Supplementary Information (SI) for Analytical Methods.

Validated green ultra-fast UPLC-MS/MS method for the determination of fedratinib in HLMs matrix: Application to in vitro and in silico metabolic stability studies

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Supplementary data

Figures

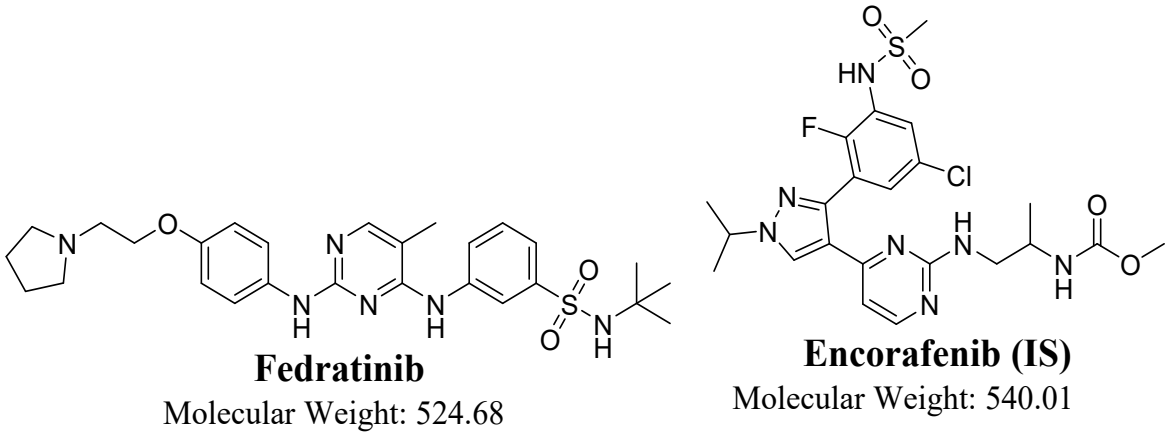

Fig. S1. The chemical structure of fedratinib and encorafenib that was employed as IS.

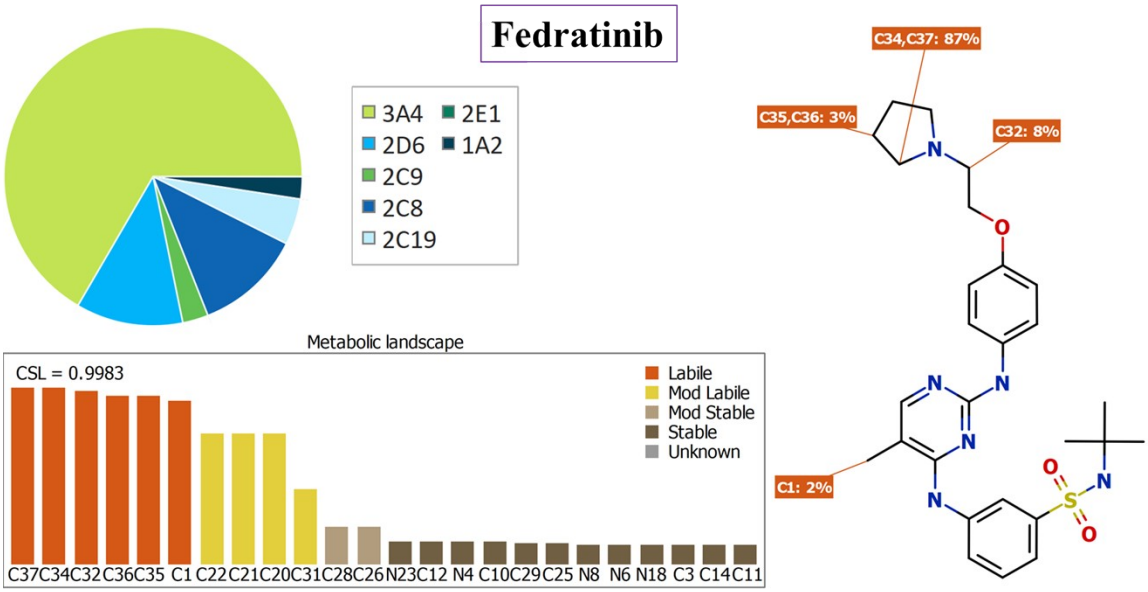

Fig. S2. The outlined CSL mark of 0.9983 implies that FDB displays a high lability to metabolic procedures. The resultant scores were calculated employing the P450 program.

