

A New Caffeine Immunoassay: Clinical and Environmental Application

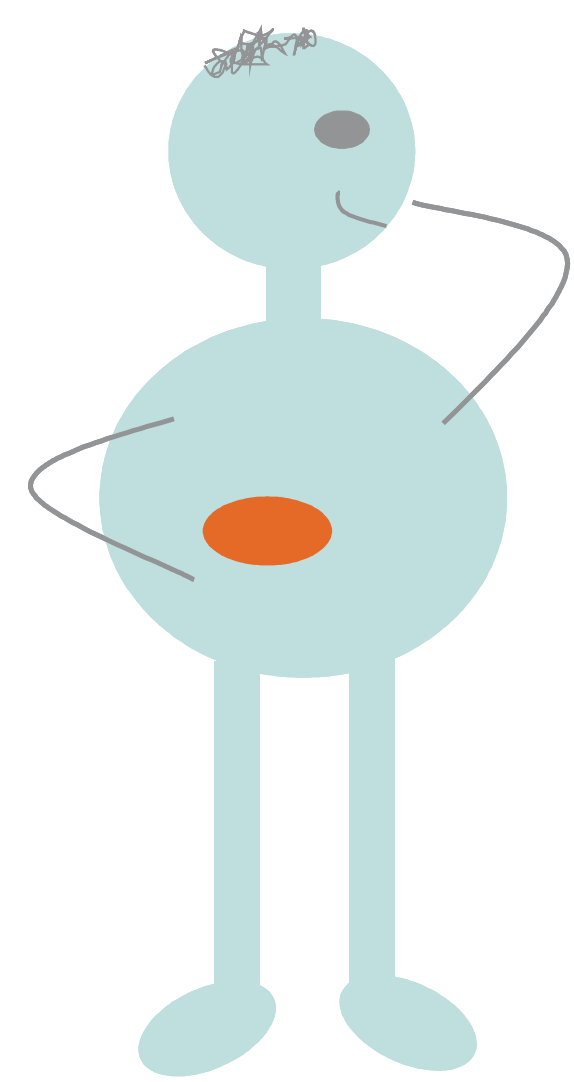
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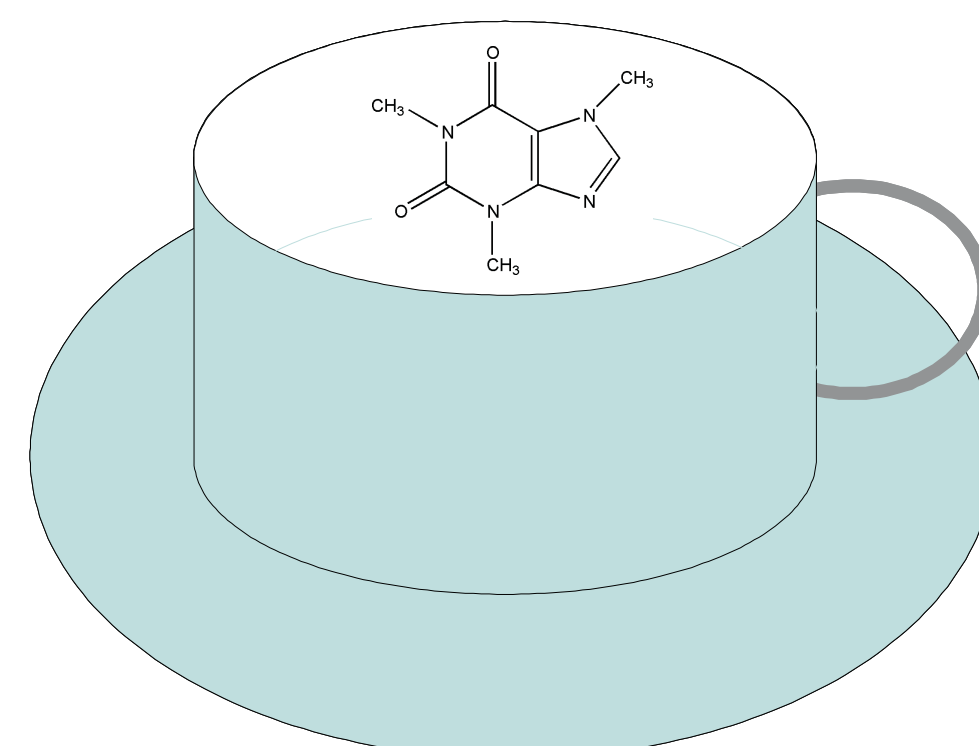
Introduction

CAFFEINE (1,3,7-trimethylxanthine) was discovered by the German chemist Friedrich Ferdinand Runge in 1819. He coined the term "kaffein", a chemical compound in "kaffee"^[1].

