A Validated Rhodamine B Fluorescence Quenching Spectrofluorimetric Method for Solriamfetol Determination in Human Plasma with Pharmacokinetic Application

Ahmed Serag [a,*], Razaz Abdulaziz Felemban [b,c], Abdulaziz Al Khzem [d], Mansour S. Alturki [d], Mohammed F. Aldawsari [e], Manal E. Alosaimi [f], Maram H Abduljabbar [g], Atiah H. Almalki [h, i, **]

[a] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Nasr City 11751, Cairo, Egypt

[b] Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Jeddah, Saudi Arabia

[c] King Abdullah International Medical Research Centre, Jeddah, Saudi Arabia

[d] Department of Pharmaceutical Chemistry, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, King Faisal Road, P.O. Box 1982, Dammam 31441, Saudi Arabia

[e] Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-kharj 11942, Saudi Arabia

[f] Department of Basic Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

[g] Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

[h] Addiction and Neuroscience Research Unit, Health Science Campus, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

[i] Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

*Corresponding author email address: (Ahmed Serag) Ahmedserag777@hotmail.com

** Corresponding author email address: (Atiah H. Almalki) ahalmalki@tu.edu.sa

2.4. Optimization of Experimental Conditions

Multiple experimental parameters that potentially influence the fluorescence quenching efficiency were systematically investigated to establish optimal analytical conditions. The optimization process employed a univariate approach wherein individual parameters were sequentially varied while maintaining other variables constant. Solriamfetol concentration was fixed at 500 ng/mL throughout the optimization procedures to ensure consistent analyte-quencher interactions.

2.4.1. Effect of pH

The pH dependency of the Rhodamine B-solriamfetol system was evaluated across a pH range of 3.0-9.0 using the Britton-Robinson buffer system. Reaction mixtures were prepared containing fixed concentrations of Rhodamine B (0.01% w/v) and solriamfetol (500 ng/mL) with varying buffer pH. The fluorescence intensity ratio (F0/F) was monitored as a function of pH to identify optimal conditions for maximum quenching efficiency.

2.4.2. Effect of Buffer Volume

Buffer volume optimization was conducted to establish appropriate ionic strength and buffer capacity for the quenching reaction. Varying volumes of Britton-Robinson buffer (pH 6.0) ranging from 0.25 to 3.5 mL were introduced into the reaction mixture while maintaining constant concentrations of Rhodamine B and solriamfetol. The spectrofluorimetric response was evaluated to determine the optimal buffer volume that provides maximum analytical sensitivity with minimal background interference.

2.4.3. Effect of Rhodamine B Concentration

The concentration of the fluorophore represents a critical parameter influencing analytical sensitivity, linear dynamic range, and potential inner-filter effects. The Rhodamine B concentration was systematically varied by adding different volumes (0.5-3.5 mL) of 0.01% w/v Rhodamine B solution to the reaction mixture while maintaining constant solriamfetol concentration. Fluorescence intensity measurements were recorded to establish the optimal fluorophore concentration that provides maximum quenching efficiency while minimizing self-quenching phenomena.

2.4.4. Effect of Reaction Time

The time required for the reaction to reach completion was studied by measuring the fluorescence response at various time intervals after mixing the reagents. The fluorescence intensity ratio (F0/F) was recorded at different time points (0-10 minutes) after combining

solriamfetol with Rhodamine B solutions at room temperature (25 ± 2°C). This experiment helped determine the minimum time needed for the reaction to stabilize, ensuring reliable and reproducible measurements during routine analysis.

All optimization experiments were performed in triplicate to ensure statistical reliability of the experimental findings. The established optimal conditions were subsequently applied for method validation and quantitative determination of solriamfetol in pharmaceutical formulations and biological matrices.

2.6. Method Validation

2.6.1. Plasma Sample Preparation Protocol

The extraction of solriamfetol from plasma samples was performed using a protein precipitation technique. Plasma samples (3 mL) were transferred to polypropylene centrifuge tubes, followed by addition of acetonitrile (3 mL) as the precipitating agent. The mixture was vortexed for 60 seconds to ensure complete protein denaturation. The samples were then centrifuged at 10,000 rpm for 10 minutes to separate the precipitated proteins. The clear supernatant was carefully transferred to clean tubes and evaporated to dryness under a gentle stream of nitrogen. The dry residue was reconstituted with 500 µL of distilled water prior to spectrofluorimetric analysis according to the general procedure described previously.

