

Supplementary material

Salt-induced homogeneous liquid-liquid microextraction of piroxicam and meloxicam from human urine prior to their determination by HPLC-DAD

Natalia Manousi¹, Sotiria V. Tsiona², Constantinos K. Zacharis^{2,*}

¹ *Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*

² *Laboratory of Pharmaceutical Analysis, Department of Pharmaceutical Technology, School of Pharmacy, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece*

*Corresponding author

Constantinos K. Zacharis

Assistant Professor

Laboratory of Pharmaceutical Analysis, School of Pharmacy,

Aristotle University of Thessaloniki (AUTH),

GR-54124, Greece

Tel: +30 2310997663

FAX: +30 2310997652

E-mail: czacharis@pharm.auth.gr

Calculation of the extraction recovery

The extraction recovery (ER %) was employed for the evaluation of the extraction efficiency of the proposed analytical scheme. This term was estimated using the following expression:

$$ER \% = \frac{C_{org} \times V_{org}}{C_{aq} \times V_{aq}} \times 100 \quad (1)$$

where C_{org} and C_{aq} are the concentrations of the analyte in the organic and aqueous phase ($5 \mu\text{g mL}^{-1}$), and V_{org} and V_{aq} are the volume of upper organic and aqueous layer, respectively. The calculation of C_{org} was performed by external standardization by direct injection of standard solutions in the range of $1 - 5 \mu\text{g mL}^{-1}$ prepared in water.

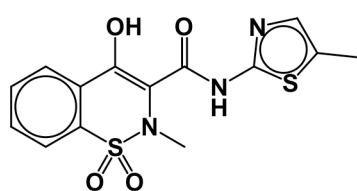
Accuracy profiles

This approach is based on the graphical report of i) the acceptability intervals that describe the required performance of the analytical method and ii) the β -expectation tolerance intervals (β -ETI). The latter represents the interval where it is expected that a proportion β of future measurements will be within the acceptance limits λ [1]. It should be stated that the accuracy profiles could be also informative for the realistic estimation of the limit of quantitation (LOQ) of the method. The mathematical expression of the analytical profile is given below:

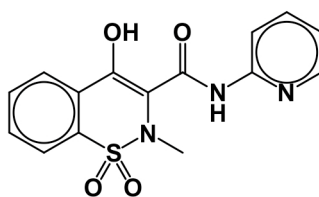
$$\left[bias(\%)_j - Q_t\left(v; \frac{1+\beta}{2}\right) \sqrt{1 + \frac{1}{pnB_j^2} s_{r,j}} ; bias(\%)_j + Q_t\left(v; \frac{1+\beta}{2}\right) \sqrt{1 + \frac{1}{pnB_j^2} s_{r,j}} \right]$$

$$\text{where } bias(\%)_j = \frac{\hat{\mu}_j - \mu_{Tj}}{\mu_{Tj}} \times 100, \quad s_{r,j} = \frac{\hat{\sigma}_{W,j}^2 + \hat{\sigma}_{B,j}^2}{\hat{\mu}_j} \times 100, \quad B_j = \sqrt{\frac{\frac{\hat{\sigma}_{B,j}^2}{\hat{\sigma}_{W,j}^2} + 1}{n \frac{\hat{\sigma}_{B,j}^2}{\hat{\sigma}_{W,j}^2} + 1}}, \quad v = \frac{(R+1)^2}{R + \frac{1}{n(p-1)} + 1 - \frac{1}{pn^2}}$$

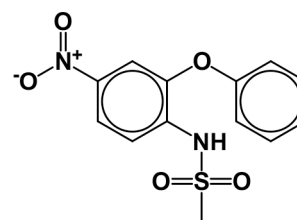
- $\hat{\mu}_j$ is the estimate of the mean results of the j^{th} concentration level
- μ_T is the unknown "true value"
- p is the number of series
- n is the number of the independent replicate per series
- $Q_t\left(v; \frac{1+\beta}{2}\right)$ is the β quantile of the t -Student distribution with v degrees of freedom
- $\hat{\sigma}_{W,j}^2$ is the within series variance
- $\hat{\sigma}_{B,j}^2$ is the between series variance



Meloxicam



Piroxicam



Nimesulide

Figure S1. Chemical structure of meloxicam (MEL), piroxicam (PIR) and the nimesulide (ISTD).

