ملتقى البحث العلمي

مركز بحوث الدراسات العلمية والطبية


بسم الله الرحمن الرحيم

Journal of Pharmaceutical and Biomedical Analysis 137 (2017) 258–267

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

ISSN: 0731-7085

Impact Factor: 3.255

Comparative pharmacokinetic profiles of selected irreversible tyrosine kinase inhibitors, neratinib and pelitinib, with apigenin in rat plasma by UPLC-MS/MS

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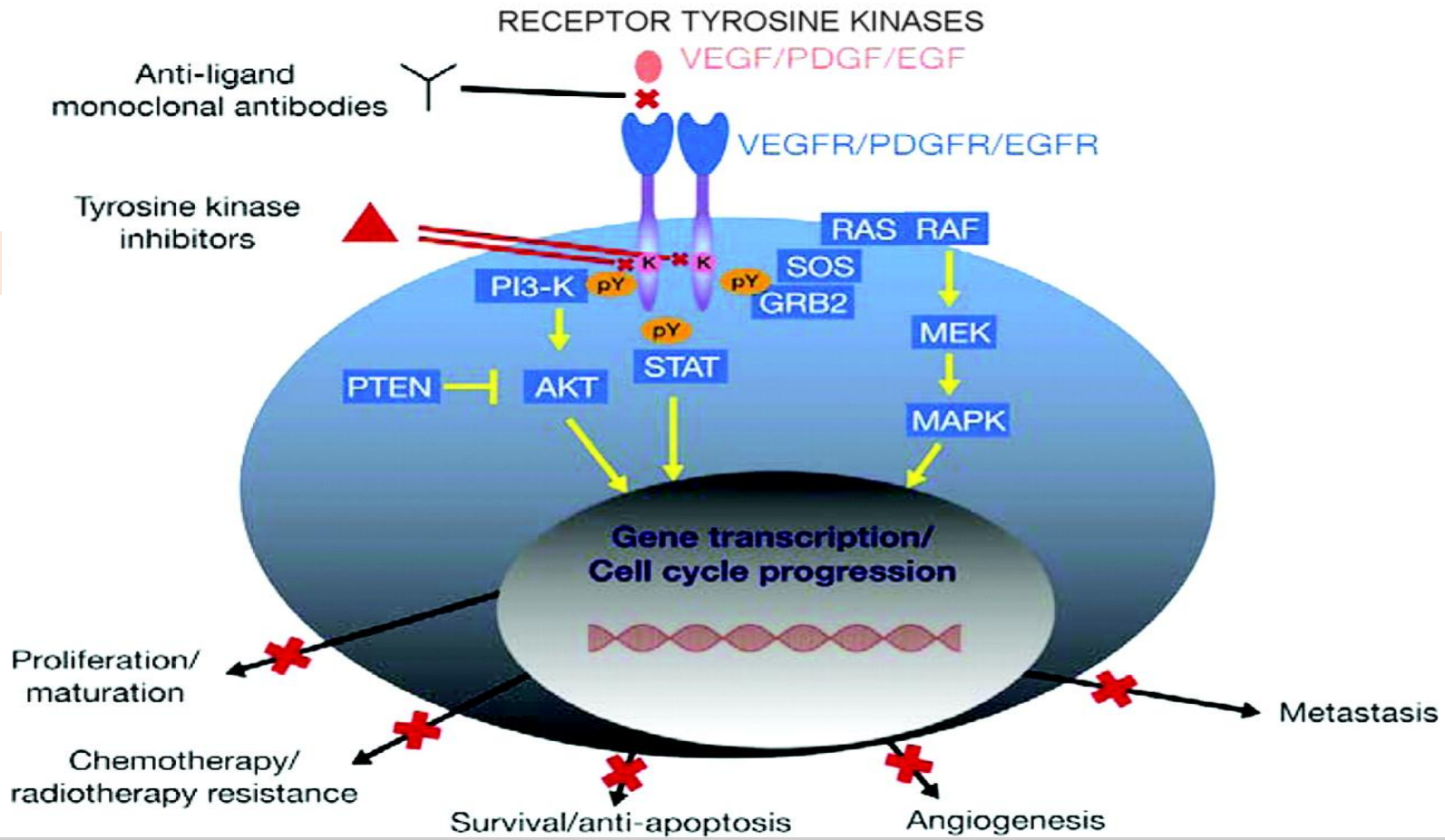
Blocking EGFR as a targeted anticancer therapy

- Monoclonal antibodies
- Small molecules
  - **Tyrosine kinase inhibitors**
  - Serine/threonine kinase inhibitors
  - Small molecule drug conjugates



Phosphorylation of tyrosine residues on the epidermal growth factor receptor (EGFR) is an important early event in signal transduction, leading to cell replication for major human carcinomas. This receptor is widely expressed in advanced cancers .

Li et al, Target Oncol, 2012

Reversible and irreversible TKIs

- ATP-competitive inhibitors inhibit protein catalytic activity in a reversible manner. Examples of which are gefitinib (GEF), and erlotinib (ERL).
- In the past decade, much progress has been made in the development of a new class of potent and selective TKIs that irreversibly inhibit their target protein .

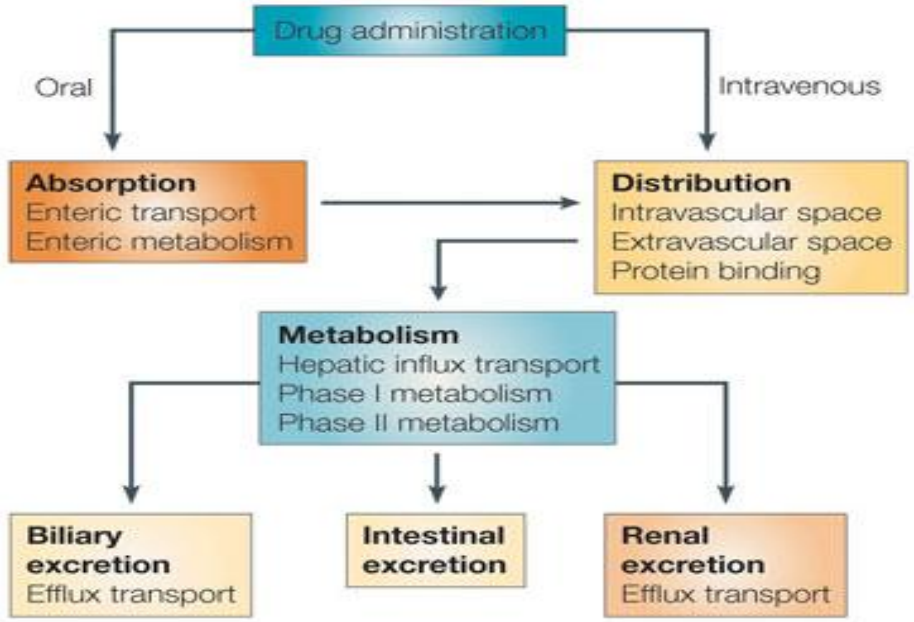
Several theoretical advantages of the second-generation irreversible EGFR TKIs over the first-generation reversible EGFR TKIs are that some have a **higher affinity** and **selectivity** for the EGFR kinase domain, and may allow a more complete blockade of the EGFR signaling pathway . ***The number of irreversible TKIs entering clinical trials studies is steadily increasing***. ***Examples of which are neratinib (NER) and pelitinib (PEL).***

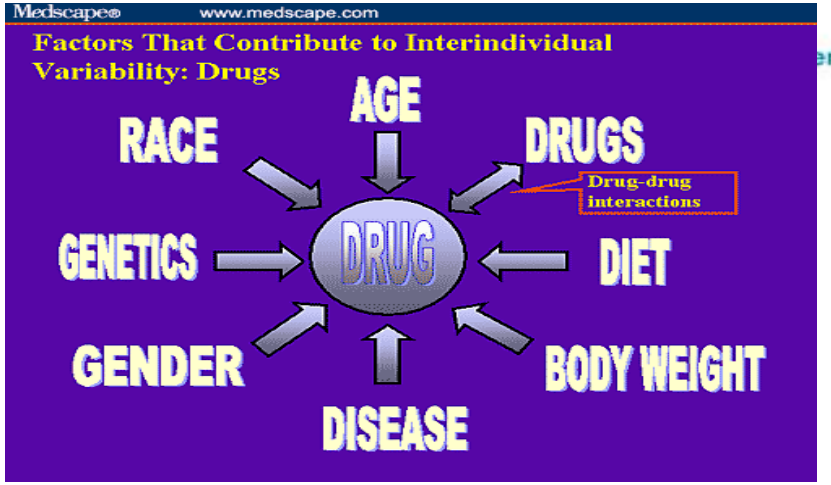
Intra/Inter-individual pharmacokinetic (PK) variability

. However, **complexity in the pharmacokinetics (PK)** of these drugs results from their **oral administration**.

Intra/Inter-individual PK variability

- **Genetic heterogeneity of drug targets**
- **Patient characteristics**
  environmental factors
  (drug–drug interactions)
  adherence to treatment.
- **Pharmacogenetic background of the patient**
  (e.g. cytochome P450 and ABC transporter polymorphisms) plays an important role in the inter-individual variability .