Ever since caffeine has been used with several purposes: as pesticide in agriculture, medicine in human therapeutics, additive in cosmetics, as a marker of human liver function^[2] and. Lately, as an environmental marker^[3].

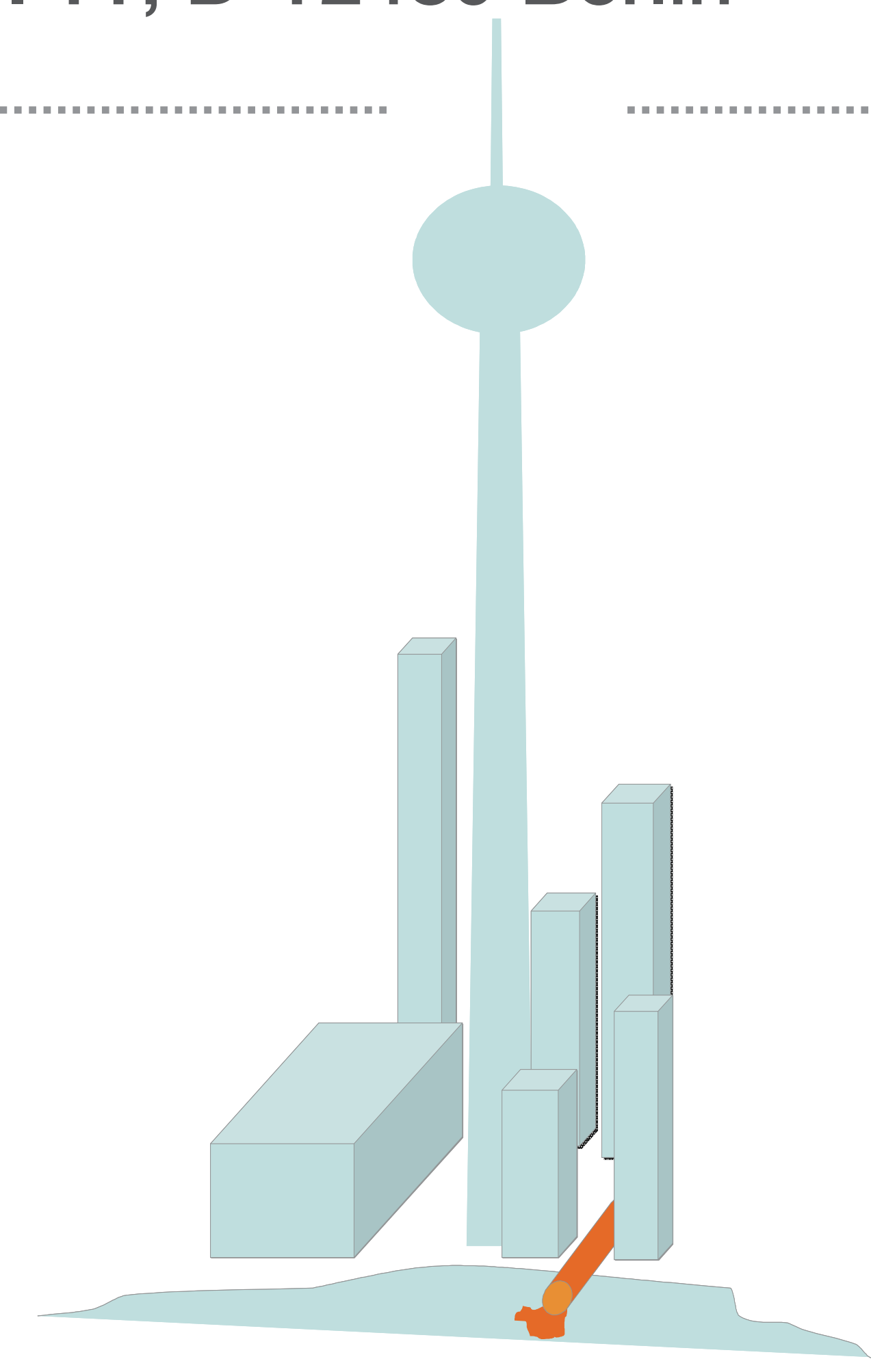


Caffeine is predominantly eliminated via N-3 demethylation to paraxanthine (1,7-dimethylxanthine). The reaction is catalyzed by cytochrome P450 (CYP1A2) and caffeine clearance is considered a "gold standard" for the enzyme's activity in humans.^[4]