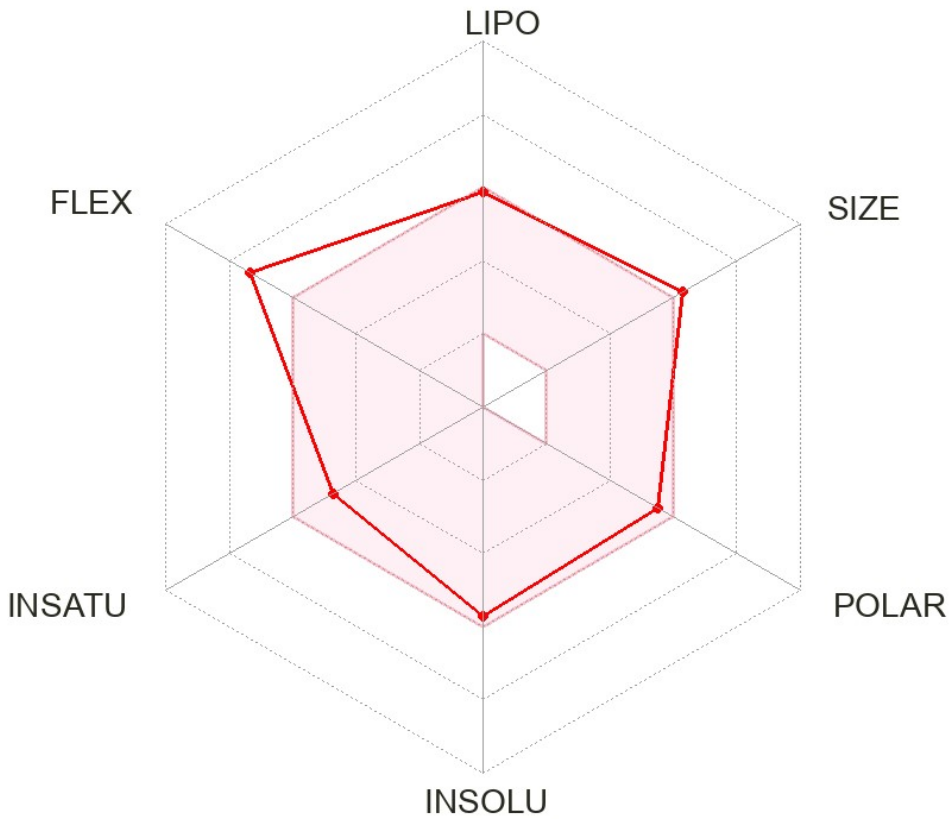

Fig. S3. The FDB ADME radar chart was employed using the in silico SwissADME program. LIPO (Lipophilicity) is estimated as XLOGP3 = +4.76. SIZE (Average molecular weight): 524.68 g/mol; INSOLU (Solubility): log S ≤ 9.51; FLEX (Flexibility): 11 rotatable bonds; POLAR (Polarity): TPSA 116.86 Å^2; Saturation (INSATU): fraction of carbons in sp^3 hybridization 0.41.