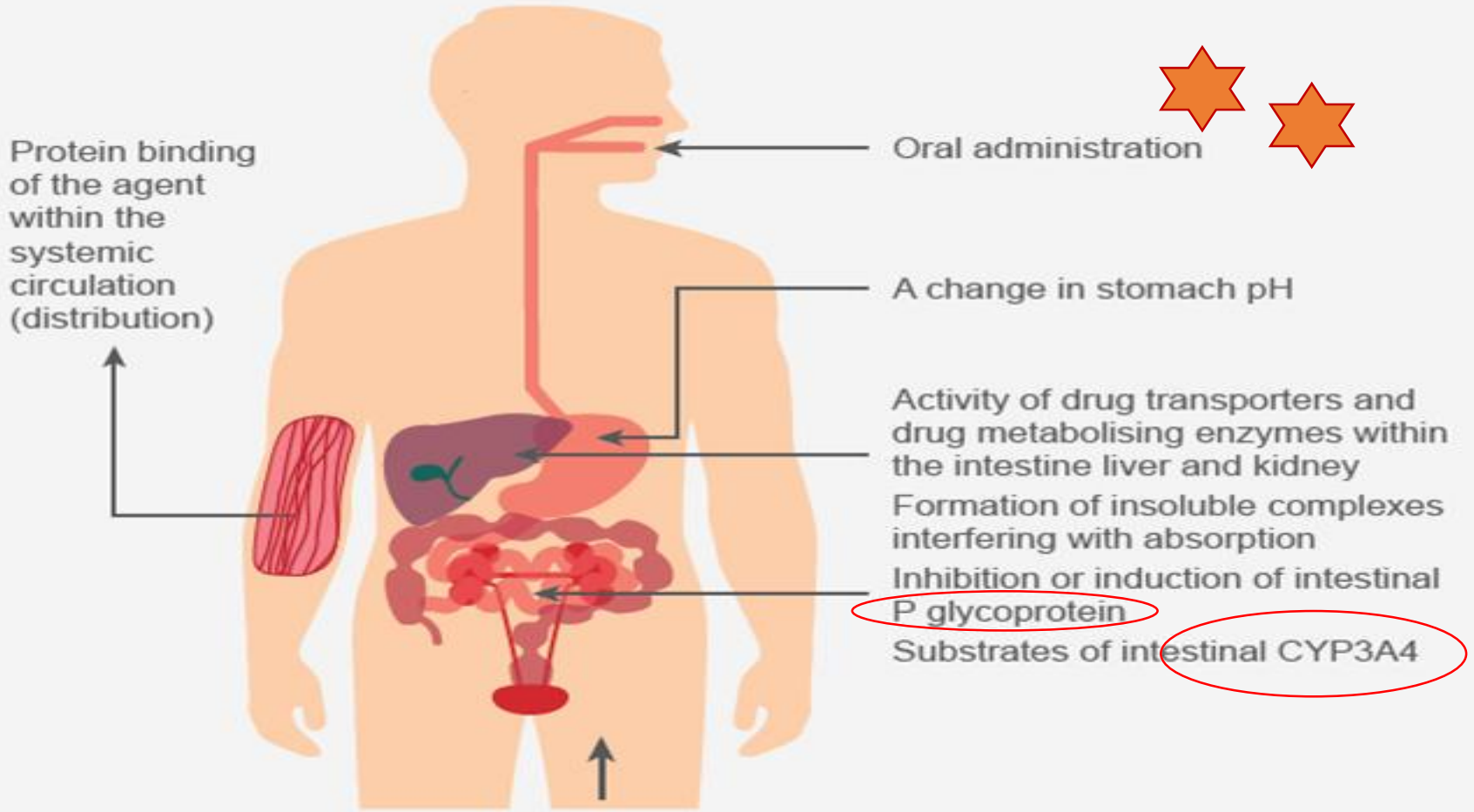




Widmer, Eur. J. Cancer, 2014

Drug-drug interactions (DDIs) with TKIs

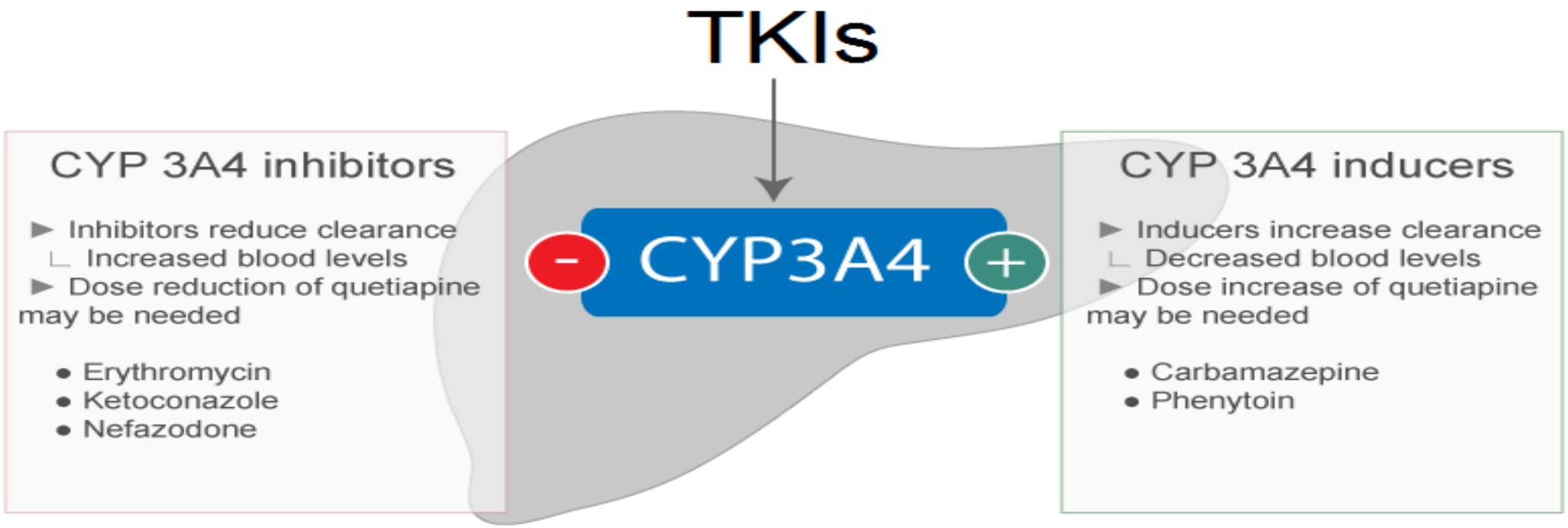
Potential sites of drug interactions with orally-administered kinase inhibitors.
Adapted from: van Leeuwen RW, van Gelder T, Mathijssen RH, Jansman FG. Drug-drug interactions with tyrosine-kinase inhibitors: a clinical perspective. Lancet Oncol 2014; 15: e315-326.



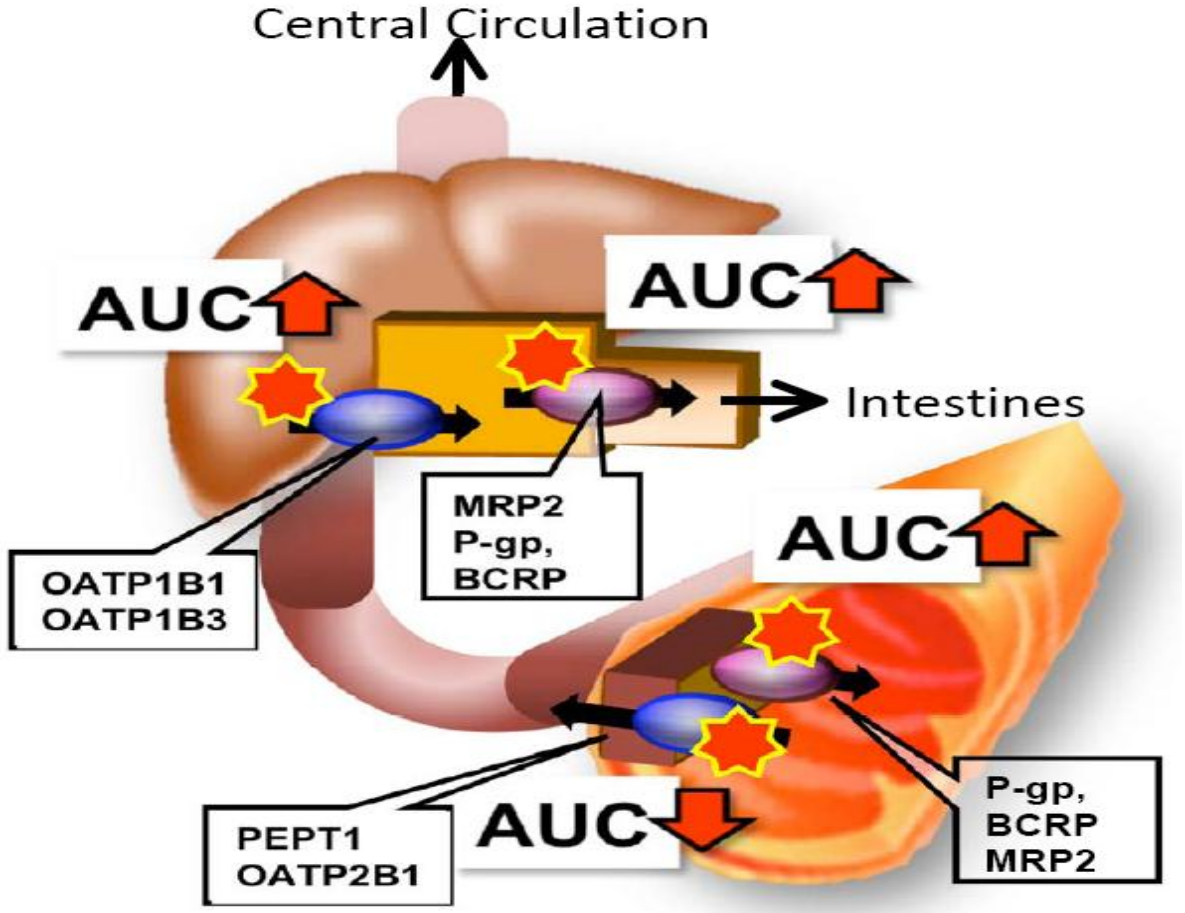
CYP3A4, cytochrome P450 3A4.