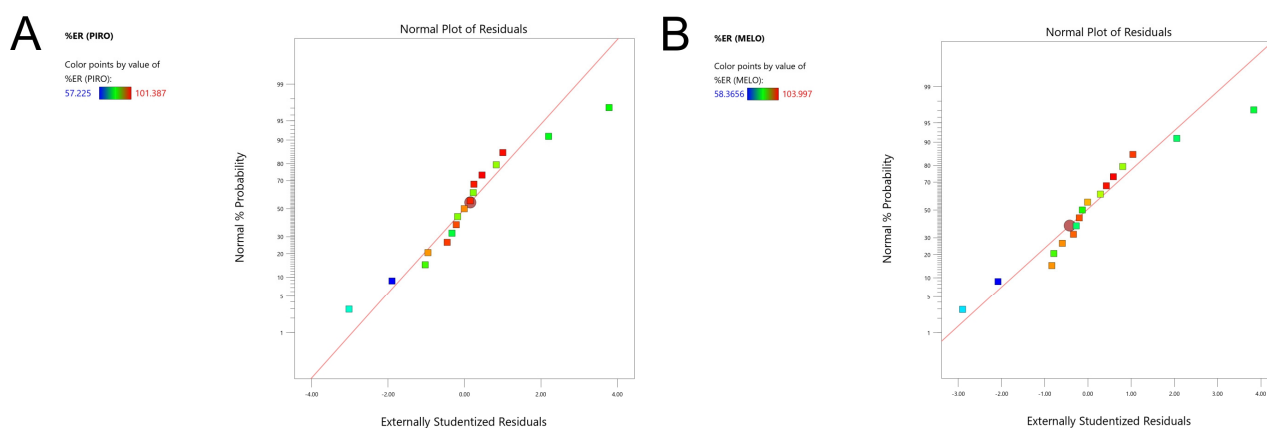


Figure S2. Normal probability plots for the % ER of A) PIR and B) MEL.

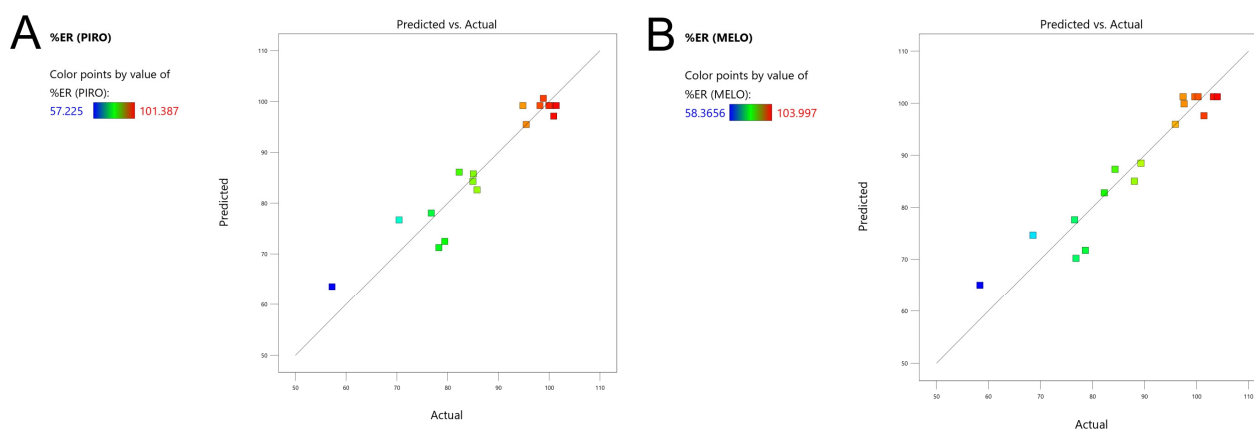


Figure S3. Residuals vs predicted plots for the of A) PIR and B) MEL.

