1	Electronic Supplementary Information
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3	Effective determination of trace residues of glibenclamide in urine samples
4	using dispersive micro solid-phase extraction and its final detection by
5	chromatographic analysis
6	
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13 Supporting information

14 This Supporting Information section includes 10 Figures and 3 Tables as follows:

Figure S1. Calibration curve and chemical structure of GB (mobile phase: methanol-water 15 (70:30, v/v); Flow rate: 1.0 mL min⁻¹; Column: KNAUER Smart line HPLC system with UV-16 VIS 2550 Detector, Zorbax SB-C₁₈ column (15 cm \times 3.9 mm id, 5 μ m particle size) under 17 ambient temperature (25 °C); λ = 230 nm); Figure S2. Effect of elution type on the extraction 18 efficiency of GB. Conditions: sample volume, 15.0 mL; eluent volume, 250 µL; extraction 19 time, 3 min; sorbent mass, 20 mg; desorption time, 2 min; pH, 6.0 at room temperature; 20 Figure S3. Standardized Pareto chart, representing the estimated effects of independent 21 variables obtained from the CCD; Figure S4. The profile optimum conditions by the CCD 22 design for the recovery of GB; Figure S5. The chromatogram obtained by DMSPE-HPLC-23 UV for urine (1) under optimum conditions: (a) non-spiked, (b) spiked with 200 μ g L⁻¹ before 24 DMSPE, and (c) extracted from urine (1) sample after DMSPE of GB; Figure S6. The 25 chromatogram obtained by DMSPE-HPLC-UV for urine (2) under optimum conditions: (a) 26 non-spiked, (b) spiked with 200 μ g L⁻¹ before DMSPE, and (c) extracted from urine (2 27 sample after DMSPE of GB; Figure S7. The chromatogram obtained by DMSPE-HPLC-UV 28 for urine (3) under optimum conditions: (a) non-spiked, (b) spiked with 200 μ g L⁻¹ before 29 DMSPE, and (c) extracted from urine (3) sample after DMSPE of GB; Figure S8. The 30 chromatogram obtained by DMSPE-HPLC-UV for urine (4) under optimum conditions: (a) 31 32 non-spiked, (b) spiked with 200 μ g L⁻¹ before DMSPE, and (c) extracted from urine (4) sample after DMSPE of GB; Figure S9. Langmuir (A) and Freundlich (B) isotherms of GB 33 extraction onto nanoparticles as a solid phase; Figure S10. Reusability of S@SnO₂-NPs-AC 34 for DMSPE of GB. The mean of three replicates was used as each extraction efficiency with 35 error bar; Table S1. Coded and un-coded values of the independent variables in CCD matrix 36 and the observed results; Table S2. ANOVA table, t-test results, F- and P-values for the 37 quadratic model; Table S3. Analytical performance of DMSPE-HPLC-UV for GB 38 determination. 39

41 Contents

42 1. Figures



45 **Figure S1.** Calibration curve and chemical structure of GB (mobile phase: methanol-water (70:30, 46 v/v); Flow rate: 1.0 mL min⁻¹; Column: KNAUER Smart line HPLC system with UV-VIS 2550 47 Detector, Zorbax SB-C₁₈ column (15 cm × 3.9 mm id, 5 μ m particle size) under ambient temperature 48 (25 °C); λ = 230 nm).





51 **Figure S2.** Effect of elution type on the extraction efficiency of GB. Conditions: sample volume, 15.0

52 mL; eluent volume, 250 μ L; extraction time, 3 min; sorbent mass, 20 mg; desorption time, 2 min; pH,

53 6.0 at room temperature.





56 Figure S3. Standardized Pareto chart, representing the estimated effects of independent variables

57 obtained from the CCD.



Figure S4. The profile optimum conditions by the CCD design for the recovery of GB.



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63 **Figure S5.** The chromatogram obtained by DMSPE-HPLC-UV for urine (1) under optimum 64 conditions: (a) non-spiked, (b) spiked with 200 μ g L⁻¹ before DMSPE, and (c) extracted from urine 65 (1) sample after DMSPE of GB.