Drug-drug interactions (DDIs) with TKIs

TKIs are mainly metabolized by CYP3A4. Thus they are susceptible to drug-drug interactions with any **co-administered drug** which has been known to be an **inhibitor or inducer of CYP450 enzymes** . **Inhibitors block the activity** of a particular CYP450 enzyme with a slow clearance of the TKI with increased frequency of side effects while **inducers increase the enzyme activity** resulting in enhancing chemotherapy clearance with a potential decrease in the effectiveness and increasing risk of relapse .



Drugs altering the activity of drug transporters (P-gp) within the intestine



Impact of transporter-based drug-drug interactions on pharmacokinetics in intestine and liver

Co-administration of natural products contained in food, which have potential **to inhibit P-gp**, may cause …**unexpected drug-induced toxicity** due to their **enhanced systemic exposure.**

Nakanishi et al, Current Drug Metabolism, 2015.

High incidence of DDIs?????

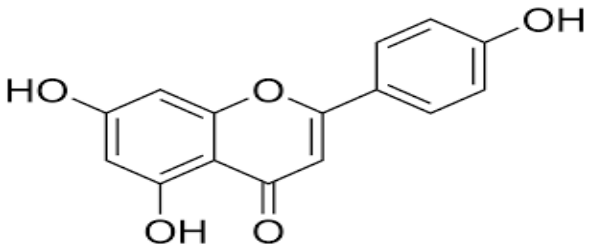
It is noteworthy to mention that cancerous patients mostly require **multiple medications** besides the anticancer agents. They include

- **Supportive care medications** used to alleviate the side effects of the particular anticancer drugs. Since many of these supportive drugs are either inhibitors or inducers of the CYP450, they could potentially affect the metabolism of co-administered TKIs
- **Complementary & alternative medicine (CAM)**
- **Anticancer medications or drugs used to treat underlying diseases.**

Administration of herbs with TKIs in some cancer cases

Herb-drug interactions with TKIs

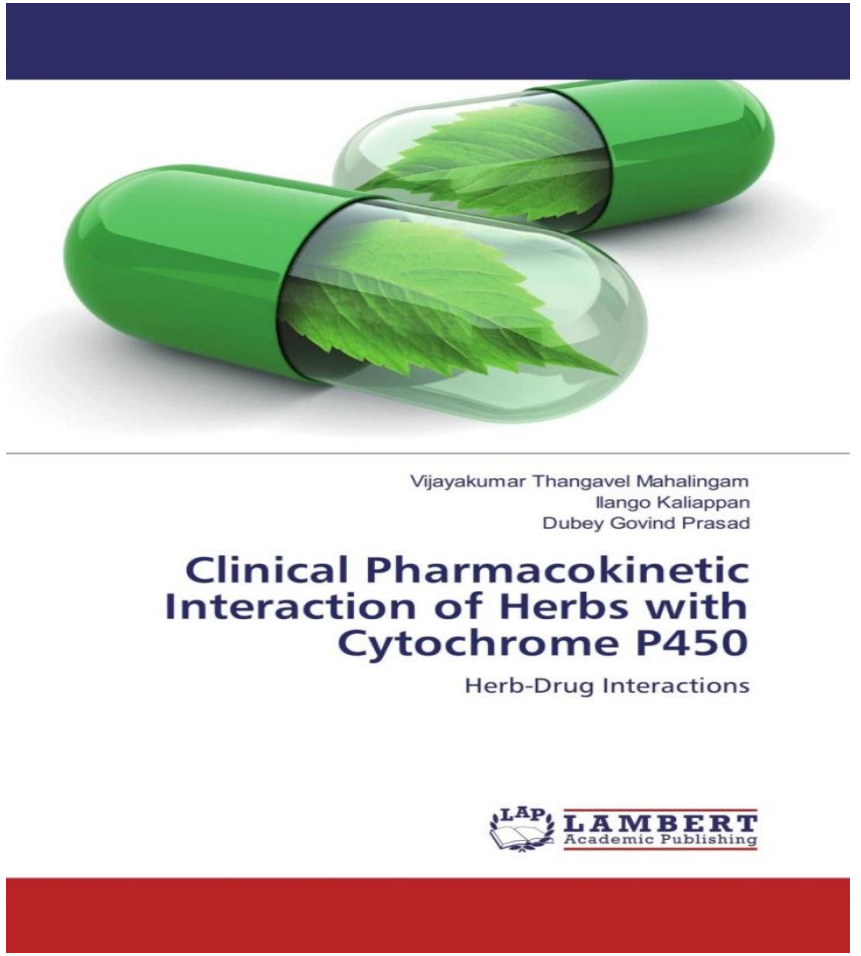
Apigenin in foods of plant origin



Flavonoids are widely used as remedies because of their spasmolytic, antiphlogistic, antiallergic, and diuretic properties. **Flavonols and flavones are flavonoids of particular importance as they were found to contain antioxidant and free radical scavenging activity in foods.**

These molecules have been shown to possess numerous anti-inflammatory, antiangiogenic, and anti-carcinogenic effects in cell culture and in various animal models.

- Apigenin, to some extent, is a potent inhibitor of the cytochrome P450 (CYP) enzyme system which is responsible for the metabolism of considerable pharmaceutical drugs.
- Given the widespread availability of apigenin, it is important to understand what effects its concomitant use may have on the disposition of medications.

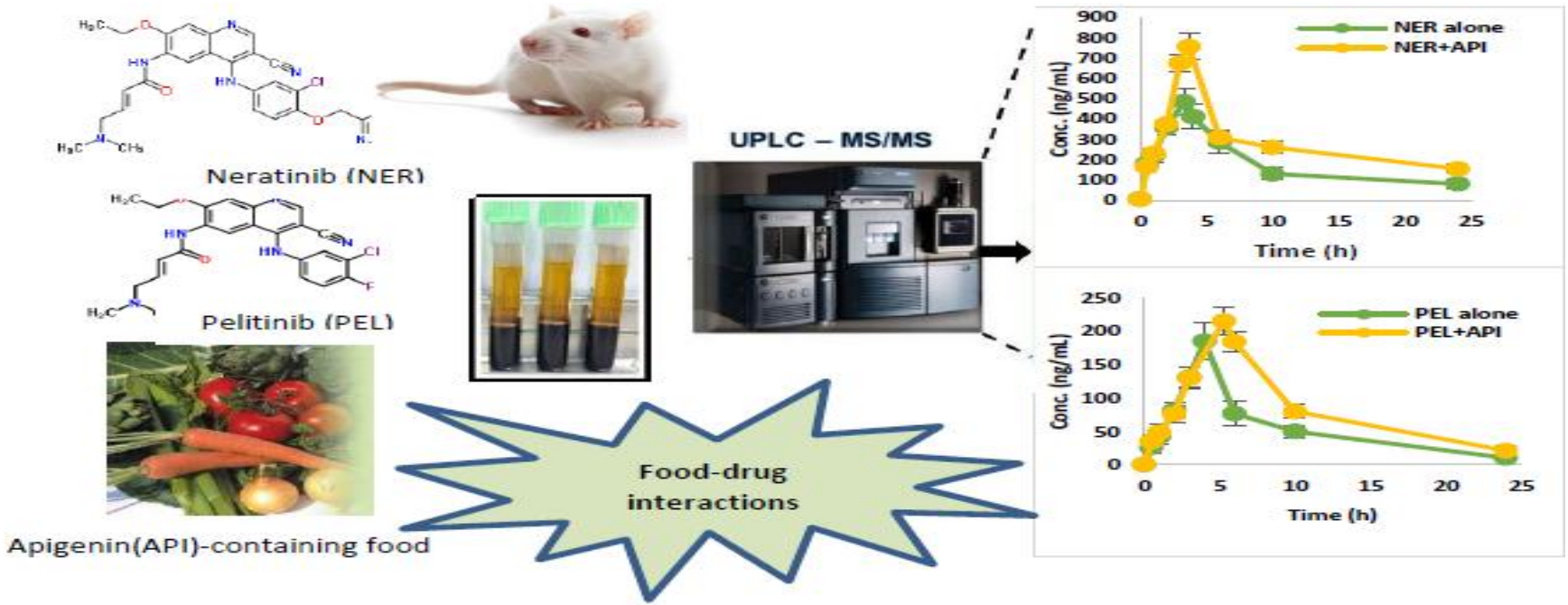


Concurrent administration of other drugs or herbal products that modulate cytochrome P450 enzymes activity may alter TKIs exposure. Therefore, a combination of flavonoids and selected TKIs is expected TO HAVE POTENT DRUG INTERACTIONS.