Several analytical methods, mostly based on chromatographic techniques, are currently in use for caffeine analysis. Besides the chromatographic analysis time itself, such techniques are usually preceded by sample preparation/concentration steps, making the entire process costly and time-consuming.

➤ **An ELISA (Enzyme-Linked Immunosorbent Assay) was developed to overcome such drawbacks.**



Caffeine is one of the most widely and frequently ingested compound throughout the world. Its presence in environmental water has been already shown, including in treated drinking water, indicating contamination of human origin.^[5]

Experimental

Assay parameters	ELISA		LC-MS/MS	
	Surface waters	Salivas	Surface waters	Salivas
Sample volume	0.5 mL	0.2 mL	500 mL	
Sample preparation	Either Solid Phase Extraction (SPE) or direct analysis	Protein precipitation	Solid Phase Extraction (SPE)	
Monoclonal antibody dilution (anti-caffeine IgG mouse)	1: 100 000	1: 10 000		
Tracer dilution (Horseradish peroxidase conjugate)	1: 75 000	1: 10 000		
Calibration range (n=8)	0.02 µg L ⁻¹ - 100 µg L ⁻¹	2.5 µg L ⁻¹ - 125 µg L ⁻¹	10 µg L ⁻¹ - 175 µg L ⁻¹	

Table 1 and 2. Summary table regarding ELISA and LC-MS/MS methods.

