Supplementary data

Evaluation of vardenafil's metabolic stability in human liver microsomes through ultra-fast UPLC-MS/MS for quantitative analysis, including assessments of greenness, ADME properties, DEREK alerts, and metabolic lability

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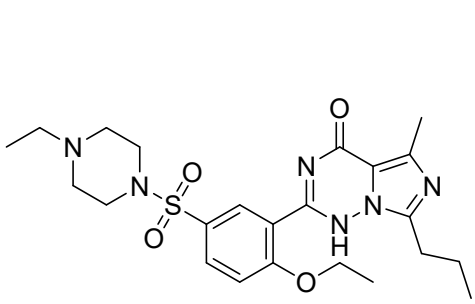
VRF in-silico ADME parameters

The SwissADME tool, developed by the Swiss Institute of Bioinformatics, is used to assess VRF ADME properties. It is accessible online at http://www.swissadme.ch/, with data retrieved on November 21, 2025 [1, 2].

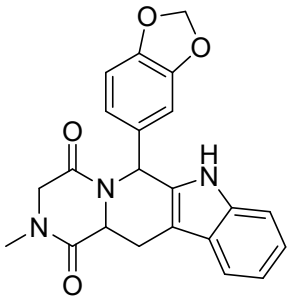
The ADME profile of VRF was evaluated using its SMILES notation, CCOc1ccc(cc1c1[nH]c(=O)c2n(n1)c(CCC)nc2C)S(=O)(=O)N1CCN(CC1)CC, through the SwissADME platform. An analysis was conducted to assess VRF's potential to exhibit drug-like characteristics by examining its ADME properties. The log P value suggested moderate water solubility (Log S = -4.65). The predicted pharmacokinetic profile for gastrointestinal absorption shows likely significant absorption, though its ability to cross the blood-brain barrier remains unknown. Drug similarity assessment adhered to Lipinski's criteria (0 violations), Veber's standards, Muegge [3, 4], and the Egan criteria. It doesn't meet the Ghose standards (No; 2 violations: MW > 480, MR > 130) [5]. The proposed bioavailability mark is 0.55. The Log Kp score, indicative of skin permeability, is -7.53 cm/s. The postulated mechanism of VRF activity involves inhibiting P-glycoprotein, a substrate. The data indicate that VRF does not inhibit CYP2C9 and CYP3A4 sub-enzymes. VRF exhibits no inhibitory influence on the CYP1A2, CYP2C19, and CYP2D6 sub-enzymes. Figure 2A illustrates the ADME radar pattern for VRF, with relevant information outlined in Table 1.

Figures:



Vardenafil

Molecular Weight: 488.60



Tadalafil

Molecular Weight: 389.40

Fig. S1. The chemical structure of vardenafil and tadalafil (IS).

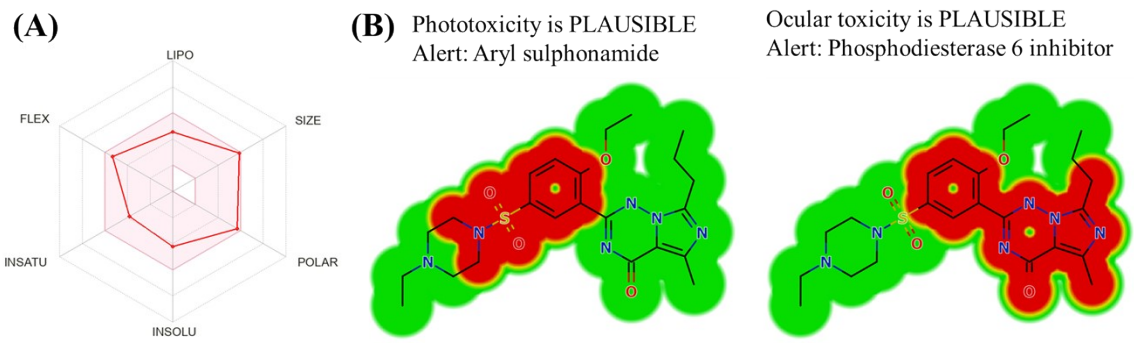


Fig. S2. Saturation (INSATU): carbons fraction in sp^3 hybridization 0.52; Molecular weight (SIZE): 488.60 g/mol; Polarity (POLAR): TPSA 121.28 Å²; Flexibility (FLEX): 8 rotatable bonds; Solubility (INSOLU): log S ≤ -6.21; Lipophilicity (LIPO) is determined as XLOGP3 = +2.46. The VRF ADME radar chart was generated using the in silico SwissADME online software (**A**). The in silico structural toxic alerts for VRF were tested using DEREK, which highlighted them in red (**B**).