Liu et al, BioMed Research International, 2013

Aim of the work

- Review of the literature revealed that, to our knowledge, no method has been found so far ***dealing with the study of the effect of food on the PK of NER/PEL.***
- Thus, this work aims at **the development and validation of a rapid and highly selective UPLC–MS/MS method for the determination of NER/PEL in rat plasma samples.**
- The validated method was successfully applied to **PK interaction studies** as a result of possible **co-administration of API, along with NER/PEL**, in the oncology practice.



I- METHOD DEVELOPMENT

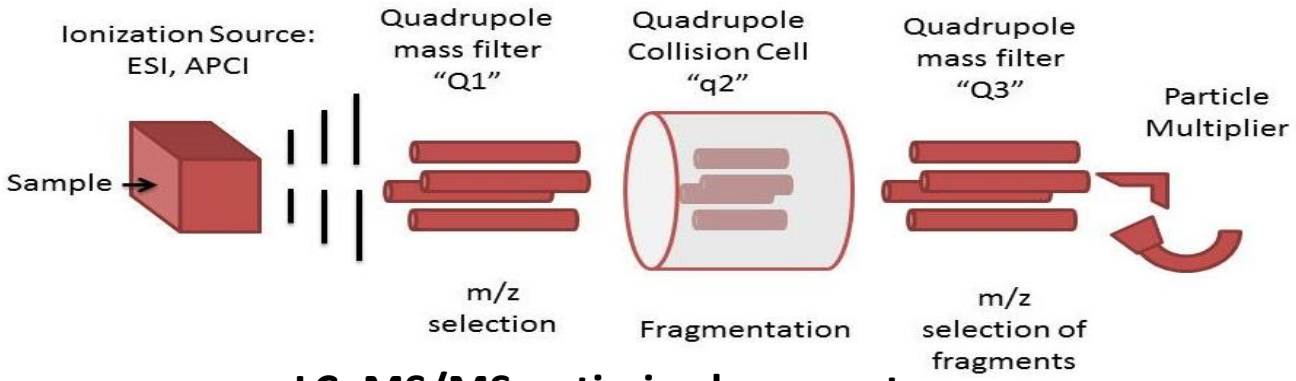
UPLC–MS/MS analysis

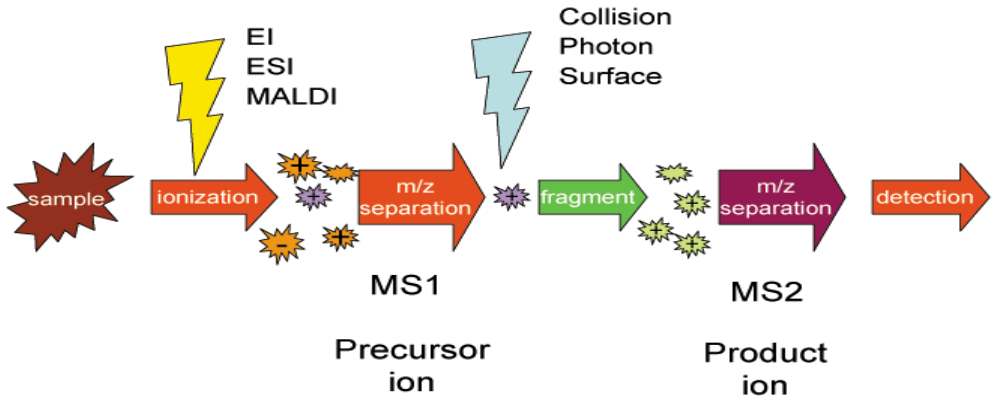
UPLC-MS/MS analysis was performed using a **Waters Model** Xevo TQ-S separation system with **a triple-quadrupole mass spectrometric detector** .The instrument was controlled by the Masslynx™ Version 4.1 software, Micromass.

Chromatographic conditions

- Acquity **UPLC BEH™ C 18 column** (100 ×1.0 mm, i.d., 1.7 µm particle size) (Waters, Ireland).
- **The mobile phase** was composed of two solvent systems namely, the aqueous phase, A (0.1 % formic acid in water) and the organic modifier, B (0.1 % formic acid in acetonitrile). Isocratic elution was applied using a mobile phase of A: B (30: 70). **Column temperature** was maintained at 45 ° C while the **auto-sampler temperature** was kept at 10 ° C throughout the runtime (2 min.). The **flow rate** was 0.2 mL/min and the **injection volume** was 5 µL using partial loop mode.

