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UPLC–MS/MS study of the effect of dandelion root extract on the plasma levels of the selected irreversible tyrosine kinase inhibitors dasatinib, imatinib and nilotinib in rats: Potential risk of pharmacokinetic interactions

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Abstract

Tyrosine kinase inhibitor treatments for chronic myeloid leukaemia based on nilotinib (NIL), dasatinib (DAS) and imatinib (IMA) have improved patient quality of life and have turned chronic myeloid leukemia from a fatal disease into a chronic disease. Dandelion is a rich source of phenolic compounds with strong biological properties, and the effects of using this plant in the treatment of different illnesses can be linked to the presence of various polyphenols found in the different parts of the plant. Thus, dandelion can potentially be used as a nutraceutical (dietary antioxidant) to prevent different disorders associated with oxidative stress, i.e. cardiovascular disorders, cancer and inflammatory processes. Mutual interference between a drug and a food constituent may result in altered pharmacokinetics of the drug and undesired or even dangerous clinical situations. In the present study, a bioanalytical ultra performance liquid chromatography–tandem mass spectrometer (UPLC–MS/MS) method was developed and validated for the quantification of DAS, IMA and NIL in rat plasma. Sample preparation was carried out using solid-phase extraction with C_{18} cartridges with a good extraction recovery of ≥94.37% for the three drugs. The method was fully validated as per the US Food and Drug Administration guidelines.

KEYWORDS

dandelion, dasatinib, drug interactions, imatinib, nilotinib, pharmacokinetics

1 | INTRODUCTION

Herbal medicine is often consumed concurrently with conventional medications because of the misconception that all-natural products are harmless. Although herbal formulations alone may not produce any adverse effects, their simultaneous use with prescribed drugs may lead to an interaction that influences both the efficacy and toxicity of the drugs (Kuhn, 2002).

Dandelion has long been used for its medicinal properties in addition to its use in soups and salads. Furthermore, the roots can be roasted and used as a coffee substitute, and the plant extracts are used as flavour components in various food products (Schutz, Kammerer, Carle, & Schieber, 2005). Dandelion plants, *Taraxacum officinale*, are members of the Asteraceae family (Schutz, Carle, & Schieber, 2006). Dandelion has been used in folklore and modern herbal medical systems owing to its antidiabetic, choleretic, antirheumatic and diuretic properties, and its anticancer, antimicrobial and anti-inflammatory effects (Martinez et al., 2015;Hassan, El-Kholy, & Galal, 2015;Tettey, Ocloo, Nagajyothi, & Lee, 2014; Wang et al., 2016). The widespread use of dandelion is due to the presence of various bioactive compounds, such as phenolic compounds, terpenes,

Abbreviations: TDM, therapeutic drug monitoring

polysaccharides and inulin, and its high content of potassium, protein, fatty acids and vitamins (Jedrejek, Kontek, Lis, Stochmal, & Olas, 2017). Furthermore, dandelion is an easily accessible food with a long history of human use and poses little risk of harm. Dandelion extracts are listed by the US Food and Drug Administration in the generally recognized as safe list for foods and supplements (Sweeney, Vora, Ulbricht, & Basch, 2005).

The efficiency of nutraceuticals in the management of haematological malignancies has been reviewed previously (Angka & Spagnuolo, 2015). A previous report indicated that patients with acute myeloid leukemia who drank dandelion root extract (DRE) had a better response with sustained low myeloblast counts compared with regular chemotherapy (Hamm, 2013). This result was explained by the fact that DRE induces extrinsic apoptosis mediated by caspase-8 in human melanoma cell lines and chronic myeloid leukemia in humans (Chatterjee, Ovadje, Mousa, Hamm, & Pandey, 2011; Ovadje, 2012). Thus, novel therapies for managing leukaemic tumours have focused on DRE as an efficient Phase I antileukaemic agent. However, the co-administration of dandelion with quinolone antibiotics, e.g. ciprofloxacin, modifies the bioavailability and distribution of ciprofloxacin in rats and may have clinical implications for ciprofloxacin dosing (Zhu, Wong, & Li, 1999). Theoretically, dandelion may increase the clearance of drugs that are UDP-glucuronosyl transferase substrates because the plant may inhibit cytochrome P4501A2 (CYP1A2) and may induce UDP-glucuronosyl transferase, a phase II enzyme (Maliakal & Wanwimolruk, 2001; Unger & Frank, 2004). Additionally, hyperkalaemia may increase the concomitant use of dandelion and potassium-sparing diuretics owing to the high potassium content in dandelion (Rodriguez-Fragoso, Reyes-Esparza, Burchiel, Herrera-Ruiz, & Torres, 2008 L).