Calibration curve

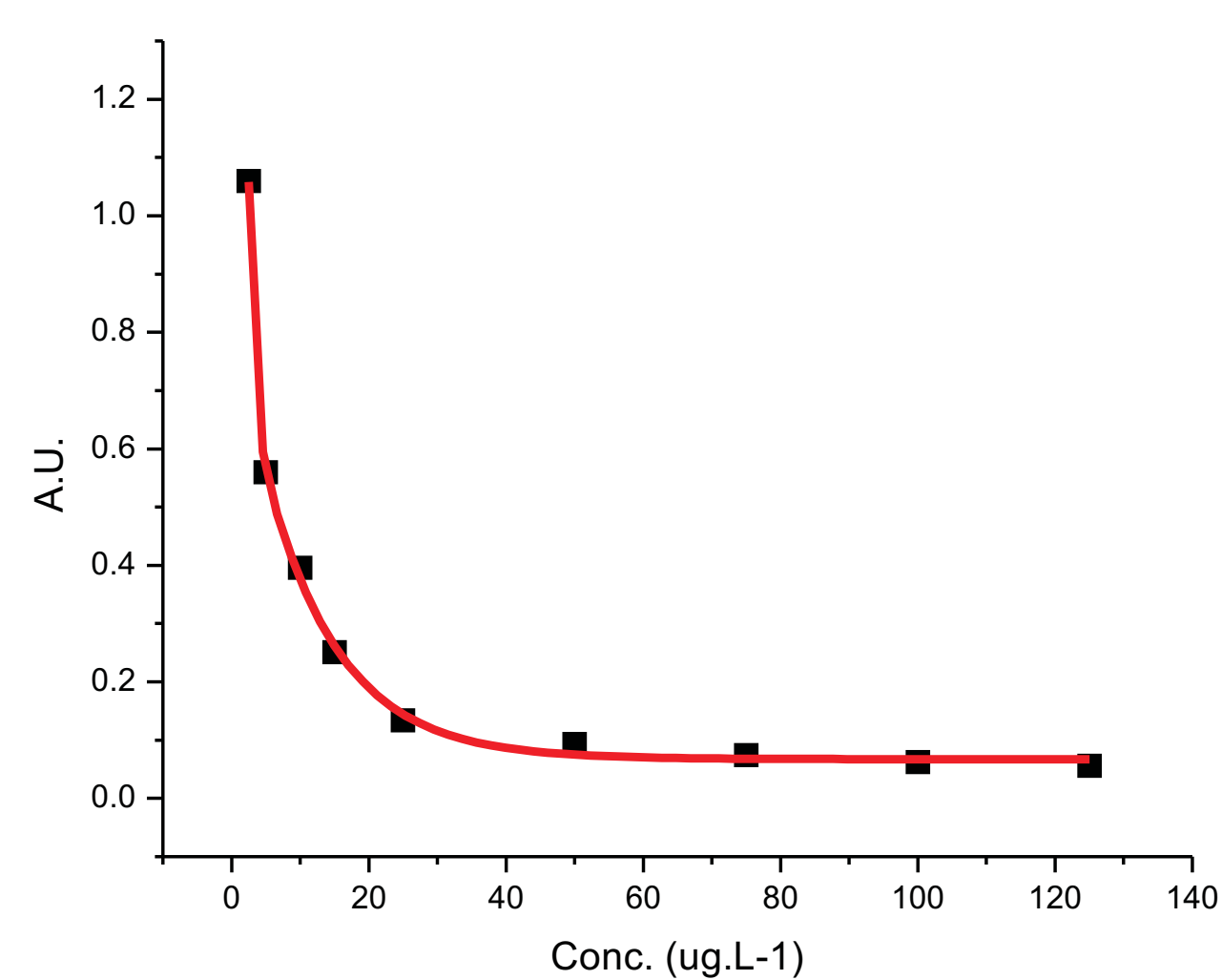


Figure 1. Calibration curve used for quantitation of salivas by ELISA. A third order exponential decay fitting was selected after residues evaluation in comparison with other models.

Cross Reactivity

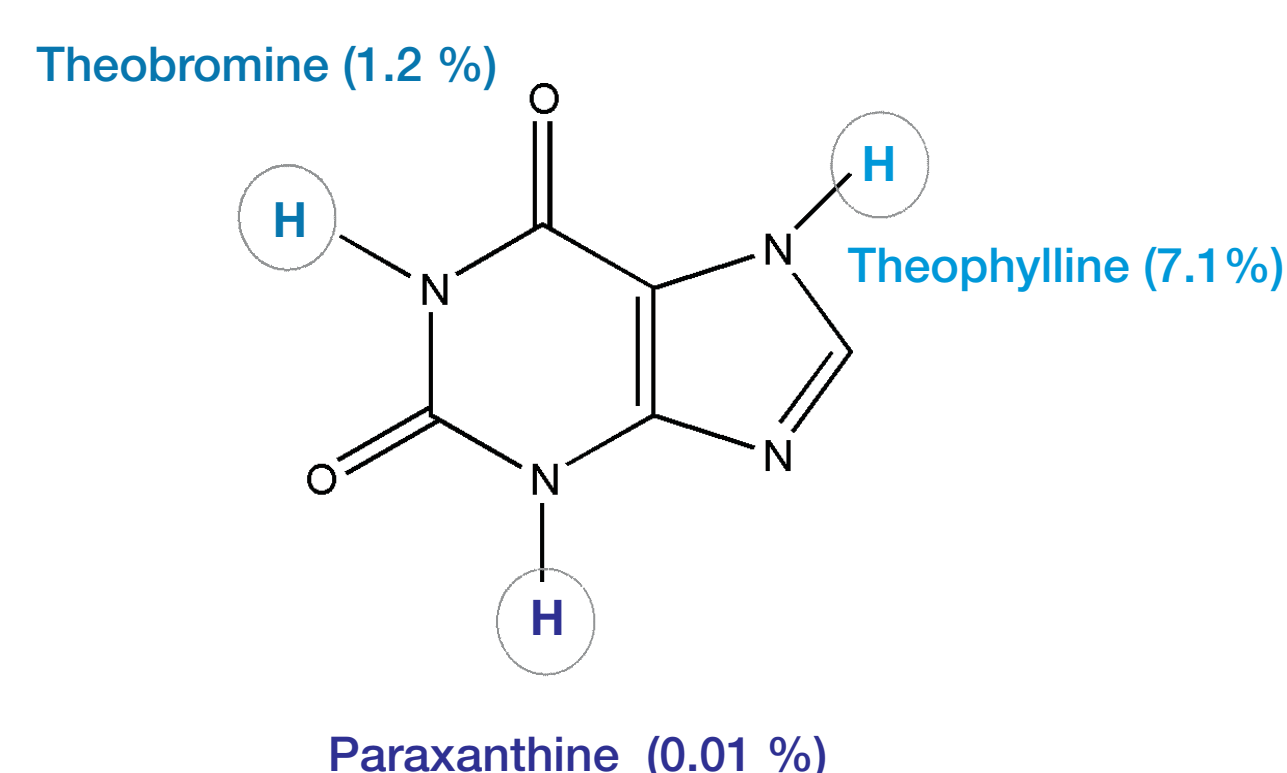


Figure 2. Antibody cross reactivity against caffeine demethylated metabolites. Paraxanthine is the most abundant metabolite produced *in vivo* (90%) and advantageously presents the lowest value.