Mass spectrometric conditions



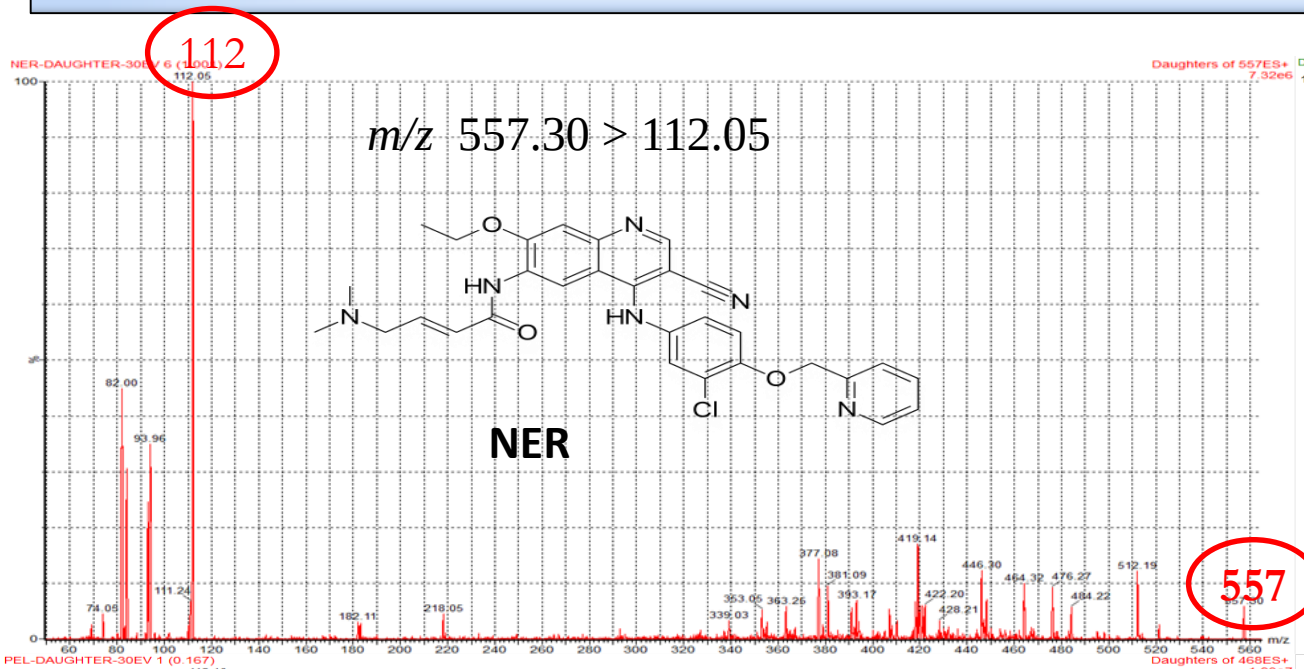


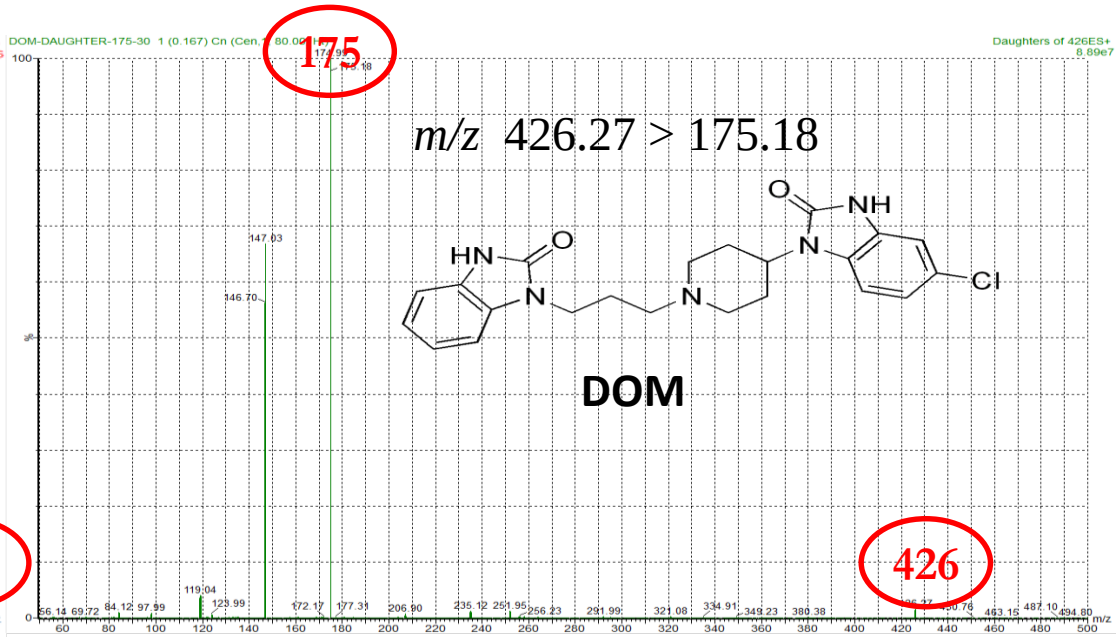
LC–MS/MS optimized parameters

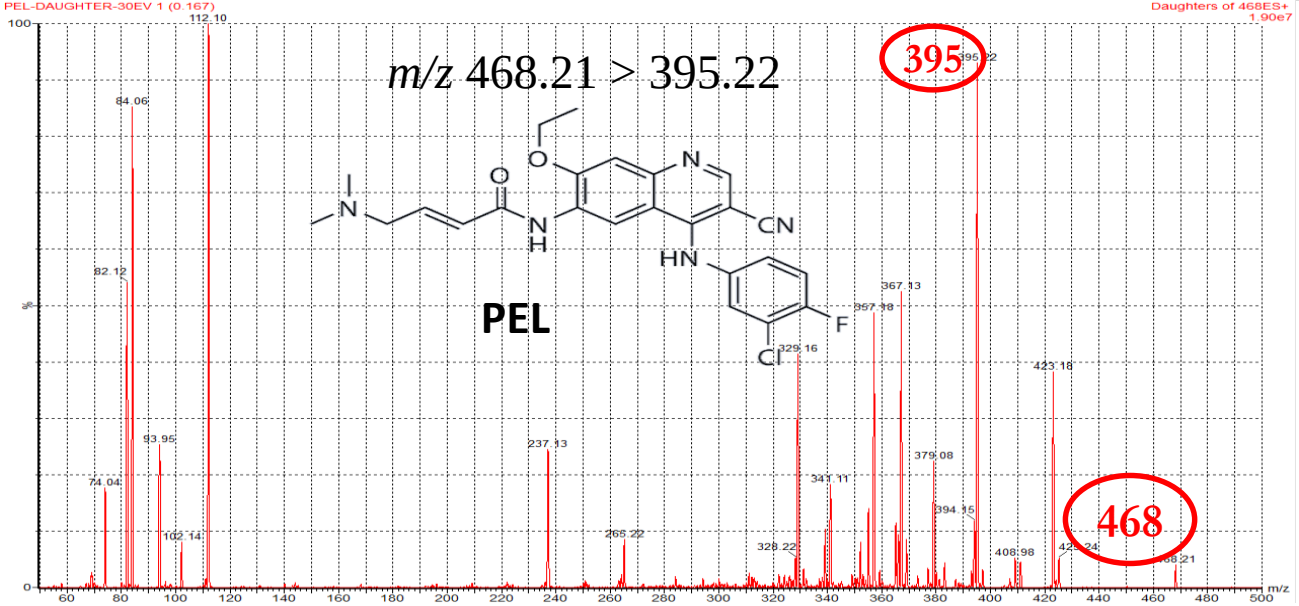
Target compound	Precursor ion $[M+H]^+$	Daughter ion	Cone voltage (V)	Capillary voltage (KV)	Collision energy (eV)	Desolvation Temperature (^{o} C)
NER	557.30	112.05	90	4	30	200
PEL	468.21	395.22	3	3	30	200
DOM (IS)	426.27	175.18	30	3.5	30	200

- **Electrospray ionization (EI) was operated in the positive ionization mode**.
- **Quantitation was performed using multiple reaction monitoring (MRM)**. Nitrogen was used as the **desolvating gas** at a flow rate of 800 L/h. **Cone gas flow** was adjusted at 150 L/h.

Product ion spectra of the studied compounds







Optimization of sample clean-up and extraction procedure

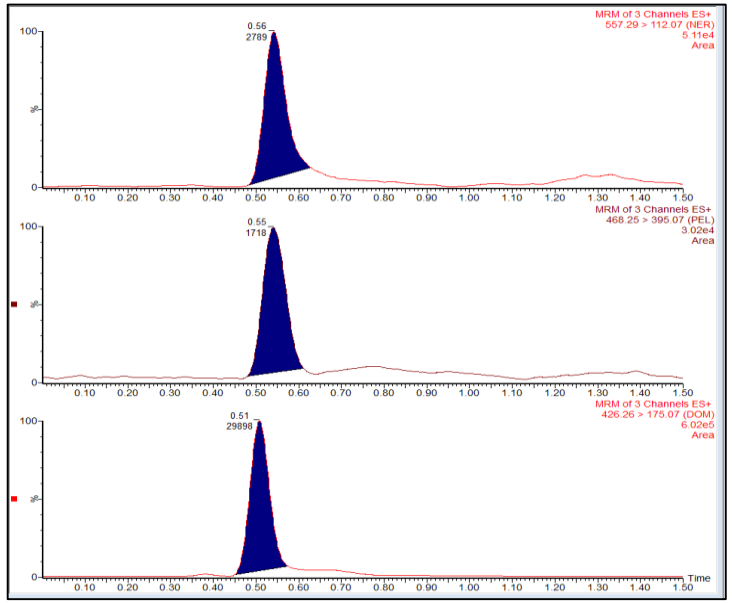
Protein precipitation with methanol

PPT + SPE

Spiking with NER, PEL

Solid-phase extraction

Evaporation to dryness
Reconstitution



UPLC-MS/MS

Methanol was used for protein precipitation. The supernatant was purified by passing through a C 18 Bond Elut cartridge used for solid phase extraction. The retained drugs were eluted with methanol and the eluate was evaporated to dryness. The residue was further reconstituted in acetonitrile then injected into the UPLC-MS/MS.

II- METHOD VALIDATION

Guidance for Industry

Bioanalytical Method Validation

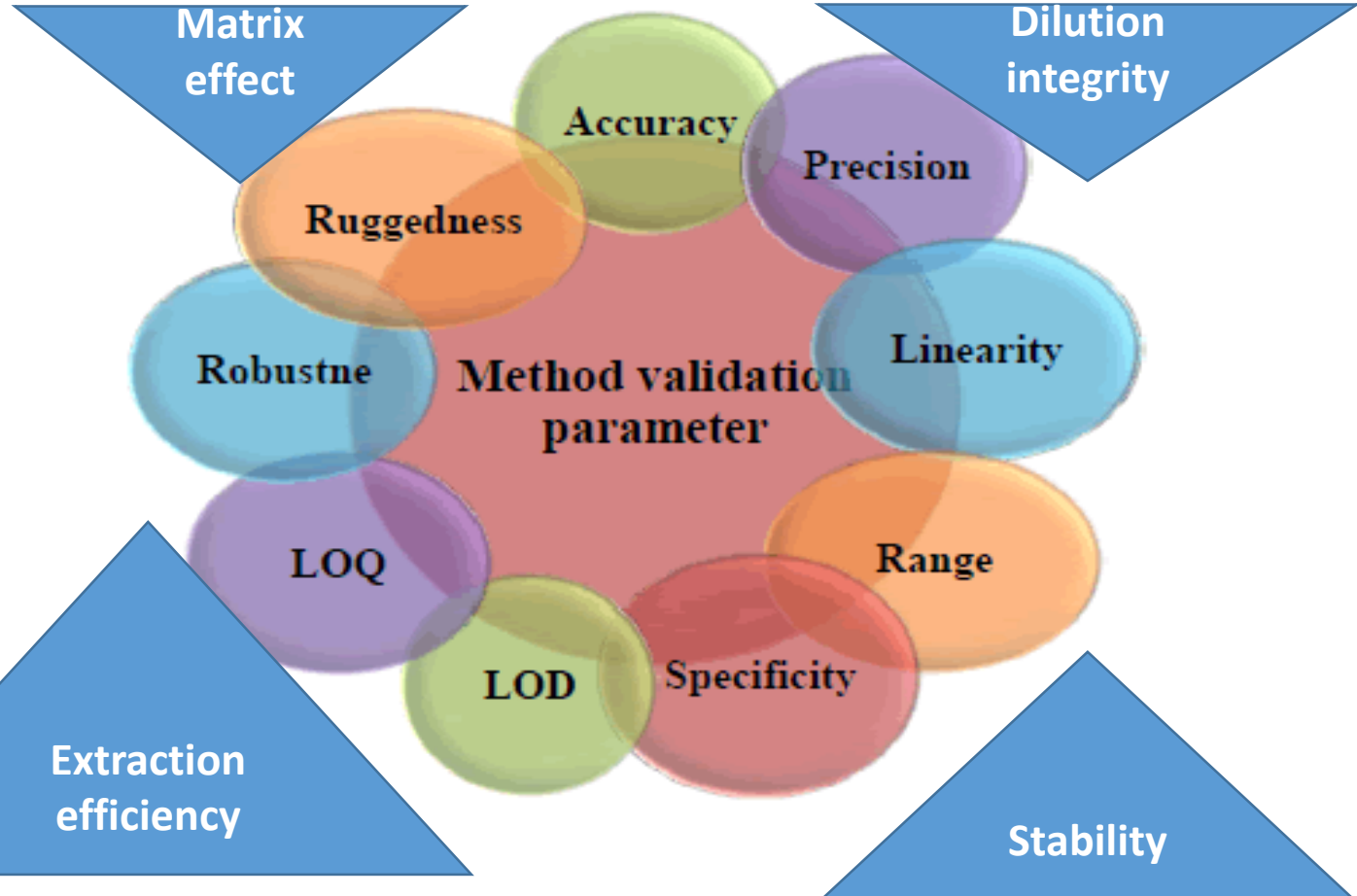
Additional copies are available from:

Drug Information Branch (HFD-210)
Center for Drug Evaluation and Research (CDER)
5600 Fishers Lane, Rockville, MD 20857 (Tel) 301-827-4573
Internet at http://www.fda.gov/cder/guidance/index.htm

or

Communications Staff (HFV-12)
Center for Veterinary Medicine (CVM)
7500 Standish Place, Rockville, MD 20855 (Tel) 301–594-1755
Internet at http://www.fda.gov/cvm

U.S Food and Drug Administration



https://www.fda.gov/downloads/drugs/guidances/ucm368107.pdf

Linearity, limits of detection and quantitation

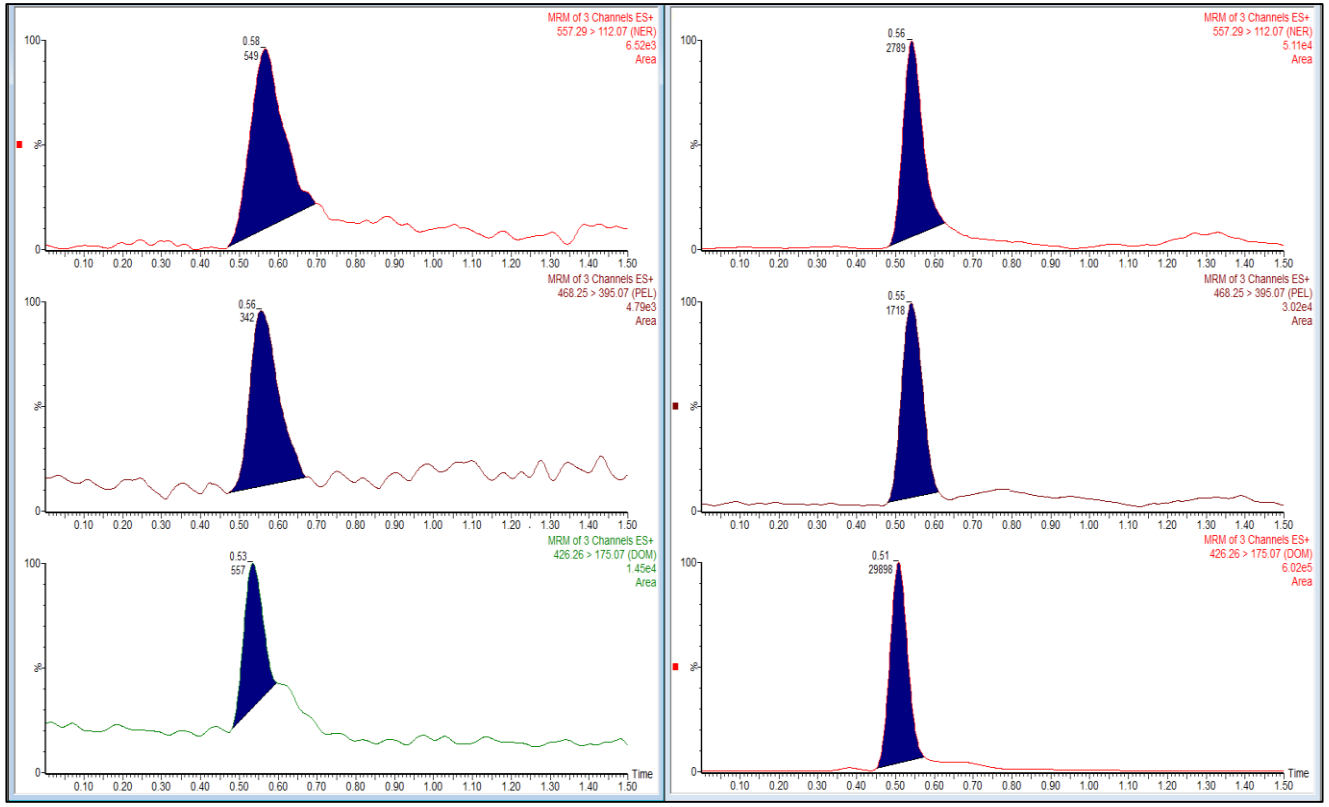
Blank

LLOQ

NER

PEL

DOM

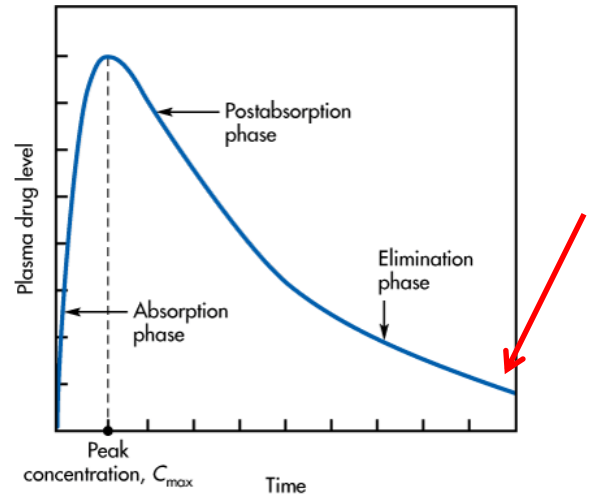


Multiple reaction monitoring (MRM) of a blank plasma, and a plasma sample spiked with a standard mixture of **NER** and **PEL** at their LLOQ levels.

Matrix-based calibration…..

- Linearity range **(0.5-200 ng/mL)**
- **LLOQ 0.5 ng/mL**
- **LLOD 0.3 ng/mL**

High method detectability, sensitivity

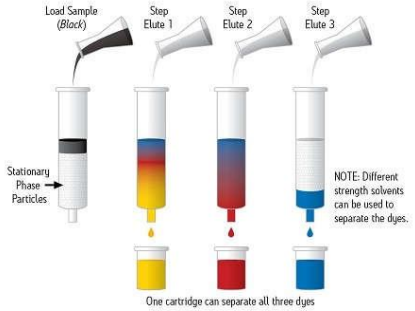


Source: Shargel L, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics, 6th Edition*: www.accesspharmacy.com

Extraction recovery, matrix effect

QC samples

	Concentration spiked (ng/ml)	Mean recovery (%) ± RSD[a]	E_r(%)[b]
Extraction recovery			
NER	0.5	93.01±5.85	-6.99
	5	92.42±3.44	-7.58
	50	93.25±6.75	-6.75
	150	94.84±0.86	-5.16
PEL	0.5	90.99±8.87	-9.01
	5	89.73±7.01	-10.27
	50	92.89±4.88	-7.11
	150	94.56±9.44	-5.44
Matrix effect			
NER	0.5	94.21±3.63	-5.79
	5	98.81±1.73	-1.19
	50	97.32±1.56	-2.68
	150	100.23±1.39	0.23
PEL	0.5	98.50±0.64	-1.50
	5	98.44±3.21	-1.56
	50	99.69±2.86	-0.31
	150	96.56±1.77	-3.44



Stability studies

Stability	Concentration added (ng/mL)	Mean recovery (%) ±RSD[a]	
		NER	PEL
Auto-sampler stability (10°C, 56 h)	5	98.88±1.00	97.85±2.67
	150	100.43±1.15	98.78±1.58
Short-term stability (25°C, 6 h)	5	98.83±3.92	99.04±2.24
	150	97.75±1.16	99.47±1.16
Long-term stability (-30°C, 30 days)	5	95.42±3.13	97.68±7.26
	150	96.30±0.93	97.17±0.80
Freeze-thaw stability (-30°C, 3 cycles)	5	98.95±0.63	100.06±3.15
	150	97.22±1.75	101.25±2.24
Refrigerator (4°C, 3 months)	5	95.04±1.81	97.73±2.88
	150	100.83±1.93	98.26±3.35

[a] Mean recovery (%) ± RSD of six determinations
[b] Percentage relative error.