Additionally, the combined consumption of home-grown teas containing dandelion, cyclosporine and sirolimus, two immunosuppressive operators, may induce a poisonous effect because of the inhibitory effects of dandelion on cytochrome 3A4 (CYP3A4) (Dufay et al., 2014). Many previous reports have focused on the broad-spectrum biological activities of dandelion. Nevertheless, the interaction between using dandelion as a dietary supplement and as a chemotherapeutic agent has not been sufficiently evaluated. Earlier studies of the pharmacokinetic (PK) interactions between tyrosine kinase inhibitors (TKIs) and drugs/herbs performed by our research group have shown that in some cases, there is a high risk of PK interactions between TKIs and co-administered herbal drugs. One of our research projects was based on the hypothesis that the erlotinib (ERL)/tamoxifen (TAM) combination could obtain the required cytotoxic effect on non-small-cell lung cancer and sought to minimize the dose-dependent side effects. However, a significant decrease in the area under the concentration–time curve (AUC; 54%) of ERL was observed upon its co-administration with TAM, suggesting a role for TAM in CYP3A4 induction with a subsequent increase in ERL clearance. On the other hand, an increase in the peak concentration (C_{max}) of TAM of (46%), with no significant increase in the AUC (10%), was observed with TAM combined with ERL compared with its single administration. This result could be related to the direct CYP3A inhibition effect of ERL leading to increased levels in plasma (Maher, Alzoman, & Shehata, 2016a). Another study studied the possibility of PK interactions of selected TKIs (gefitinib GEF and erlotinib ERL) taken in combination with corticosteroids (dexamethasone and prednisolone) and the antiemetic ondansetron in rat plasma samples (Maher, Alzoman, & Shehata, 2016b). The C_{max} and AUC for GEF/ERL decreased with the simultaneous administration of prednisolone, dexamethasone and ondansetron. Additionally, we reported a decrease in ERL and lapatinib bioavailability with green tea co-administration, while significantly increased neratinib and pelitinib plasma levels were observed with co-administration of apigenin. Such food–drug interactions are due to alterations in the activity of metabolizing enzymes and/or transporter proteins; therefore, patients taking TKIs should preferably avoid drinking green tea or taking apigenin during treatment with TKIs (Maher, Alzoman, Shehata, & Abahussain, 2017a; Maher, Alzoman, Shehata, & Abahussain, 2017b). Thus, particular attention should be paid to avoiding the concomitant administration of nutraceuticals with TKIs during the treatment regimen.

Among the most commonly used antileukaemic TKIs are dasatinib (DAS), imatinibmesylate (IMA) and nilotinib (NIL), and their concomitant intake with dandelion has been profoundly beneficial in the treatment of chronic myeloid leukaemia. Therefore, a synergistic effect of dandelion with these antileukaemic TKIs has been postulated.

The bioavailability of DAS, IMA and NIL, as well as of other TKIs, is mainly affected by the function of ABC transporters andCYP3A metabolizing enzymes (Van Leeuwen, van Gelder, Mathijssen, & Jansman, 2014; Widmer et al., 2014). The clinical significance of the interaction between dietary supplements and medications can range from statistically insignificant and minor to clinically significant and major (Zarowitz, 2010). Because dandelion has some effect on CYP3A4liver enzymes that are involved in drug metabolism, its PK interactions with co-administered TKIs are likely to have a negative effect on bioavailability (Dufay et al., 2014). A review of the available literature revealed that the effects of dandelion on the PK of DAS, IMA, and NILs have not yet been investigated.