2.6.2. Validation Parameters

The spectrofluorimetric methodology underwent comprehensive validation according to International Council for Harmonization (ICH) M10 guidelines for bioanalytical procedures, encompassing the following analytical parameters:

2.6.2.1. Linearity and Sensitivity

Linearity was established through analysis of seven discrete concentration levels spanning 25-1000 ng/mL in plasma matrix. Calibration curves were constructed by plotting ratio of fluorescence intensity (F0/F) against corresponding solriamfetol concentrations, where F0 and F represent Rhodamine B fluorescence intensities in the absence and presence of solriamfetol, respectively. Linear regression analysis was employed to determine calibration equations and correlation coefficients.

The sensitivity was assessed by determining the limit of detection (LOD) and limit of quantification (LOQ) using the statistical approach based on the calibration curve parameters according to the equations: LOD = 3.3σ/S and LOQ = 10σ/S, where σ represents the standard

deviation of the blank and S represents the calibration curve slope. The practical LOQ was established as the lowest concentration that could be quantified with acceptable precision and accuracy.

2.6.2.2. Accuracy and Precision Assessment

Accuracy and precision were evaluated at four concentration levels (25, 75, 500, and 750 ng/mL) by analyzing five replicates (n=5) within a single day (intraday) and on three consecutive days (interday). Precision was expressed as relative standard deviation (RSD%), and accuracy was calculated as the percentage recovery of the known added amount of analyte in the sample. The acceptance criteria for precision and accuracy were in accordance with ICH M10 guidelines.

2.6.2.3. Selectivity Evaluation

The selectivity was assessed by analyzing the quenching effect of potential interferents commonly found in plasma including electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, PO_4^{3-}), biomolecules (tryptophan, tyrosine, glutamic acid, albumin, glucose, creatinine, uric acid), and pooled plasma. Additionally, the selectivity evaluation was extended to encompass structurally related compounds including phenylpropanolamine and phenylalanine, which share the phenylpropylamine scaffold with solriamfetol, as well as commonly co-administered medications in narcolepsy and obstructive sleep apnea patients including modafinil, fluoxetine, and amlodipine. The quenching effect percentage (QE%) was determined for each substance and compared with solriamfetol to evaluate potential interference in biological matrices. The analysis was performed under identical experimental conditions to verify the specificity of the fluorescence quenching mechanism for solriamfetol quantification.

2.6.2.4. Robustness Determination

The robustness of the analytical method was evaluated by deliberately introducing small variations in critical experimental parameters including buffer pH (±0.2 units), buffer volume (±0.1 mL), and Rhodamine B volume (±0.1 mL). The impact of these modifications on analytical performance was assessed to determine method reliability under typical laboratory condition variations.

2.6.2.5. Cross-validation with reference LC-MS/MS method

Cross-validation was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as the reference method according to the validated procedure described by Ratnakumari et al. [12]. The LC-MS/MS system comprised an Agilent 6470 Triple Quadrupole system (Agilent

Technologies, Santa Clara, CA, USA) equipped with an electrospray ionization source operating in positive ionization mode. Chromatographic separation was achieved using an Xterra MS C18 column (150 × 1.0 mm, 3.5 µm, Waters Corporation, Milford, MA, USA) with isocratic elution using a mobile phase consisting of 5 mM ammonium formate in methanol and 50% methanol in acetonitrile (90:10, v/v) at a flow rate of 0.5 mL/min. Detection was performed by multiple reaction monitoring (MRM) in positive ionization mode using mass transitions of m/z 195.1→179.3 for solriamfetol and m/z 254.1→177.2 for modafinil as the internal standard. Twelve spiked plasma samples prepared at four QC concentration levels (LLOQ QC, LQC, MQC, and HQC) were independently analyzed by both techniques under identical sample preparation conditions. Method comparison was evaluated using Pearson correlation analysis, linear regression, Bland-Altman agreement assessment, and equivalence testing with 90% confidence intervals using 80–125% acceptance criteria in accordance with ICH M10 bioanalytical method validation guidelines.

Table S1: Stern Volmer and thermodynamic parameters for rhodamine B-solriamfetol interaction at different temperatures.