Accuracy and precision

QC samples

Dilution integrity

Error, RSD within 15% for conc. other than LLOQ (20%)

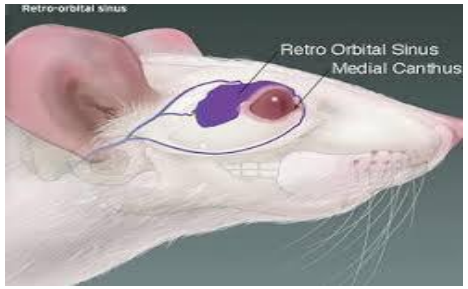
Accuracy and Precision					
		Intra-day (n=6)		Inter-day (n=18)	
	Concentration added (ng/mL)	Mean recovery (%) ±RSD	E_r(%)	Mean recovery (%) ±RSD	E_r(%)
NER	0.5	98.07±2.85	-1.93	98.57±4.69	-1.43
	5	98.26±4.99	-1.74	95.47±4.89	-4.53
	50	92.18±6.33	-7.82	97.67±4.87	-2.33
	150	100.72±1.56	0.72	97.98±2.76	-2.02
PEL	0.5	97.44±4.84	-2.56	101.34±2.70	1.34
	5	90.08±2.19	-9.92	99.09±2.70	-0.91
	50	91.08±5.60	-8.92	94.18±6.10	-5.82
	150	99.67±1.52	-0.33	91.38±10.02	-8.62

Dilution integrity (n=6)					
		1:2		1:5	
NER	300	98.38±2.67	-1.62	99.61±2.69	-0.39
PEL	300	99.49±1.62	-0.51	97.88±4.28	-2.12

III- APPLICATION TO PHARCOKINETIC STUDY

- **Group I** • Control
- **Group II** • NER (30 mg/kg)
- **Group III** • PEL (10 mg/kg)
- **Group IV** • API (100 mg/kg) + NER (30 mg/kg)
- **Group V** • API (100 mg/kg) + PEL (10 mg/kg)

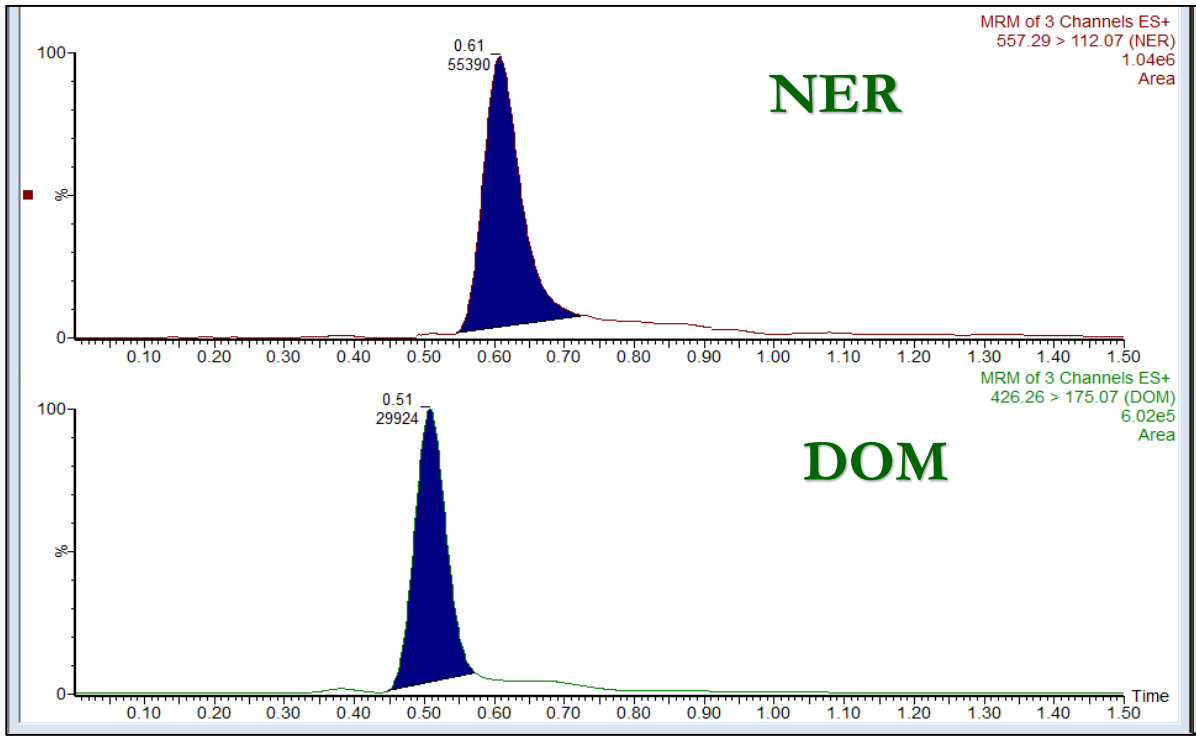
Oral route



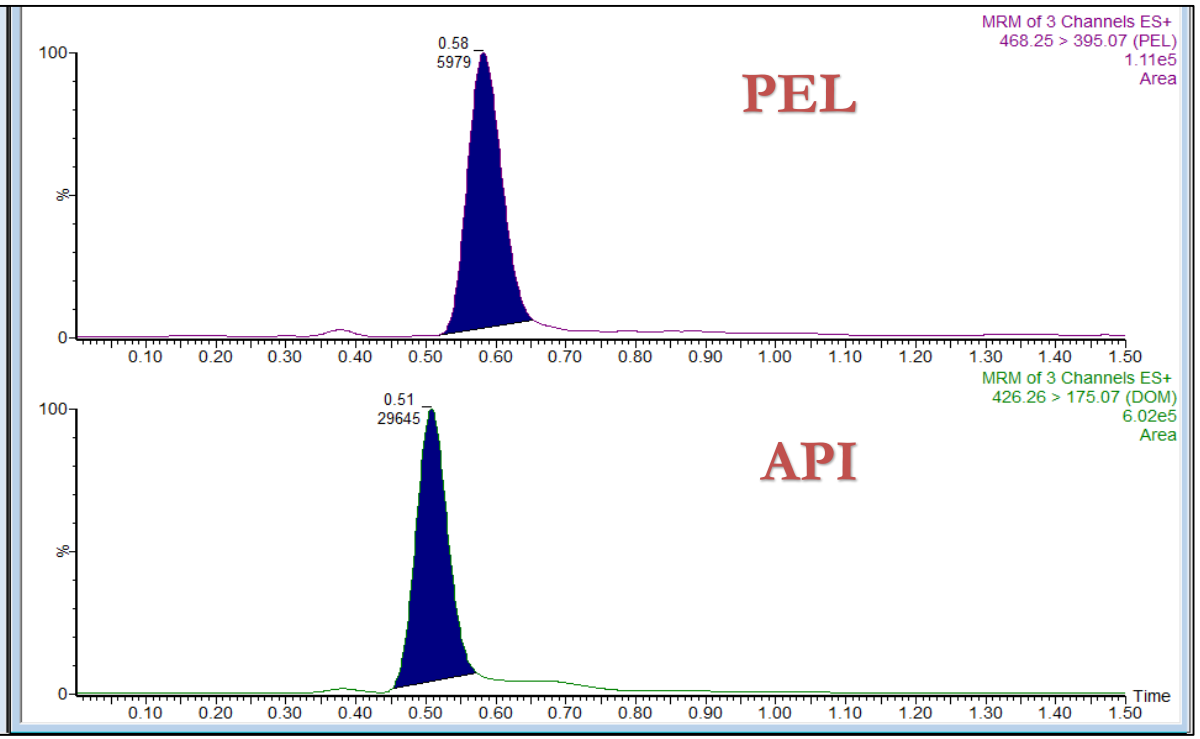
Blood samples (0.3 mL) from control and treated groups were collected from orbital plexus in EDTA-K2 tubes. **Blood samples were collected at different time intervals (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 10.0, and 24.0 h) following drug administration**, spiked with a constant volume of 0.1 mL of DOM, IS (5 ng/mL). Plasma samples were then treated by SPE then analyzed by UPLC-MS/MS.

