Evaluation of the Greiner Bio-One saliva collection device for the analysis of cortisol with UPLC-MS/MS

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Introduction

Salivary cortisol (C) reflects unbound (free) serum C concentration and has therefore become a valuable diagnostic tool in endocrinology. However, collecting oral fluid (OF) from xerostomic individuals with absorption-based systems like the Salivette Cortisol (S; Sarstedt) can be cumbersome. In this study we compared the stimulating liquid-based Saliva Collecting System (G; Greiner Bio-One) to the S applying a sensitive UPLC-MS/MS method for C quantification.

Methods

Subjects: 20 volunteers took part in 2 series of OF collection: series A: 10 volunteers each collected 3 consecutive OF samples using the same collection device (n = 60 samples); series B: all individuals collected 4 consecutive OF samples using the 2 different devices in different order (n = 80 samples). In both series total collection time never exceeded 15 min.
Sample collection: OF samples were collected using the G and S device according to the manufacturer. OF concentration [%] of the OF/buffer mixture collected with the G was quantified spectrophotometrically on an Olympus AU680 using the G saliva quantification kit. **Instrument:** Waters Acquity UPLC system connected to a XEVO TQ-S detector. **Sample preparation:** to 100 µL sample 10 µL internal standard containing 5 ng/ml cortisol-d4 in MeOH was added followed by 50 µl sulfosalicylic acid (20%), 600 µl ACN and 100 µL 10 M ammonium acetate. After vortexing and centrifugation the supernatant was mixed with 10 µL ethylene glycol and evaporated under nitrogen at 45 °C. The residue was dissolved with 80 µL H_2O and 10 µl MeOH. 5 µL was injected into the UPLC. **UPLC conditions:** separation was performed on a Waters 2.1 x 150 mm BEH Phenyl 1.7 µm column kept at 60°C. Mobile phase A consisted of 20 mM ammoniumformate and 0.1% formic acid solved in H_2O. Mobile phase B was 0.1% formic acid solved in MeOH. Gradient separation was conducted within 6 min at a flow rate of 0.55 mL/min with 85% A and 15% B at the beginning and 0% A and 100% B at the end. **Detector settings:** data were acquired with an ESI source operating in the positive ionization, MRM mode. Capillary voltage was set to 0.2 kV, ion source temperature was 150 °C, and desolvation gas was heated to 650 °C and delivered at a flow rate of 1000 L/h. Cone gas (N_2) was set to 150 L/h and the collision gas (Ar) was maintained at 0.18 mL/min. Cone voltages: cortisol 20 V. **Calibration:** 0.025, 0.05, 0.075, 0.1, 0.125, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 5.0, 10.0, 20.0 [ng/ml]. **Quality control:** from Referenzinstitut für Bioanalytik (RfB), matrix: serum, QC low: target value 365.1 nmol/L = 132 ng/ml, QC high: target value 824.8 nmol/L = 299 ng/ml, both contols were diluted 1:100. Homemade control sample: pooled OF, target value 0.61 nmol/L = 0.22ng/ml. **LOQ:** 0.025ng/ml.

Conclusions

-- The UPLC-MS/MS method is sensitive, proved to be robust and allowed high throughput for routine analysis.
-- Consecutive OF sampling with the same collection device in an individual resulted in similar C concentrations.
-- Using both devices subsequently in an individual revealed good agreement of the C concentration for G and S samples.
-- The G is of equal use when compared to the S. A big advantage is the standardised short collection time especially when working with xerostomic patients.

UPLC-MS/MS method

Fig.1 Cortisol: Chromatogram and calibration

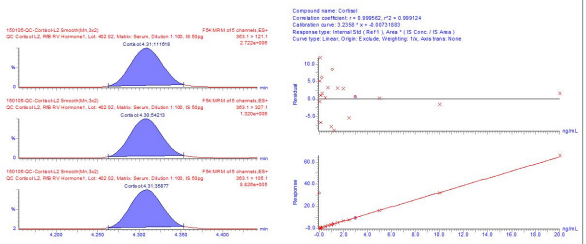
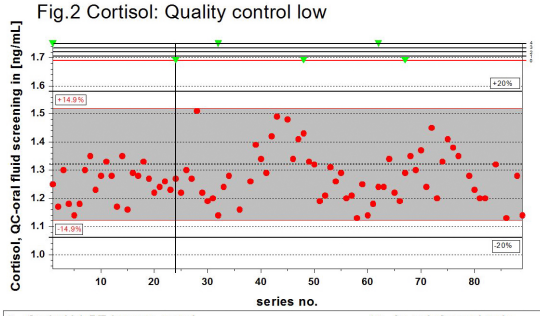


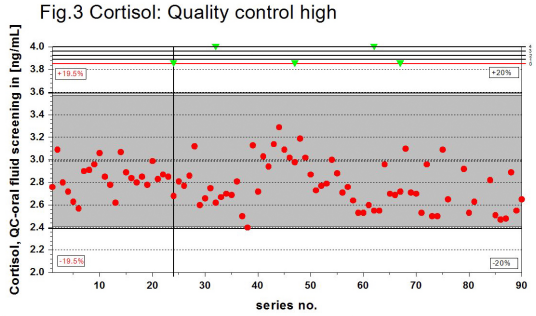
Fig.2 Cortisol: Quality control low



- Cortisol-L1, RfB-hormone control
  Target value: (±20%) 1.06-1.32-1.58 [ng/mL]
  Allowed variation of the single value (according to RiliBÄK): 14.9%
  1.12-1.32-1.52