Temperature (K)	Ksv (10^5 L/mol)	K_a (10^5 L/mol)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/(mol·K)
298	7.95	9.25	-34.05	-17.57	55.27
303	7.25	8.23	-34.33		
313	6.19	6.59	-34.88		

Table S2: Intraday and interday accuracy and precision for solriamfetol quantification in human plasma according to ICH M10 guidelines.

Concentration (ng/mL)	***Intraday***		***Interday***	
	Accuracy (% R ± SD) [a]	*Precision (*RSD%*)*	*Accuracy (% R ± SD)* [a]	*Precision (*RSD%*)*
25	104.84 ± 3.875	3.696	101.19 ± 4.333	4.282
75	96.54 ± 3.596	3.725	98.12 ± 4.470	4.556
500	96.42 ± 2.539	2.633	97.31 ± 3.423	3.518
750	103.47 ± 1.754	1.695	101.60 ± 2.156	2.122

[a] Average of five determinations

Table S3: Quantitative assessment of matrix effect across diverse plasma sources at multiple concentration levels for solriamfetol quantification

	Plasma 1			Plasma 2			Plasma 3		
Concentration (ng/mL)	Recovery %	(CV%)	(%Bias)	Recovery %	(CV%)	(%Bias)	Recovery %	(CV%)	(%Bias)
75	96.64	3.866	-3.36	96.04	3.038	-3.96	96.57	3.558	-3.43
500	102.70	2.298	2.70	95.99	3.116	-4.01	95.08	2.859	-4.92
750	102.35	1.595	2.35	102.99	2.918	2.99	99.81	1.969	-0.19

Table S4: Robustness study of the developed Rhodamine B-based spectrofluorimetric method for solriamfetol determination through systematic perturbation of critical analytical parameters.

Parameter	Modification	% Recovery ± SD
	5.8	100.85 ± 0.864
Buffer (pH)	6 (optimum)	98.22 ± 0.916
	6.2	99.42 ± 1.670
	1.4	101.21 ± 1.040
Buffer volume (mL)	1.5 (optimum)	101.34 ± 1.099
	1.6	98.95 ± 1.549
	0.9	99.42 ± 0.917
Rhodamine B volume (mL)	1 (optimum)	101.43 ± 0.729
	1.1	101.42 ± 1.145

Table S5: Statistical parameters for cross-validation between the developed spectrofluorimetric method and LC-MS/MS reference method for solriamfetol determination in human plasma.

Parameter	Result	Acceptance Criteria
Correlation Coefficient (r)	0.9993	$r > 0.95$
R-squared	0.9985	$R^2 > 0.90$
Regression Slope	1.000 (95% CI: 0.973–1.027)	0.95–1.05
Mean Bias	-7.24 ng/mL	—
Mean Bias (%)	-7.25%	±15%
Equivalence Test (90% CI)	87.0%–97.8%	80–125%
Bias Significance	$p = 0.07$	$p > 0.05$