Application to pharmacokinetic studies

NER/API

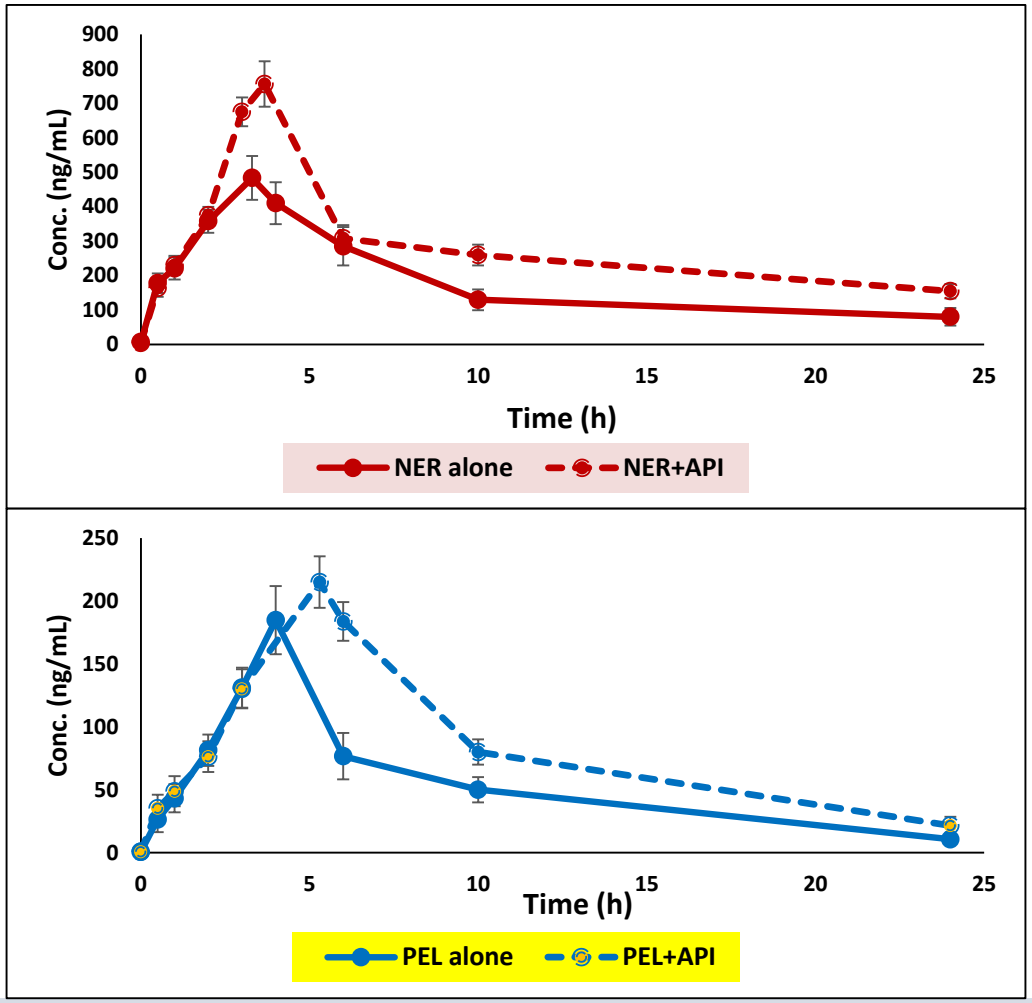


PEL/API



Multiple reaction monitoring (MRM) of plasma sample of treated rats collected 3 h after the oral administration of a combination of **NER (30 mg/kg) and API (100 mg/kg)** and plasma sample of treated rats collected 4 h after the oral administration of a combination of **PEL (10 mg/kg) and API (100 mg/kg)**.

Pharmacokinetic studies



Drug	Pharmacokinetic parameter			
	C_{max} (ng/mL) ±SD	t_{max} (h) ±SD	$t_{½}$ (h) ±SD	AUC (ng.h/mL) ±SD
Group I NER (30 mg/kg)	**483.34**±63.41	3.33±0.58	7.00±1.50	**4295**±99.100
Group II PEL (10 mg/kg)	**184.78**±27.05	4.00±1.25	5.80±0.22	**1290**±112.21
Group III NER(30 mg/kg) +API (100 mg/kg)	**755.64**±65.91	3.67±0.58	5.20±0.66	**6787**±158.55
Group IV PEL (10 mg/kg) +API (100 mg/kg)	**214.94**±20.53	5.30±1.15	9.20±2.33	**1970**±212.02

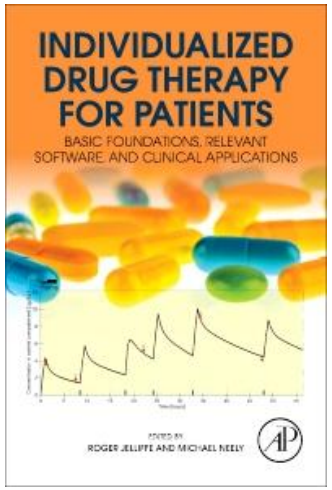
The concentration–time profile of the studied drugs in rats after oral administration of **NER (30 mg/kg) or PEL (10mg/kg), and API (100 mg/kg),** compared with single administration of each drug alone at the same dose levels.

Thus API-induced increased bioavailability of NER/PEL could be attributed to the inhibitory effect of API on both CYP3A4 enzymes as well as P-gp efflux proteins.

Conclusion

In the present study, **a UPLC-MS/MS method** has been developed and validated for the quantification of the two irreversible TKIs, NER and PEL, in plasma samples. The proposed method has many advantages over the previously reported methods for the determination of either NER or PEL including **higher sensitivity**, **smaller sample volume**, and **shorter analysis time**.

The applicability of the developed SPE-UPLC-MS/MS method was extended to studying the **possibility of PK interaction between NER/PEL and the widely used flavonoid, API.** The results showed that API could affect the CYP-mediated metabolism of NER/PEL with increased drug plasma levels and enhanced drug-induced toxicity. **Thus TDM of these drugs**, when co-administered with API, is very important for the sake of public health.



Particular care should be paid with the intake of CAM medication with TKIs

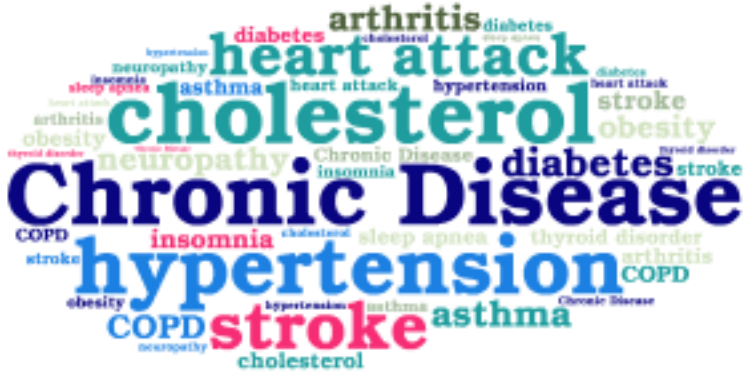
Thus TDM of these drugs, **when co-administered with API**, is very important for the sake of public health.

futurework

Although this study was conducted **in rats** and not on humans and that differences in their PK pattern could exist, the possibility of occurrence of PK interactions between NER/PEL and API could exist when shifting to **clinical studies**.

Extend the study to different herb-TKIs PK interactions

Chronic diseases & drug metabolism

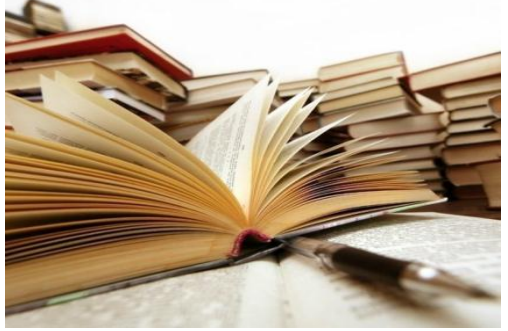


Acknowledgements

Acknowledgments

This research project was supported by a grant from the "**Research Center of the Center for Female Scientific and Medical Colleges**," Deanship of Scientific Research, King Saud University.

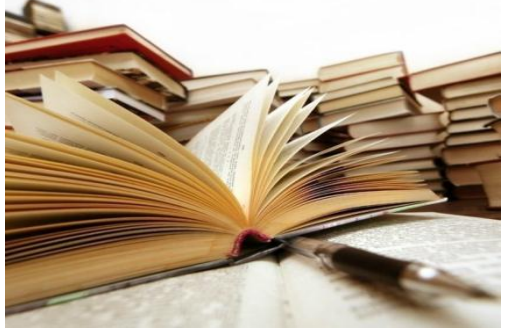
REFERENCES



- **H. M. Maher**, N. Z. Alzoman, S. M. Shehata, Simultaneous determination of ***selected tyrosine kinase inhibitors*** with corticosteroids and antiemetics in rat plasma by solid phase extraction and ultra-performance liquid chromatography–tandem mass spectrometry: Application to pharmacokinetic interaction studies, ***J. Pharm. Biomed. Anal.*** 124 (2016) 216–227

- **H. M. Maher**, N. Z. Alzoman, S. M. Shehata , Simultaneous determination of ***erlotinib and tamoxifen*** in rat plasma using UPLC-MS/MS: application to pharmacokinetic interaction studies, ***J.*** **Chromatogr.** ***B.*** 1028 (2016) 100–110.

- **H.M. Maher**, N. Z. Alzoman, S. M. Shehata, A. O. Abahussain, UPLC–ESI–MS/MS study of the effect of green tea extract on the oral bioavailability of ***erlotinib and lapatinib*** in rats: Potential risk of pharmacokinetic interaction, J. Chromatogr. B, 30-40 (2017) 1049–1050



- A. Thomas-Schoemann, B. Blanchet, C. Bardin, G. Noé, P. Boudou-Rouquette, M. Vidal, F. Goldwasser, Drug interactions with solid tumour-targeted therapies, Crit. Rev. Oncol. Hemat. 89 (2014) 179–196
- R.W. van Leeuwen, T. van Gelder, R. H. Mathijssen, F. G. Jansman, Drug–drug interactions with tyrosine-kinase inhibitors: a clinical perspective, Lancet Oncol. 15 (2014) e315–26.
- N. Widmer, C. Bardin, E. Chatelut, A. Paci, J. Beijnen, D. Levêque, G. Veal, A. Astier, Review of therapeutic drug monitoring of anticancer drugs part two – Targeted therapies q, Eur. J. Cancer 50 (2014) 2020– 2036
- H. H. Cao, J. H. Chu, H. Y. Kwan, T. Su, H. Yu, C. Y. Cheng, X. Q. Fu, H. Guo, T. Li, A. K. Tse, G.X. Chou, H.B. Mo, Z.L. Yu, Inhibition of the STAT3 signaling pathway contributes to apigenin mediated anti-metastatic effect in melanoma, Sci. Rep. 25 (6) 21731, DOI: 10.1038/srep21731
- A. I. Alvarez, R. Real, M. Pérez, G. Mendoza, J. G. Prieto, G. Merino, Modulation of the activity of ABC Transporters (P-Glycoprotein, MRP2, BCRP) by flavonoids and drug response, J. Pharm. Sci. 99 (2) (2010) 598-617.


Thank
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