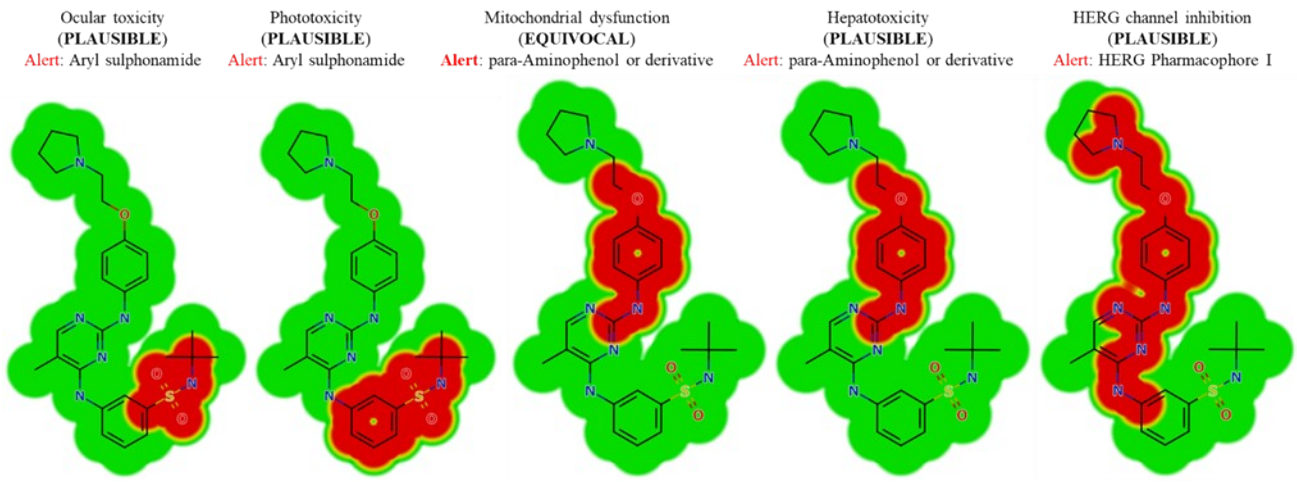

Fig. S4. The FDB structural alarms were characterized applying the DEREK program and highlighted in red color.

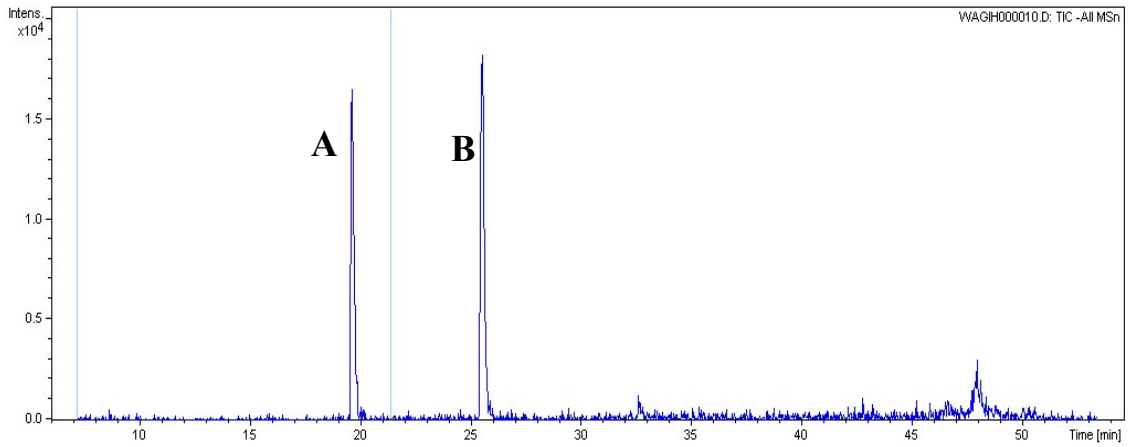

Fig. S5. Two segments chromatogram for p-hydroxy phenytoin (A) and phenytoin (B).