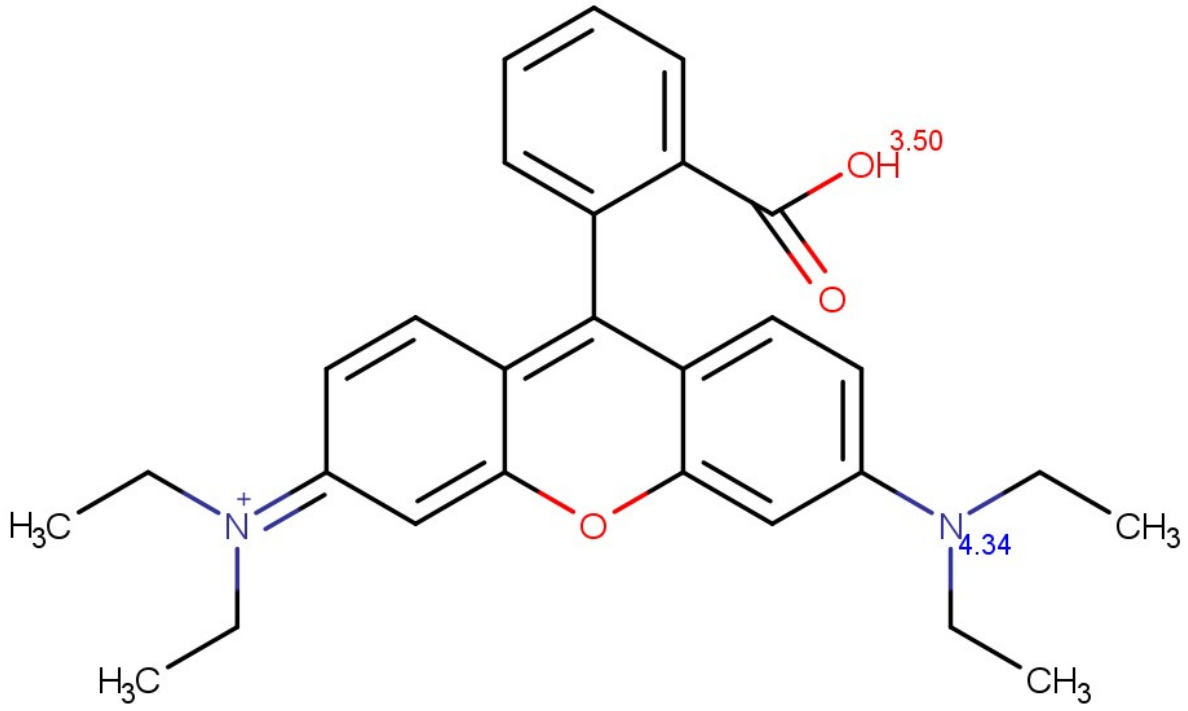

Fig. S1: Structural representation of Rhodamine B showing the zwitterionic form with annotated pKa values for the carboxyl group (3.50) and diethylamino moiety (4.34) as calculated by Marvin Sketch software.

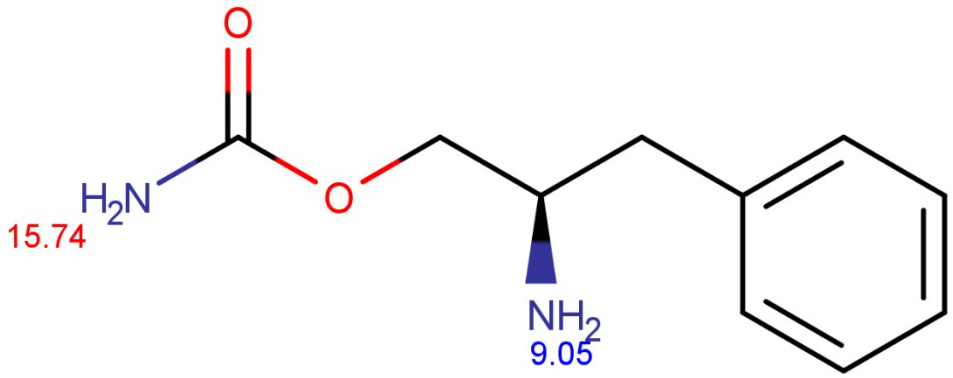

Fig. S2: Chemical structure of solriamfetol ([R]-2-amino-3-phenylpropylcarbamate hydrochloride) with annotated pKa values for the primary amine (9.05) as determined by Marvin Sketch computational analysis.