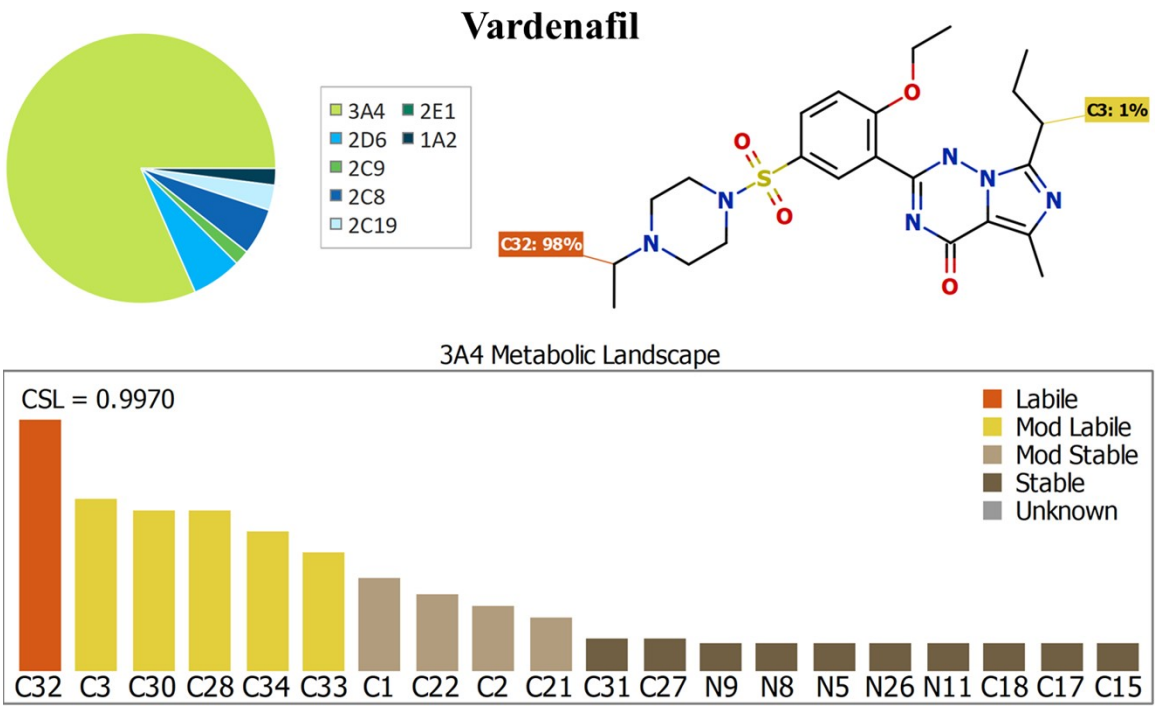


Fig. S3. The metabolic lability data revealed a CSL value of 0.9970, indicating that VRF is highly metabolically labile (unstable). The outcomes were estimated using the P450 tool.