0 = end of control cycle
1 = new IS and new calibration
2 = new calibration
3 = new IS
4 = new QC dissolved

Fig.3 Cortisol: Quality control high



- Cortisol-L2, RfB-hormone control
  Target value: (±20%) 2.39-2.99-3.59 [ng/mL]
  Allowed variation of the single value (according to RiliBÄK): 19.5%
  2.41-2.99-3.57

0 = end of control cycle
1 = new IS and new calibration
2 = new calibration
3 = new IS
4 = new QC dissolved

Results

A. Consecutive sampling with the same collection devices

Fig.4 3 OF samples collected with Greiner

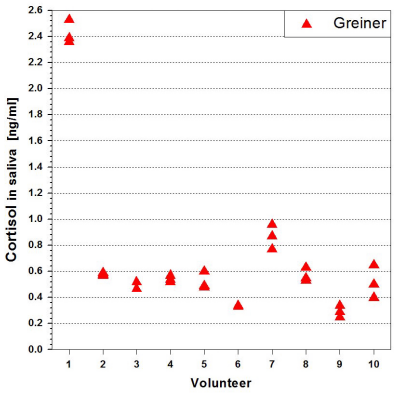
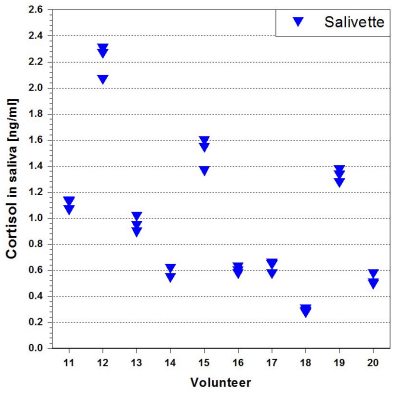


Fig.5 3 OF samles collected with Salivette



B. Consecutive sampling with the two collection devices

Fig.6 Greiner and Salivette: 4 samples / 2 devices

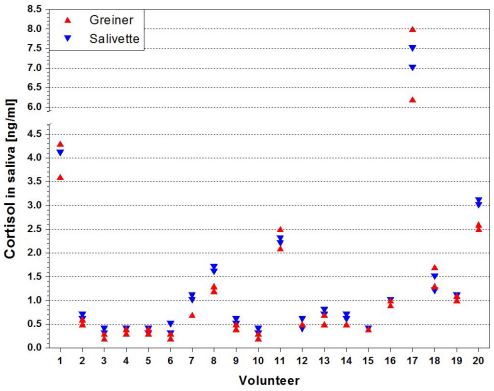


Fig.7 Mean C values of Greiner and Salivette

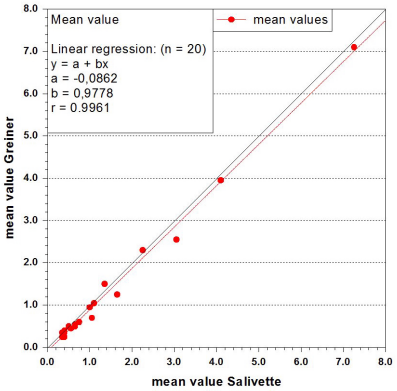
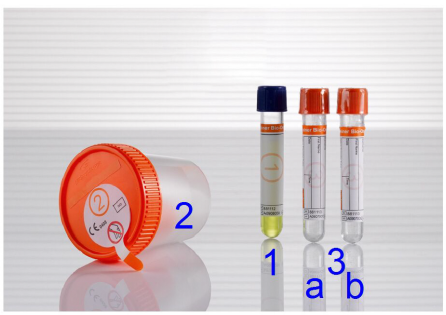


Fig.8 Sampling order

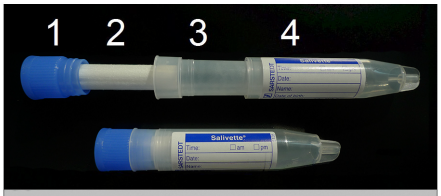
Volunteer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
sampling order	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S
Greiner (G), Salivette (S)	G	S	S	G	G	S	S	G	S	G	S	G	G	G	G	S	S	G	S	G
	S	G	G	S	S	G	G	S	G	S	G	S	S	S	S	G	S	G	G	S
	S	G	S	G	G	S	S	G	S	G	S	S	S	S	S	G	S	G	G	S

Fig.9 Saliva Collection System - Greiner Bio-One



(1) rinsing oral cavity with Saliva Extraction Solution (SES) for 2 minutes
(2) spitting OF/SES into beaker
(3) transfer OF/SES into evacuated tubes (a+b sample) containing bacteriocids and send to lab
(4) after centrifugation amylase and OF concentration are determined on an Olympus AU680

Fig.10 Salivette for cortisol analysis - Sarstedt



(A) open the Salivette
(B) put the swab in the oral cavity
(C) chew the swab gently for 1 minute at least
(D) return the saturated swab to the insert
(E) close the insert with the stopper

1. stopper
2. synthetic swap
3. suspended insert
4. centrifuge vessel