UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Adsorption of the model drug testosterone on laboratory tips

S. Bodé, B. Baert, B. De Spiegeleer*

Drug Quality & Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium.

To whom correspondence is addressed: bart.despiegeleer@ugent.be

(2007-118 PSWC 2007, Amsterdam)

Introduction

Quantitative drug analysis requires careful evaluation of each step of the process, including liquid handling. Loss by adsorption is an analytically problematic issue, especially for hydrophobic compounds like testosterone. Therefore, we investigated the operational adsorption characteristics of this steroid in different solvents and using different laboratory tips. In addition, the adsorption characteristics of 6 other steroids were investigated and initial QSPR established.

Experimental

Solvent : 10mM phosphate buffered saline (PBS), 5% bovine serum albumin (BSA) in PBS and AcCN/H_2O (50/50, V/V)

Laboratory tips : o Glass

o Plastic: untreated standard PP tips (3 different suppliers), siliconized, developmental (proprietary) NO:1 to NO:5

Methodology : o 50-200 µL of steroid solutions

o Pipetting 1 to 3 times

o Resulting solution HPLC: ✓ Isocratic AcCN/H_2O (HCOOH 0.1% m/V)

✓ UV detection

Results and discussion

1. Influence of BSA and organic modifier AcCN in solvent (untreated plastic tips)

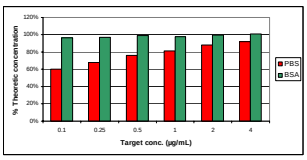

Figure 1: Testosterone recovery BSA vs. PBS

- Protocol is adequate to significantly detect adsorption phenomena.
- Solvent plays important role: BSA and AcCN containing aqueous solutions did not exhibit adsorption at all.
- Testosterone dissolved in PBS shows a significant adsorption.
- As expected, a concentration effect is also confirmed.

Table 1: Recovery of testosterone glass vs. PP tip, PBS vs. AcCN/H_2O (%)

Target conc. (µg/mL)	Glass tips		PP tips	
	PBS	ACN/H_2O	PBS	AcCN/H_2O
80	102.25	102.77	93.00	94.63
8	102.18	101.66	87.09	94.71
0.8	99.80	103.60	70.07	90.71

2. Influence of laboratory tip (PBS solution)

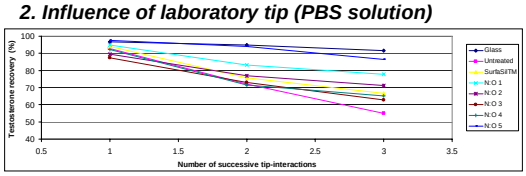

Figure 2: Testosterone recovery of seven tip types and glass

- Difference between investigated materials is observed.
- Glass shows negligible adsorption, untreated plastic laboratory-tips the highest adsorption.
- Other tips behave between these two extremes.

3. Other Steroids

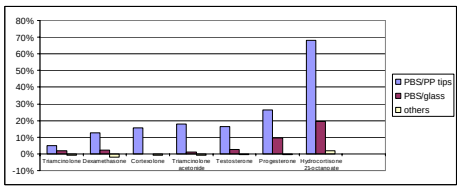

Figure 3: Mean adsorption values

Mean adsorption values are presented in Figure 3 resulting in different classes:

- PBS/PP tips: mean adsorption values on PP tips of three different suppliers.
- PBS/glass: adsorption on glass tips in PBS.
- Others: mean adsorption on PP tips of three different suppliers and on glass in 5% (m/V) BSA and AcCN/H_2O 50/50 (V/V).
- Overall experimental error: Standard deviation over all "others" values = 1.8% (n= 53)

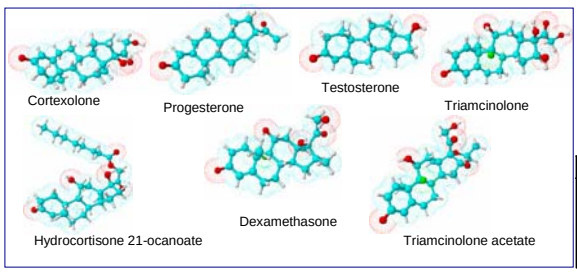

Figure 4: Molecular structures of the 7 steroids

QSPR using MLR resulting in significant descriptors:

1) LFER (Abraham) descriptors: - McGowan characteristic volume (V)
   - Solute H-bond basicity (B)
2) Constitutional descriptors: - Number of H-atoms (nH)
   - Mean electrotopological state (Ms)
3) Molecular descriptors: - Ghose-Crippen molar refractivity (AMR)
   - Ghose-Crippen octanol-water partition coefficient (AlogP)
   - Hydrophilic factor (Hy).

Table 2: QSPR models

Descriptors	Model	S	R^2	F	R^2cv_{LOO}	FIT	AIC
LFER	-0.774 + 0.515V - 0.296B	0.0252	0.990	202	0.912	36.8	1.59E-03
Constitutional	-0.531 + 0.0342nH - 0.128Ms	0.0284	0.988	159	0.938	28.9	2.02E-03
Molecular	-0.960 + 0.00779AMR + 0.137ALOGP + 0.0934 Hy	0.0179	0.996	270	0.938	23.5	2.88E-03

Conclusions

1. Significant adsorption of testosterone in PBS on untreated laboratory plastic tips; less adsorption on developmental tips.
2. No significant adsorption of testosterone in BSA or AcCN containing solutions on any investigated tip types.
3. Above conclusions also for other steroids to different extent; 4 groups: 1) Triamcinolone: no biopharmaceutical significant adsorption (4.8% ± 0.5%, n = 3)
   2) Dexamethasone, cortexolone, triamcinolone-acetonide, testosterone (15.7% ± 2.7%, n = 12)
   3) Progesterone (26.2% ± 2.1%, n = 3)
   4) Hydrocortisone 21-octanoate (= 68.1% ± 12.9%, n = 3)
4. QSPR established by LFER, constitutional and molecular descriptors: 1) Large V-coefficient: greater dispersion force interaction and higher energy required size cavity.
   2) Increase in H-bond basicity decreases the plastic-tip interaction.
   3) The number of hydrogens positively influences the adsorption (*e.g.* hydrophobic, as more carbons)
   4) The higher the polarizability (expressed as Ms) often due to more polar hetero-atoms, the lower the adsorption.
   5) Higher hydrophobicity (AlogP) corresponds with higher adsorption, which is fine-tuned by the opposite hydrophilicity factor (Hy).
   6) A positive effect of the Ghose-Crippen molar refractivity (~ volume) gives a positive adsorption.

Overall, molecular volume (V, AMR) and hydrophobicity (AlogP modulated by Hy, nH) positively influence adsorption. Basic hetero-atoms (B, Ms) oppositely decreases adsorption.