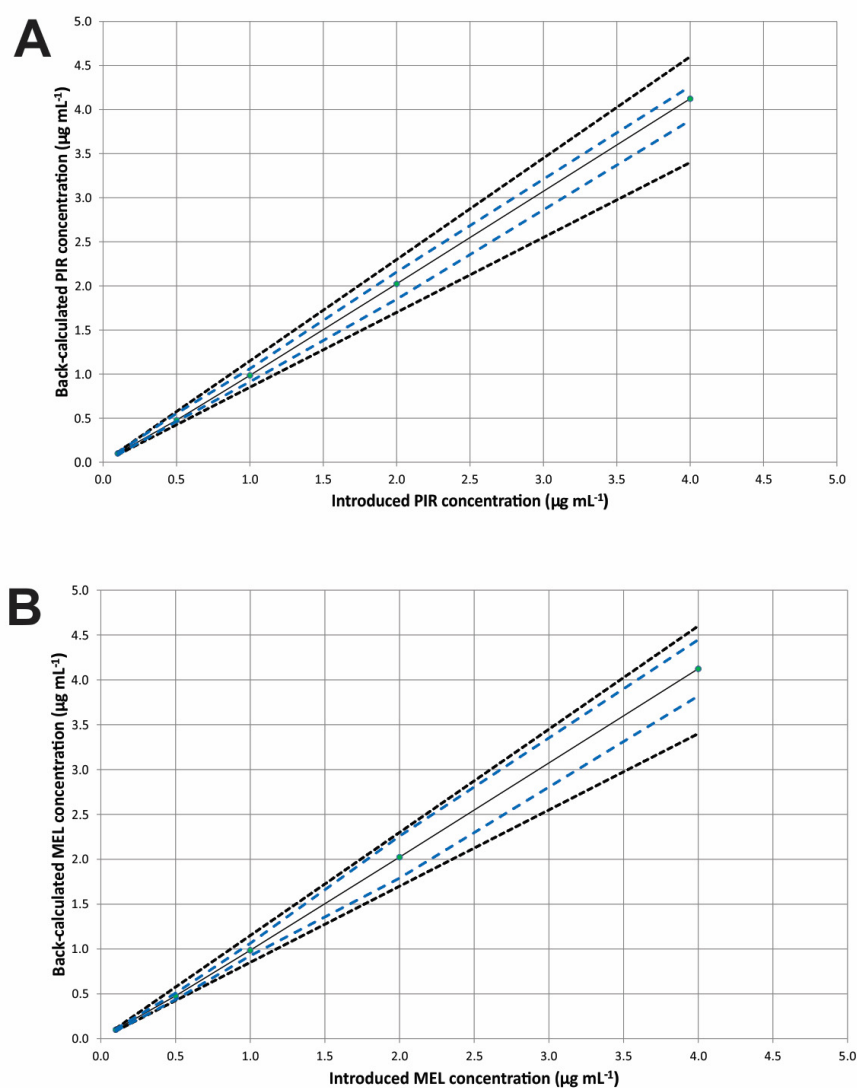


Figure S4. Linearity profile of A) MEL and B) PIR. The plain blank line corresponds to the identity line ($Y = X$), the blue dashed line represents the accuracy profile (β -ETI) and the dotted curves illustrate the acceptance limits $\lambda \pm 15\%$ expressed in $\mu\text{g mL}^{-1}$.

Table S1. Analysis of variance (ANOVA) for BBD for ER% of MEL.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2602.59	6	433.77	17.91	< 0.0001	significant
A-V(ACN)	316.42	1	316.42	13.06	0.0047	
B-V(S)	53.75	1	53.75	2.22	0.1672	
C-Na2SO4 volume	10.48	1	10.48	0.4325	0.5256	
BC	361.74	1	361.74	14.93	0.0031	
A ²	326.44	1	326.44	13.48	0.0043	
B ²	1451.55	1	1451.55	59.93	< 0.0001	
Residual	242.21	10	24.22			
Lack of Fit	213.07	6	35.51	4.87	0.0736	not significant
Pure Error	29.14	4	7.29			

Table S2. Analysis of variance (ANOVA) for BBD for ER% of PIR.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2319.37	6	386.56	15.57	0.0002	significant
A-V(ACN)	420.53	1	420.53	16.93	0.0021	
B-V(S)	118.66	1	118.66	4.78	0.0537	
C-Na2SO4 volume	24.27	1	24.27	0.9774	0.3462	
BC	233.75	1	233.75	9.41	0.0119	
A ²	242.76	1	242.76	9.77	0.0108	
B ²	1214.38	1	1214.38	48.90	< 0.0001	
Residual	248.35	10	24.84			
Lack of Fit	221.69	6	36.95	5.54	0.0596	not significant
Pure Error	26.66	4	6.66			

Table S3. Validation results for the determination of MEL in human urine.

Validation criteria			
Response function (unweighted linear regression)	Slope	Intercept ($\times 10^3$)	r^2
$(k^a = 3; m = 5; n = 3)$ (0.1 – 4.0 $\mu\text{g mL}^{-1}$)			
Day 1	0.8946	– 0.0619	0.9997
Day 2	0.8815	– 0.0109	0.9990
Day 3	0.8722	– 0.0312	0.9994
Precision ($k = 3; n = 3$)			
C ($\mu\text{g mL}^{-1}$)	s_r (%) ^b	s_R (%) ^c	
0.1	3.9	2.9	
0.5	1.6	3.5	
1	2.2	3.0	
2	1.7	2.8	
4	2.8	2.0	
Trueness ($k = 3; n = 3$)			
C ($\mu\text{g mL}^{-1}$)	Relative bias (%)		
0.1	– 1.7		
0.5	+ 1.6		
1	– 1.3		
2	+ 0.2		
4	+ 1.7		
Accuracy ($k = 5; n = 3$)			
C (%)	Relative β -ETI (%)		
0.1	[– 14.63, 11.22]		
0.5	[– 7.43, 10.68]		
1	[– 8.67, 6.17]		
2	[– 7.56, 7.94]		
4	[– 3.09, 6.56]		
Linearity ($k = 3; n = 3; m = 5$) (0.1 – 4.0 $\mu\text{g mL}^{-1}$)			
Slope	1.035		
Intercept	– 0.032		
r^2	0.9999		
LOD ($\mu\text{g mL}^{-1}$)	0.03		
LLOQ ($\mu\text{g mL}^{-1}$)	0.1		

^a k : number of experiments, m : calibration levels and n : replicates. ^b s_r (%): relative standard deviation for repeatability. ^c s_R (%): relative standard deviation for intermediate.

Reference

1. Hubert, P.; Nguyen-Huu, J.J.; Boulanger, B.; Chapuzet, E.; Chiap, P.; Cohen, N.; Compagnon, P.A.; Dewe, W.; Feinberg, M.; Lallier, M.; et al. Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal - Part I. *J. Pharm. Biomed. Anal.* **2004**, *36*, 579–586.