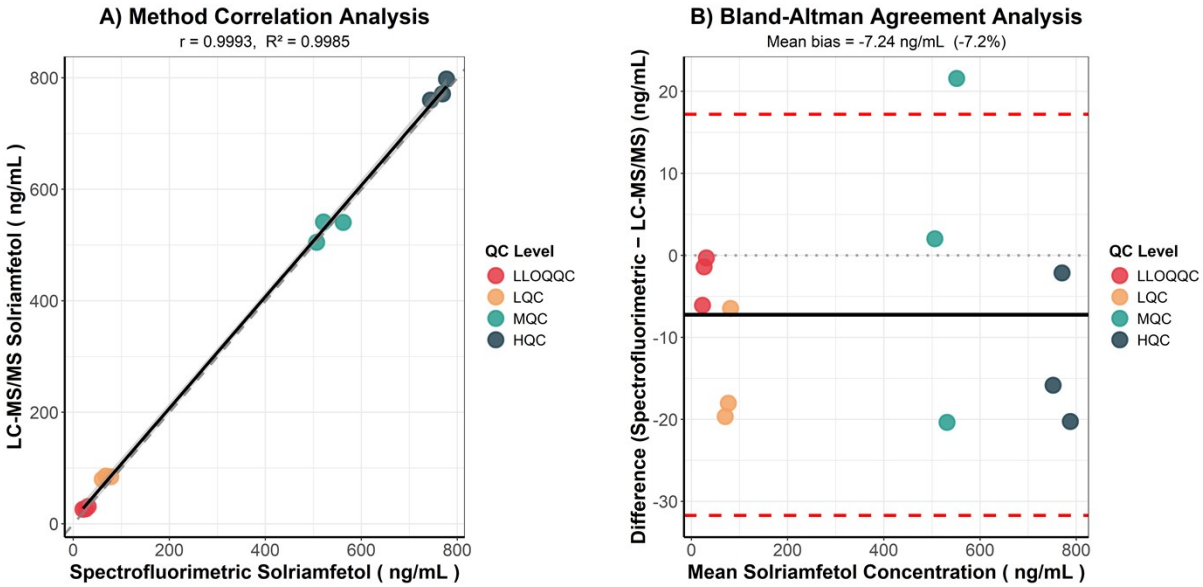

Fig. S3: Cross-validation of the developed spectrofluorimetric method against LC-MS/MS for solriamfetol determination in spiked human plasma: (A) Method correlation analysis (r = 0.9993, R^2 = 0.9985, slope = 1.000); (B) Bland-Altman agreement analysis showing mean bias of -7.24 ng/mL (-7.2%) with data points distributed within the limits of agreement (red dashed lines), confirming analytical equivalence between both methods (p = 0.07).

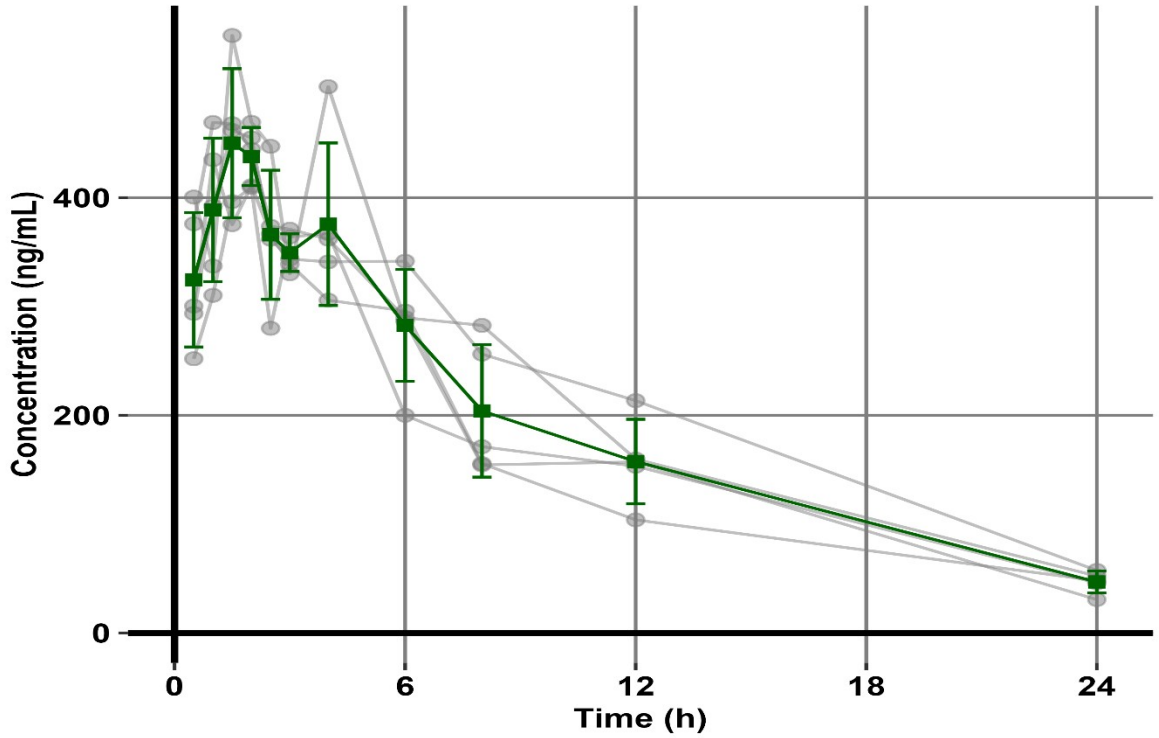

Fig. S4: Mean plasma concentration-time profile following oral administration of solriamfetol (75 mg) to healthy volunteers (n=5), characterized by rapid absorption followed by multi-exponential elimination phase.