LC–MS/MS methods have been widely applied to measure selected TKIs in plasma (Andriamanana, Gana, Duretz, & Hulin, 2013; Cao et al., 2015; Haouala et al., 2009; Moreno, Wojnicz, Steegman, Cano-Abad, & Ruiz-Nuno, 2013; Zhang et al., 2014). The current work aims to develop and validate a rapid and highly selective UPLC–MS/MS method for the determination of DAS, IMA and NIL in rat plasma samples. This method was successfully applied to the pharmacokinetic study of DAS, IMA and NIL either alone or with dandelion.

2 | EXPERIMENTAL SECTION

2.1 | Chemicals

Erlotinib, used as the internal standard (IS), was purchased from Pfizer Inc. (NY, USA). Dasatinib, IMA and NIL were supplied by HaoyuanChemexpress Co. Ltd (Shanghai, China).

Dandelion root extract capsules were obtained from Natural Factors, Canada. Each capsule contains 300 mg ofdandelion root powder

(*T. officinale*) HPLC-grade methanol and acetonitrile (Panreac, EU) for LC–MS were used in this study. Formic acid (Sigma Aldrich, Chemie GmbH, Steinheim, Germany) was used in the analysis.

Ultrapure water was used throughout the protocols and was obtained using the Milli-Q Advantage system. The purification was performed using a 0.22 µm Millipore filter from Millipore (Molsheim, France).

2.2 | UPLC–MS/MS assay

Chromatographic analysis was performed using a UPLC–MS/MS Model XevoTQ-S separation system (Waters, Singapore). The system was equipped with a binary solvent manager and a sample manager (Acquity™ Ultra-performance LC). A triple-quadrupole mass spectrometry detector (StepWave™ Ultra-performance LC) with electrospray ionization (Zspray™ESI–APCI–ESCi, Ultra-performance LC) and multiple reaction monitoring (MRM) mode was used. MassLynx™ version 4.1 software (Micromass, Manchester, UK) was used to process and manipulate the data.

UPLC separation was achieved using an Acquity UPLCBEH™ C_{18} analytical column (100 × 1.0 mm i.d., 1.7 µm particle size; Waters, Dublin, Ireland). The mobile phase consisted of acetonitrile and 50% water + 50% methanol (55:45, v/v), each with 0.1% formic acid. The mobile phase was delivered at 0.2 ml/min, and 5 µl of each sample solution was injected in partial loop mode. The column and autosampler temperature were maintained at 45and 4°C, respectively.

The electrospray ionization (ESI) source used with MS/MS detection was operated in positive ion mode. The optimized MS parameters included a source temperature of 150°C and a dwell time of 0.025 s. The desolvating gas, cone gas and collision gas flow rates were adjusted at 800, 150 and 0.15 ml/min, respectively. Additionally, the MS analyser was set to the resolutions of low mass (LM) of 2.8 and high mass (HM) of 14.86 for both ion energies 1 and 2. Quantitation was performed using MRM of the transition from the protonated precursor ion $[M + H]^+$ to the product ion at *m/z* 488.03 → 400.92 (DAS), *m/z* 494.11 → 394.03(IMA), *m/z* 530.02 → 289.00 (NIL) and *m/z* 394.29 → 278.19 (ERL).

2.3 | Preparation of calibration standards and quality control samples

DAS, IMA, NIL and ERL IS stock solutions (1 mg/ml) were prepared in methanol. Serial dilutions were used to prepare a standard curve ranging from 25 to 500 ng/ml for DAS and NIL and from 50 to 1000 ng/ml for IMA. For the TKI in rat plasma quantification study, 50 µl of blank plasma was spiked with standard DAS, IMA and NIL solutions, using eight different volumes of the study drugs to cover the desired concentration range. Fifty microlitres of 50 ng/ml ERL (IS) was added to all spiked samples for a total volume of 1 ml.