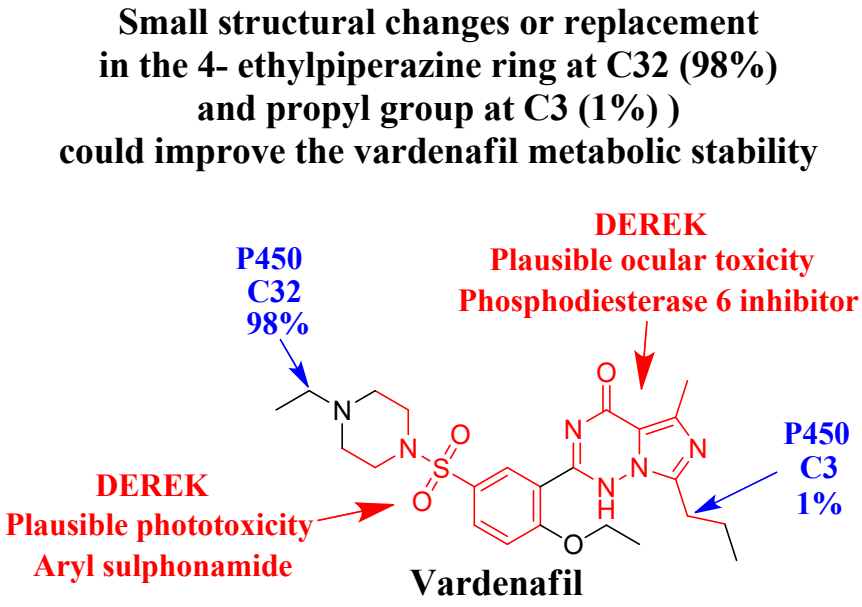


Fig. S4. The VRF structure shows the P450 metabolic sites marked in blue, with the 4-ethylpiperazine ring at C32 (98%) and the propyl group at C3 (1%) also marked in blue, which are accountable for VRF's metabolic lability. However, VRF DEREK alert predictions (red) indicate that Aryl sulphonamide is expected to exhibit plausible phototoxicity.

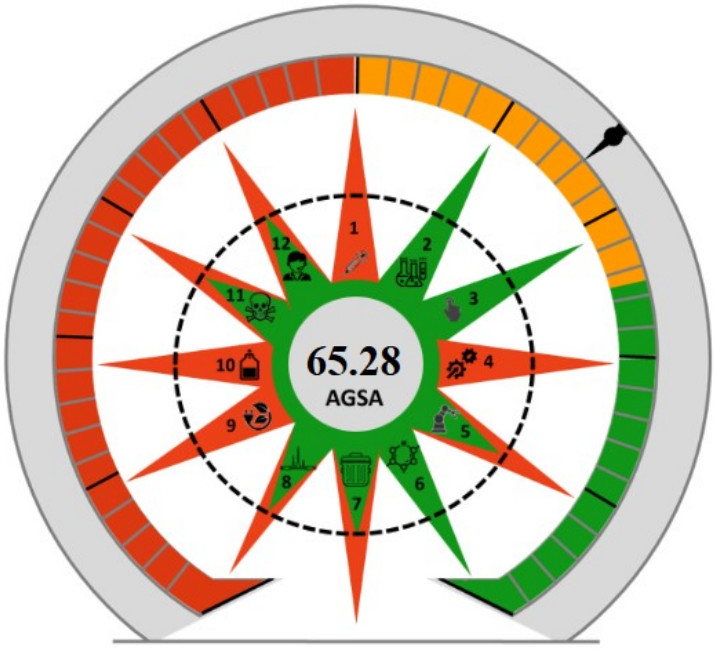


Fig. S5. The greenness evaluation results for the established technique (UPLC-MS/MS) using the AGSA, freely available online software, yield a score of 65.28, indicating a good degree of greenness.

Tables:

Table S1. The ADME characteristics of VRF were evaluated using the online SwissADME tool.