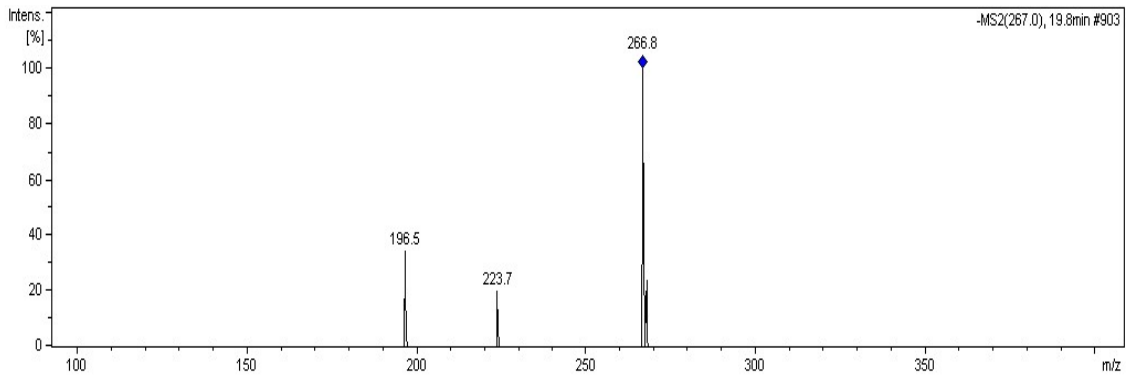

Fig. S6. Mass spectra for p-hydroxy phenytoin.

Tables

Table S1. The ADME parameters of FDB were assessed applying the online SwissADME program.

Physicochemical features	
Formula	$C_{27}H_{36}N_6O_3S$
Heavy atoms num.	37
Molecular weight	524.68 g/mol
Rotatable bonds number	11
Arom. heavy atoms number	18
Fraction Csp3	0.41
Number of H-bond acceptors	7
Number of H-bond donors	3
TPSA	116.86 Å²
Molar Refractivity	151.66
Lipophilicity	
XLOGP3 (Log Po/w)	4.76
Log Po/w (SILICOS-IT)	2.95
Log Po/w (iLOGP)	4.21
WLOGP (Log Po/w)	5.52
MLOGP (Log Po/w)	2.06
Consensus Log Po/w	3.90
Druglikeness	
Ghose	No; 3 violations: #atoms>70, MW>480, MR>130,
Lipinski	Yes; 1 violation: MW>500
Egan	Yes
Muegge	Yes
Veber	Rotors>10: No; 1 violation
The score of bioavailability	0.55

Water Solubility	
Solubility	9.86e-04 mg/ml ; 1.88e-06 mol/l
Log S (ESOL)	-5.73
Class	Moderately soluble
Solubility	5.96e-05 mg/ml ; 1.14e-07 mol/l
Log S (Ali)	-6.94
Class	Poorly soluble
Solubility	3.69e-07 mg/ml ; 7.03e-10 mol/l
Log S (SILICOS-IT)	-9.15
Class	Poorly soluble
Medicinal Chemistry	
PAINS	0 alert
Leadlikeness	No; 3 violations: XLOGP3>3.5, MW>350, Rotors>7
Brenk	0 alert
Synthetic accessibility	4.18
Pharmacokinetics	
P-gp substrate	No
GI absorption	Low
BBB permeant	No
CYP1A2 inhibition	No
CYP2D6 inhibition	Yes
CYP3A4 inhibition	Yes
CYP2C19 inhibition	Yes
Skin permeation (Log Kp)	-6.12 cm/s
Inhibition of CYP2C9	Yes

Table S2. Optimized features of the present UPLC-MS/MS method for FDB and ENB.

	Extraction Recovery		Mobile Phase		Stationary Phase		
	Protein Precipitation Using ACN	Solid Phase Extraction	ACN	Methanol	C18 Column	C8 Column	HILLIC Column
FDB	High (101.96%± 5.99%)	Low (80.37%)	0.26 min	0.36 min	0.26 min	0.21 min	0.12
	Precise (RSD < 5.88%)	Not precise	Optimum peak	Tailed	Good shape	Tailed peaks	Unretained peak
ENB	High (101.61 ± 3.32 and)	Low (72.61%)	0.62 min	0.41 min	0.62 min	0.43 min	0.14
	Precise (RSD < 3.18%)	Not precise	Optimum peak shape	Overlapped	Perfect shape	Perfect shape	Unretained peak

Table S3. UPLC-MS/MS optimized features.