Quality control samples were prepared by spiking 50 µl volumes of plasma with ERL (IS) and four concentrations of each drug in a linear range as follows: very low (lower limit of quantitation, LLOQ, 25 ng/ml for DAS and NIL and 50 ng/ml for IMA), low (100 ng/ml for DAS and NIL and 200 ng/ml for IMA), medium (250 ng/ml for DAS and NIL and 500 ng/ml for IMA) and high (400 ng/ml for DAS and NIL and 800 ng/ml for IMA).

2.4 | Sample preparation

Plasma samples (blank and spiked) were prepared by solid-phase extraction (SPE). Plasma samples were vortexed for 5 min at 6000 rpm. After centrifugation, the supernatant of each sample was separated, followed by washing of the residue with 0.5 ml of methanol. The clear combined supernatant and washing solutions were purified by pouring onto Strata® C_{18}-E (55 µm, 70 Å; 200 mg/3 ml) cartridges that had been preconditioned with methanol followed by ultrapure water. The retained drugs were then eluted with 0.5 ml of methanol that was evaporated to dryness under nitrogen using a nitrogen evaporator. The obtained residue was then reconstituted in 0.5 ml of acetonitrile, and 5 µl of each sample was injected into the UPLC–MS/MS system for analysis.

2.5 | Study design and animals

All experiments were carried out in accordance with the ethical guidelines for experimental studies with animals as per the Research Ethics committee, King Saud University (Ethics Reference number KSU-SE-19-13). Thirty male Wistar rats weighing 250 ± 30g were provided by the animal house, Women Student-Medical Studies and Sciences Sections, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were housed under standard laboratory animal conditions for a regular 12 h day–night cycle. The environment was maintained at 24–27°C and a relative humidity of 40–60%. The rats were fasted for at least 12 h with free access to water before the day of the experiment. The rats were randomly divided into six groups of five rats each. An appropriate weight of each drug was triturated in

TABLE 1 LC–MS/MS optimized parameters for the determination of the studied drugs

Target compound	Precursor ion $[M + H]^+$	Daughter ion	Cone voltage (V)	Capillary voltage (kV)	Collision energy (eV)	Desolvation temperature (°C)
DAS	488.03	400.92	10	3.8	33	200
IMA	494.11	394.03	10	4.0	30	200
NIL	530.02	289.00	30	4.0	30	200
ERL (IS)	394.29	278.19	25	3.5	30	200

DAS, Dasatinib; IMA, imatinib; NIL, nilotinib; ERL, erlotinib.

aqueous methyl cellulose (0.5% w/v). The drug suspensions (0.25 ml) were administered using a gavage needle throughout the study. Rats in groups I, III, and V were given DAS 25 mg/kg, IMA 25 mg/kg or NIL 25 mg/kg, respectively. Rats in groups II, IV and VI were first given an oral dose of DRE at 500 mg/kg, and 30 min later, the rats were subjected to an oral dose of DAS 25 mg/kg, IMA 25 mg/kg or NIL 25 mg/kg, respectively.

2.6 | Blood collection

A 0.3 ml blood sample from the retro-orbital sinus of each rat was collected into a heparinized tube. The blood samples were collected following the administration of the drugs at 0 (prior to dosing), 0.5, 1, 2, 3, 5, 6 and 24 h. The blood samples were then immediately centrifuged at 4500 rpm for 30 min at 4°C. The plasma was stored at