Outlook

Is the assay selective enough to distinguish caffeine in other matrices?

Can the assay sensitivity be further improved by means of a different detection mode, like fluorescence spectroscopy?

Literature

- [1] B.A. Weinberg, B.K. Bealer, The world of caffeine The science and culture of the world's most popular drug, in Prologue, Routledge, 1st Edition, 2001, London, UK
- [2] T. Wang *et al.*, Klinische Wochen-Schrift, 1985, 63, 1124-1128
- [3] I. J. Buerge *et al.*, Environmental Science & Technology, 2003, 37, 691-700
- [4] J.A. Carrillo *et al.*, Therapeutic drug monitoring, 2000, 22, 409-417

Results and Discussion

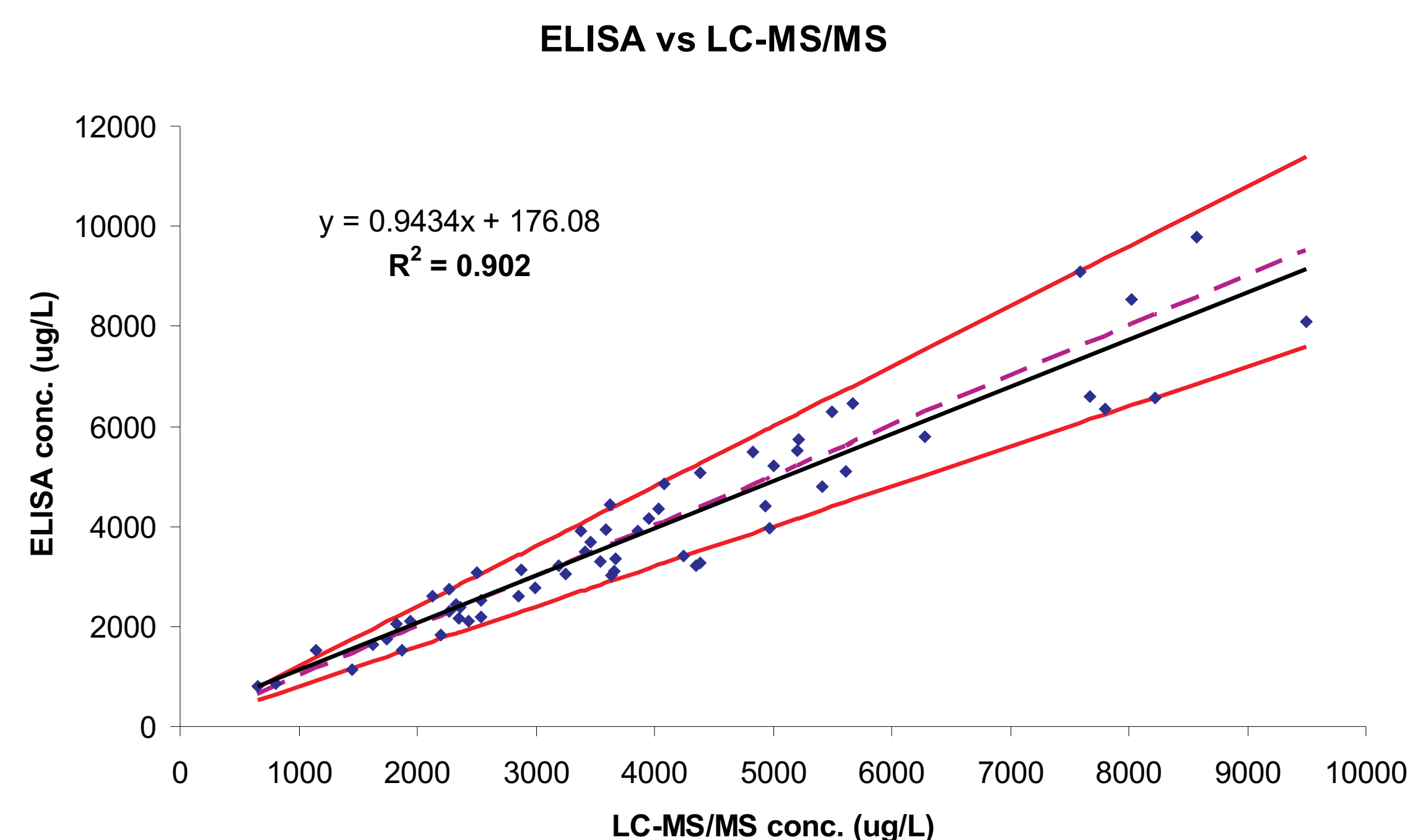


Figure 3. Caffeine concentrations in sixty saliva samples analyzed by both methods. The samples were provided by ten regular caffeine consumers throughout one day, before caffeine intake and thereafter at regular intervals. The red lines represent $\pm 20\%$ of deviation from the ideal curve with slope one (dashed line), and the bold line represents the trendline of all values (navy-blue rhombi).