Physicochemical characteristics		**Water Solubility**	
Heavy atom number.	34	Solubility	2.96e-02 mg/ml; 6.06e-05 mol/l
Formula	$C_{23}H_{32}N_6O_4S$	Log S (ESOL)	-4.22
Rotatable bond number	8	Class	Moderately soluble
Num of aromatic heavy atoms	15	Solubility	1.09e-02 mg/ml; 2.23e-05 mol/l
Molecular weight	488.60 g/mol	Log S (Ali)	-4.65
Num. of H-bond acceptors	8	Class	Moderately soluble
		Solubility	2.98e-04 mg/ml; 6.10e-07 mol/l
Num. of H-bond donors	1	Class	Poorly soluble
Fraction Csp3	0.52	Log S (SILICOS-IT)	-6.21
TPSA	121.28 Å²	**Medicinal Chemistry**	
Molar Refractivity	138.53	Synthetic accessibility	4.26
Lipophilicity		Leadlikeness	No; 2 violations: MW>350, Rotors>7
Log Po/w (XLOGP3)	2.46	Brenk	0 alert
Consensus Log *Po/w*	2.58	PAINS	0 alert
Log Po/w (iLOGP)	3.80	**Pharmacokinetics**	
Log Po/w (SILICOS-IT)	2.42	P-gp substrate	Yes
Log Po/w (MLOGP)	1.82	Skin permeation (Log Kp)	-7.53 cm/s
Log Po/w (WLOGP)	2.39	Permeable to BBB	No
Druglikeness		CYP2D6 inhibition	No
Ghose	No; 2 violations: MW>480, MR>130	GI absorption	High
Lipinski	Yes; 0 violations	CYP1A2 inhibition	No
Egan	Yes	CYP2C19 inhibition	No
Muegge	Yes	CYP2C9 inhibition	Yes
The score of bioavailability	0.55	CYP3A4 inhibition	Yes
Veber	Yes		

Table S2. MRM mass spectrometric features for VRF and TDF (IS) as adjusted by the IntelStart software.

Compound	Formula/Mass	Transition	Parent m/z	Cone Voltage	Daughters	Collision Energy	Ion Mode
VRF MRM	$C_{23}H_{32}N_6O_4S$	1	489.32	16	151.04	76	ES+
		2	489.32	16	72.01	64	ES+
TDF MRM	$C_{22}H_{19}N_3O_4$	1	390.17	78	268.13	14	ES+
		2	390.17	78	135.04	34	ES+

Table S3. The twelve principles of the AGSA software for the UPLC-MS/MS method.

Principles	Choices	Answer	Points
1	**Direct Analysis**		
	What is the extent of sample treatment required before analysis?	Extensive treatment (e.g., extraction, filtration, concentration)	1
2	**Minimum Sample Size**		
	What is the sample size required for the analysis?	Less than 0.1 gram	3
3	**In-Situ Measurements**		
	Where are the measurements performed?	Measurements are performed directly at the sampling site (in situ)	3
4	**Integration of Analytical Processes**		
	What is the level of integration of analytical processes?	Separate processes requiring multiple steps and instruments	1
5	**Automation and Miniaturization**		
	What is the degree of automation of the method?	Semi-automated	1
	What is the degree of miniaturization of the method?	Semi-miniaturized	1
6	**Avoid Derivatization**		
	Does the method require derivatization?	No derivatization required	3
7	**Minimum Waste and Efficient Waste Management**		
	What is the volume of waste generated by the method?	Less than 100 milliliters per sample	1.5
	How is analytical waste managed in the method?	Proper waste disposal is performed	1
8	**High Throughput**		
	How many analytes are determined by the method in a single run?	2-3 analytes	2
9	**Low Energy Consumption**		
	What is the amount of energy consumed by the analytical process?	>1.5 KW/sample	1
10	**Use of Renewable Reagents**		
	What is the source of reagents used in the method?	Non-renewable, synthetic reagents	1
11	**Low Toxicity**		
	What is the toxicity level of reagents used in the method?	3-5 pictograms	2
12	**Operator Safety**		
	What level of safety is provided for the operator during the procedure?	Moderate-risk procedures requiring some PPE	2

References

1. M. W. Attwa, A. S. Abdelhameed and A. A. Kadi, *Heliyon*, 2024, **10**.
2. M. W. Attwa, A. S. Abdelhameed and A. A. Kadi, *Medicina*, 2024, **60**, 1626.
3. I. Muegge, *Medicinal Research Reviews*, 2003, **23**, 302-321.
4. M. Motiwale, H. Verma, O. Silakari and B. Sapra, *Computational Drug Delivery: Molecular Simulation for Pharmaceutical Formulation*, 2024, 39.
5. M. W. Attwa, A. S. Abdelhameed and A. A. Kadi, *Medicina*, 2024, **60**, 1914.