** in saliva samples
- This method showed **good analytical features**: showing low LODs, good linearity and RSDs ≤ 10 %
- The method was applied to real samples from different volunteers, obtaining relative recovery values between 86 and 111 %
- This modification, unlike the previous M-SA-DSPE applications, **avoids the use of external agitators** (i.e. vortex, ultrasounds etc.) employing the **minimum extraction times**

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