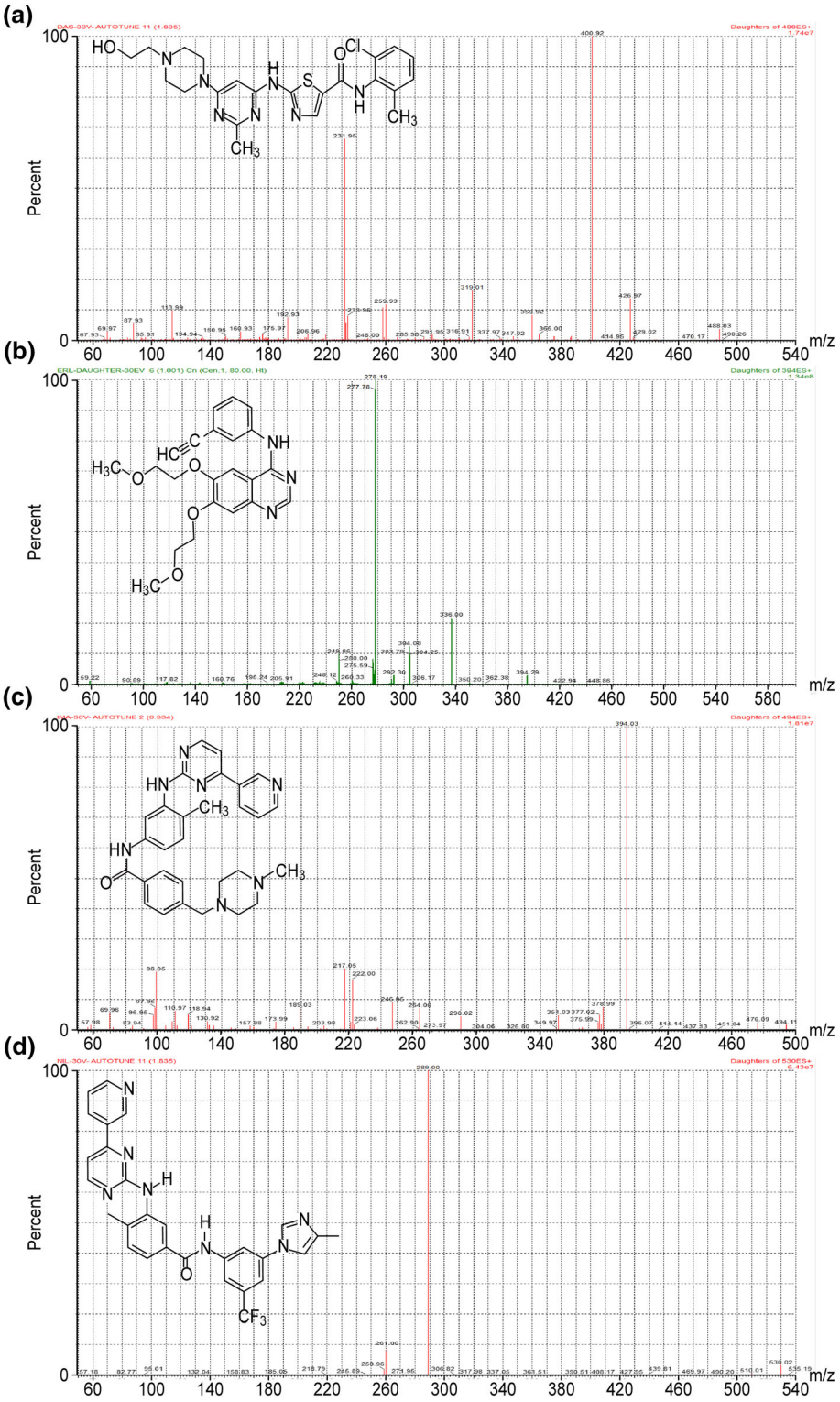

FIGURE 1 Product ion spectra of (a) dasatinib (DAS), (b) erlotinib (ERL), (c) imatinib (IMA) and (d) nilotinib (NIL)

−20°C until analysis. A total of 50 µl of ERL IS (50 ng/ml) was added to a 50 µl volume of each plasma sample. All of the samples were then treated as described in Section 2.4.

