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Bovine mammary gland peptibolomics: Adsorption of peptides at the sample drying step

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INTRODUCTION

Variable recovery of peptides is a well-known but often neglected phenomenon that impacts quantitative peptide analysis. Only recently, few studies have systematically investigated the factors influencing the adsorption of peptides, however, without including the drying step in the sample preparation.[1,2]

The sample concentrating and drying step can be a significant source of peptide loss, mainly due to adsorption.[3] Efficient and careful removal of solvent from samples by centrifugal evaporation or freeze-drying methods is an important step in peptidomics. The recovery of peptides has not yet been fully investigated with these sample drying methods. Moreover, the surface adsorption of the peptides by the container and efforts to reduce this adsorption by organic additives is only scarcely elaborated until now. In this experiment, the recovery of five model peptides was investigated applying different organic additives in function of the two applied solvent evaporation processes and vials (polypropylene and glass).

EXPERIMENTAL

EXPERIMENT 1: EFFECT OF ADDITIVES ON MODEL PEPTIDES RECOVERY:

A mixture of model peptides (0.2 µg/µl each), *i.e*. bovine insulin, buserelin, goserelin, leucine-enkephalin, and mouse obestatin prepared by water/acetonitrile mixture (95:5 V/V) was pipetted into total recovery glass vials containing the five separate additives, *i.e.* DMSO, DMF, PEG 400, mannitol (0.5% W/V aqueous solution) and n-nonyl-β-D-glucopyranoside (0.05% W/V aqueous solution). 95 µl of model peptides mixture was added to vials containing the five µl of selected additives. Without drying involved, the samples were analyzed by the HPLC method (**Table 1**).

EXPERIMENT 2: EFFECT OF ADDITIVES ON MODEL PEPTIDE RECOVERY INCLUDING DRYING AND VIAL MATERIAL EFFECTS:

A 0.2 µg/µl mixture of model peptides was prepared in water. Five µl of additives were pipetted into vials made of polypropylene and glass containing 95 µl of the model peptides mixture. After drying the samples by centrifugal evaporation or freeze-drying, 95 µl of water/acetonitrile mixture (95:5 V/V) was added to the samples (recovery values were corrected for water-drying loss in case of mannitol and n-nonyl-β-D-glucopyranoside aqueous solutions, water, DMSO, and DMF). Samples were analyzed in triplicate (n=3) by the HPLC (**Table 1**).

Column	Vydac Everest RP-C18 250 mm × 4.6 mm, 5 µm, 300 Å
Column temperature	30°C
Flow rate	1 ml/min
Mobile phase constituents	A = Water with 0.1% m/V formic acid B = Acetonitrile with 0.1% m/V formic acid
Gradient	Isocratic for 1 min: 90% A and 10%B Linear gradient to 40% B at 60 min
Sample temperature	Ambient
Injection volume	30 µL
Detection	UV @ 275 nm

Table 1: HPLC method

RESULTS AND DISCUSSION

- The drying action resulted in a decreased recovery of model peptides (**Table 2**).
- Maximum amounts of model peptides were recovered for the following vial type-evaporation technique combinations: polypropylene-lyophilization and glass-centrifugal evaporation (**Figure 1**).
- n-nonyl-β-D-Glucopyranoside is the best additive for recovery of peptides during centrifugal evaporation as well as during freeze-drying (**Figure 2**).
- Recovery of goserelin and buserelin was significantly lower on glass vials when LyoVac was used, while this was not consistent using SpeedVac (**Figure 3**).

Peptide	Additive	Recovery (%)			
		SpeedVac		LyoVac	
		Plastic	Glass	Plastic	Glass
Leucine-enkephalin	Mannitol	90.3 ± 3.5	98.9 ± 4.2	89.0 ± 2.9	94.6 ± 3.4
	Water	97.6 ± 9.1	97.2 ± 8.3	93.2 ± 7.4	96.2 ± 9.1
	DMSO	96.5 ± 5.5	93.2 ± 4.7	85.1 ± 4.7	91.4 ± 3.4
	DMF	89.8 ± 3.5	101.9 ± 1.0	90.9 ± 1.6	93.3 ± 3.3
	PEG 400	89.4 ± 3.3	97.8 ± 3.6	83.4 ± 5.2	93.3 ± 3.1
	n-β-D-Glucopyranoside	95.9 ± 1.6	100.9 ± 2.9	91.4 ± 2.0	95.0 ± 1.4
Goserelin	Mannitol	92.3 ± 4.6	92.3 ± 6.7	99.2 ± 3.1	64.3 ± 3.6
	Water	84.4 ± 4.5	102.2 ± 8.8	100.2 ± 4.7	73.5 ± 5.3
	DMSO	88.8 ± 5.1	100.6 ± 5.7	96.1 ± 5.5	72.1 ± 4.5
	DMF	89.7 ± 6.8	90.8 ± 7.2	92.4 ± 5.8	72.8 ± 6.5
	PEG 400	85.7 ± 8.2	92.8 ± 7.8	71.4 ± 6.6	54.0 ± 7.7
	n-β-D-Glucopyranoside	93.1 ± 1.3	92.9 ± 8.3	90.4 ± 2.5	90.7 ± 2.5
Bovine insulin	Mannitol	62.8 ± 0.8	83.6 ± 4.2	81.7 ± 4.0	94.7 ± 5.3
	Water	82.4 ± 5.1	83.9 ± 6.0	90.3 ± 6.1	94.8 ± 2.4
	DMSO	92.6 ± 7.1	92.5 ± 7.4	94.1 ± 8.4	103.6 ± 9.2
	DMF	74.1 ± 2.7	89.2 ± 4.5	91.8 ± 5.1	98.6 ± 3.8
	PEG 400	95.8 ± 5.5	81.0 ± 5.2	64.7 ± 2.1	95.7 ± 3.3
	n-β-D-Glucopyranoside	95.4 ± 7.1	92.1 ± 6.7	89.3 ± 5.0	88.9 ± 4.9
Buserelin	Mannitol	91.9 ± 4.4	93.4 ± 1.4	85.0 ± 3.1	71.9 ± 0.9
	Water	86.4 ± 3.8	91.6 ± 8.6	89.5 ± 1.5	82.4 ± 2.7
	DMSO	86.4 ± 1.8	93.4 ± 1.2	103.7 ± 2.1	81.7 ± 2.1
	DMF	91.1 ± 2.4	90.1 ± 1.2	94.2 ± 6.7	77.7 ± 0.9
	PEG 400	79.0 ± 2.2	105.7 ± 3.3	94.1 ± 2.4	79.2 ± 5.0
	n-β-D-Glucopyranoside	96.2 ± 1.7	92.0 ± 3.8	92.0 ± 2.4	95.1 ± 1.7
Mouse obestatin	Mannitol	95.3 ± 3.1	93.7 ± 4.4	90.8 ± 6.0	95.2 ± 4.7
	Water	90.8 ± 2.8	91.1 ± 11.0	91.2 ± 3.0	88.5 ± 4.0
	DMSO	99.8 ± 3.7	91.2 ± 6.1	95.8 ± 4.0	91.1 ± 2.7
	DMF	101.4 ± 7.8	90.2 ± 7.4	101.3 ± 8.0	91.8 ± 7.0
	PEG 400	101.8 ± 8.3	93.2 ± 3.4	82.7 ± 4.4	87.2 ± 1.5
	n-β-D-Glucopyranoside	97.2 ± 2.6	102.5 ± 3.6	92.6 ± 2.8	96.1 ± 2.3