➤ Bland-Altman analysis and independent two-sided *t*-test (equal variances, $p=0.05$, 118 degrees of freedom) were also performed and confirmed the outcome visualized in the figure above.

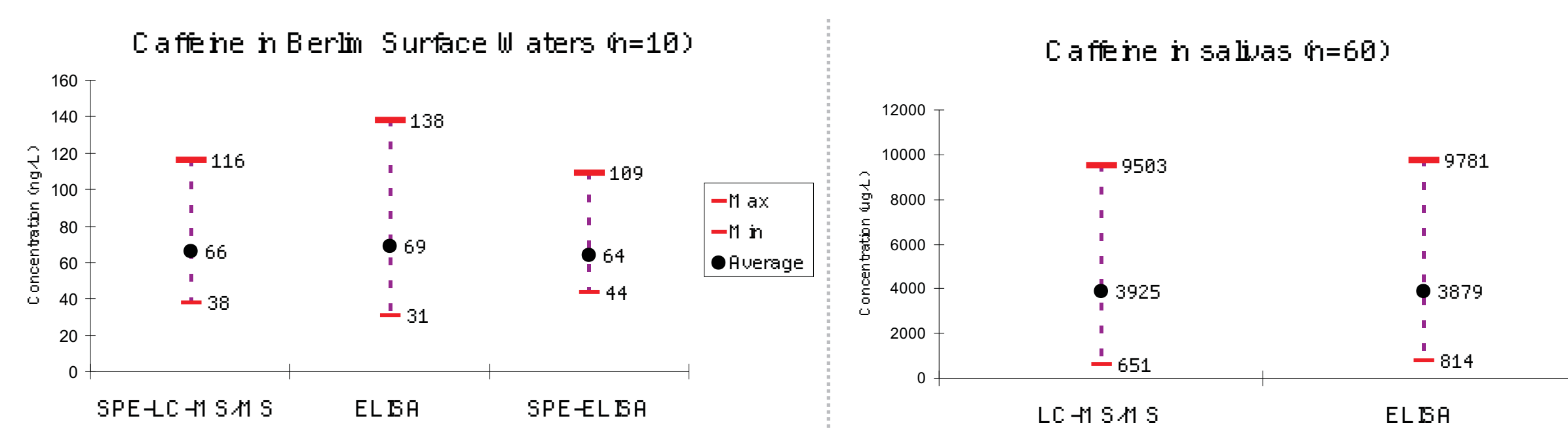


Figure 4. Variation between the methods found for saliva and surface water samples. The surface waters were analyzed directly by ELISA (1000fold less concentrated than the extracts injected into the LC-MS/MS). The SPE extracts were additionally analyzed by ELISA to provide an easier comparison between methods in the same concentration range.

➤ The immunoassay provides results equivalent to those from the LC-MS/MS method regarding the studied matrices and the intended purpose.

➤ On a single ELISA plate 72 samples are analyzed within 3 hours. Even excluding the SPE procedure, the same number of samples requires 64 hours using LC-MS/MS.

Acknowledgement

We would like to thank all colleagues who provided the saliva samples and Kristin Petsch of WG Immunochemical Methods for repeating the analyses, thus independently confirming the results.