68 **Figure S6.** The chromatogram obtained by DMSPE-HPLC-UV for urine (2) under optimum 69 conditions: (a) non-spiked, (b) spiked with 200 μ g L⁻¹ before DMSPE, and (c) extracted from urine (2 70 sample after DMSPE of GB.



Figure S7. The chromatogram obtained by DMSPE-HPLC-UV for urine (3) under optimum conditions: (a) non-spiked, (b) spiked with 200 μ g L⁻¹ before DMSPE, and (c) extracted from urine (3) sample after DMSPE of GB.



Figure S8. The chromatogram obtained by DMSPE-HPLC-UV for urine (4) under optimum
conditions: (a) non-spiked, (b) spiked with 200 µg L⁻¹ before DMSPE, and (c) extracted from urine
(4) sample after DMSPE of GB.







used as each extraction efficiency with error bar.

2. Tables

90 Table S1. Coded and un-coded values of the independent variables in CCD matrix and the observed91 results.

Independent variables			Unit	Levels ($\alpha = 2$)						
				-α	Low (-	-1)	Center	(0)	High (+1)	+α
(X ₁) pH			-	2.0	4.0		6.0		8.0	10
(X ₂) Sorbent mass			mg	5.0	10		15		20	25
(X ₃) Contact time			min	0.5	2.0		3.5		5.0	6.5
(X ₄) Elution volume			μL	100	200		300		400	500
			Factors						ER% GB	1
Run	X1	X ₂		X3			X4		Response	
1	8.0	20		5.0)		200		64.196	
2	8.0	20		2.0)		200		77.543	
3	8.0	10		5.0)		400		60.722	
4	4.0	20		2.0			400		96.245	
5	8.0	10		2.0			400		97.508	
6	4.0	10		5.0			200		74.951	
7	4.0	20		5.0			400		58.729	
8	4.0	10		2.0			200		41.391	
9	2.0	15		3.5	3.5		300		39.948	
10	10	15		3.5	5		300		88.901	
11	6.0	5.0)	3.5	5		300		79.610	
12	6.0	25		3.5	5		300		85.451	
13	6.0	15		0.5	5		300		85.332	
14	6.0	15		6.5	5		300		55.571	
15	6.0	15		3.5	5		100		45.471	
16	6.0	15		3.5	5		500		87.037	
17	6.0	15		3.5	5		300		79.149	
18	6.0	15		3.5	5		300	80.531		
19	6.0	15		3.5	5		300		77.792	

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	5712.61	14	408.04	275.21	< 0.0001
X ₁	1198.20	1	1198.20	808.15	< 0.0001
X ₂	17.06	1	17.06	11.51	0.0194
X ₃	806.72	1	806.72	544.11	< 0.0001
X4	863.87	1	863.87	582.65	< 0.0001
X ₁ X ₂	49.03	1	49.03	33.07	0.0022
X ₁ X ₃	266.53	1	266.53	179.77	< 0.0001
X ₁ X ₄	6.84	1	6.84	4.61	0.0845
X ₂ X ₃	283.66	1	283.66	191.32	< 0.0001
X ₂ X ₄	299.74	1	299.74	202.17	< 0.0001
X ₃ X ₄	1116.64	1	1116.64	753.14	< 0.0001
X ₁ ²	316.41	1	316.41	213.41	< 0.0001
X2 ²	16.39	1	16.39	11.06	0.0209
X ₃ ²	110.65	1	110.65	74.63	0.0003
X4 ²	242.78	1	242.78	163.75	< 0.0001
Residual	7.41	5	1.48		
Lack of Fit	2.83	2	1.41	0.92	0.4867
Pure Error	4.59	3	1.53		
Corr. Total	5720.03	19			

93 Table S2. ANOVA table, F- and P-values for the quadratic model.

Table S3. Analytical performance of DMSPE-HPLC-UV for GB determination.

Quantitative analysis	
Sample volume (mL)	15.0
extraction solvent (mL)	0.250
Linear range (µg L ⁻¹)	0.60-5500
Limit of detections (LODs) (µg L ⁻¹)	0.09-0.16
limit of quantification (LOQs) (µg L ⁻¹)	0.30-0.52
Reproducibility (RSD, %)	<7.0
Repeatability (RSD, %)	<6.0