Table 2: Peptide loss during sample evaporation

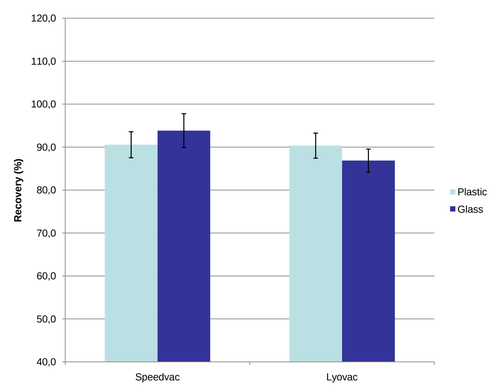

Figure 1
Effect of drying method on model peptides recovery

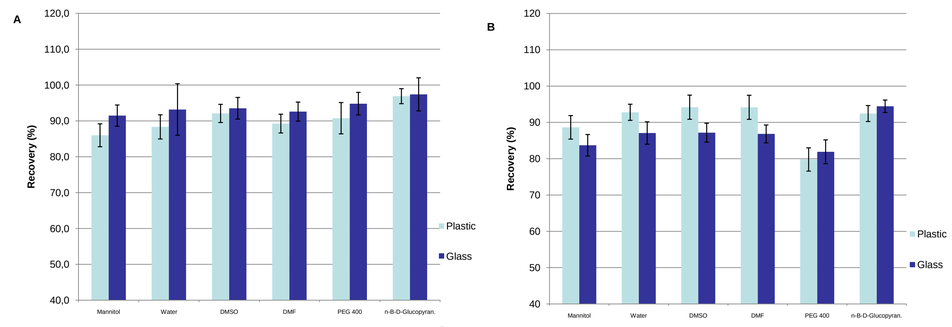

Figure 2
Effect of additives using SpeedVac (A) and LyoVac(B)

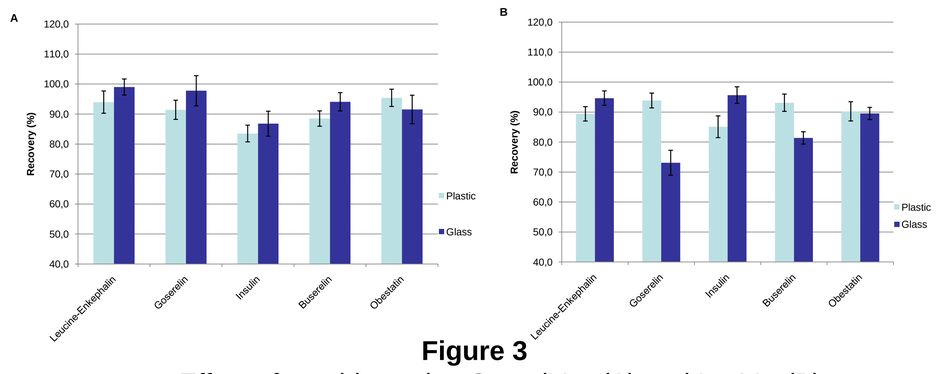

Figure 3
Effect of peptides using SpeedVac (A) and LyoVac(B)

CONCLUSIONS

Drying step (*e.g.* as part of the sample preparation) can cause significant peptide loss. Applying n-nonyl-β-D-glucopyranoside as an additive along with choosing appropriate vial material (*i.e.* polypropylene for LyoVac and glass for SpeedVac methods*)* can prevent or decrease peptide loss during the selected solvent removal evaporation procedure.

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