UPLC conditions		MS/MS (TQD MS) conditions	
Isocratic mobile phase	0.1% HCOOH in H_2O (55%; pH: 3.2)	ESI	The flow rate of cone gas is 100 liters per hour.
	45% ACN		Positive mode
	Injection volume: 5.0 µl		The voltage of the RF lens is measured to be 0.1 volts.
	Flow rate: 0.4 mL/min.		The capillary voltage utilized in this experiment was set at 4 kilovolts (KV).
Eclipse SB C18 reversed column	30.0 mm long		The voltage of the extractor is 3.0 volts.
	2.1 mm i.d.		The drying gas used in this experiment is nitrogen, which is maintained at a temperature of 350 °C. The flow rate of nitrogen is set at 100 L/hr.
	1.8 µm particle size	Mode	MRM
	T: 22.0 ± 2.0 °C	Collision cell	Argon gas at 0.14 mL/min

Table S4. MRM-tuned parameters for the determination of FDB and ENB.

	Time in min.	Rt in min.	Analyte	MRM features
Mass spectra segment	0.0 to 0.5	FDB (0.26)	FDB	525→ 98 (CE [a] 42 and CV [b] 58)
				525→ 84 (CE: 40 and CV: 58)
	0.5 to 1.0	ENB (0.62)	ENB (IS)	540 → 359 (CV: 36 and CE: 56)
				540 → 112 (CV: 32 and CE: 56)

[a] Collision energy, [b] cone voltage.

Table S5. The greenness calculation of the established UPLC-MS/MS method.

Standards	Mark	Weight
1. Utilize direct approaches of analysis to circumvent the necessity for sample treatment.	0.3	2
2. The aims are to accomplish a limited sum of samples and a minimal sample size.	0.98	2
3. Whenever feasible, it is advisable to conduct observations on site.	0.66	2
4. The amalgamation of operational procedures and analytical approaches leads to conserving energy and a reduction in reagent usage.	1.0	2
5. One should choose automated and miniaturized approaches.	0.75	2
6. Avoidance of derivatization is recommended.	1.0	2
7. It is important to prevent the generation of a crucial amount of analytical surplus and confirm that appropriate measures are in place for its control.	1.0	2
8. Methods that analyze many analytes or parameters simultaneously are favored over methods that analyze one analyte at a time.	1.0	2
9. Energy consumption should be reduced.	0.0	2
10. It is sensible to arrange reagents that are origenated from natural resources.	0.5	2
11. It is important to remove or substitute toxic reagents.	1.0	3
12. The level of safety for operators should be enhanced.	0.8	2

Table S6. MoGAPI scores for the proposed UPLC-MS/MS approach.

Category	
Sample preparation	
Collection (1)	**Offline (Red 1)**
Preservation (2)	**Chemical or physical (Yellow 2)**
Transport (3)	**None (Green 3)**
Storage (4)	**Under special conditions (Red 1)**
Type of method: direct or indirect (5)	**Extraction required (Red 1)**
Scale of extraction (6)	**Microextraction (Yellow 2)**
Solvents/reagents used (7)	**Non-green solvents/reagents used (Red 1)**
Additional treatments (8)	**None (Green 3)**
Reagents and solvents	
Amount (9)	**<10 mL (<10 g) (Green 3)**
Health hazard (10)	**Slightly toxic, slightly irritant; NFPA health hazard score is 0 or 1 (Green 3)**
Safety hazard (11)	**Highest NFPA flammability, instability score of 0 or 1. No special hazards. (Green 3)**
Instrumentation	
Energy (12)	**>1.5 kW h per sample**
Occupational hazard	**Hermetization of the analytical process**

Category	
(13)	**(Green 3)**
Waste (14)	**<1 mL (<1 g) (Green 3)**
Waste treatment (15)	**Degradation, passivation (Yellow 2)**
ADDITIONAL MARK: QUANTIFICATION	
No oval in the middle of GAPI: ***Procedure only for qualification (1)***	