2.7 | Calculation of pharmacokinetic parameters

The plasma concentration–time profiles of the three studied TKIs (DAS, IMA, NIL) with and without DRE were processed using the PKSolver 2.0 Add-in to Excel 2010. Noncompartmental analysis was used. Various PK parameters were calculated, including the C_{max}, time required to reach C_{max} (T_{max}), elimination half-life ($t_{1/2}$), area under the curve from time 0 to the last sampling time t (AUC_{0-t}) and from time 0 to infinity ($AUC_{0-\infty}$), mean residence time from time 0 to the last sampling time t (MRT_{0-t}) and from time 0 to infinity ($MRT_{0-\infty}$), clearance (CL) and volume of distribution (V_d).

For each treated group, all of the PK parameters were statistically significant compared with the corresponding values obtained for its respective control; groups II, IV and VI were compared with groups I, III and V, respectively, using Student's t-test with P-values of 0.05.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of UPLC–MS/MS conditions

For maximum specificity and sensitivity, the UPLC–MS/MS analytical method was optimized. For the simultaneous determination of DAS, IMA, NIL and ERL (IS) (100 ng/ml), the different tandem mass spectrometry conditions were optimized, including the intensity and the relative abundance of the precursor ions and the intensity of a particular fragment ion. Positive ESI mode was selected for the analysis of the drugs because it yielded a higher response than negative mode. The studied drugs were detected by monitoring protonated precursor to product ions by MRM mode at m/z 488.03(DAS), m/z 494.11(IMA), m/z 530.02 (NIL) and m/z 394.29 (ERL), while the product ions were at m/z 400.92 (DAS), m/z 394.03 (IMA), m/z 289.00 (NIL) and m/z 278.19 (ERL). Various detection parameters (e.g. source temperature, cone and capillary voltage, flow rate of the desolvation gas and desolvation temperature) were selected to obtain the highest response. The source temperature of 150°C and desolvation gas flow rate of 80 L/h resulted in the highest response of the protonated precursor ions. Additionally, an increase in the relative abundance of the precursor ions was associated with an increase in the cone voltage or capillary voltage until the optimal values were obtained. Furthermore, the collision energy was optimized. The intensity of a particular fragment ion increased with increasing collision gas energy until the optimal values were obtained. The optimized MS/MS conditions of the studied drugs are summarized in Table 1. Figure 1 shows the full-scan product ion spectra of the protonated precursor ions $[M + H]^+$ for DAS, IMA, NIL, and ERL.

For the chromatographic elution of standard mixtures of DAS, IMA, NIL and ERL (IS), various mobile phases composed of mixtures of different ratios of acetonitrile (30–90%), water and formic acid (0.05–0.15%) were evaluated. Experimental trials of different proportions of acetonitrile (30–90%) and water with 0.1% formic acid revealed that, with the exception of ERL, a slight tailing was observed for all ratios of acetonitrile in all peaks. If methanol was incorporated into the water content at different ratios, there was some improvement in the peak shape of all of drugs, except for IMA, which showed a slight decrease in the peak intensity for all of the drugs. Sharp and symmetrical peaks of all drugs were obtained with the acetonitrile fraction of 55% and 45% water–methanol (50:50 v/v). Formic acid was essential to increase the ionization in positive ESI mode, which results in an increase in signal response. The formic acid content in the mobile phase was studied in the range of 0.05–0.15%. It was observed that a decrease in the retention time of all of the drugs was associated with an increase in the formic acid content in the mobile phase until 0.1%, above which there was tailing in the IMA peak. The study revealed that optimum system suitability parameters were achieved when using 0.1% formic acid content in the mobile phase. Final analysis was performed with the mobile phase consisting of acetonitrile and water–methanol (50:50 v/v) and 0.1% formic acid (55:45, v/v) for the entire runtime of 2 min. ERL was selected as the IS because ERL had suitable retention and chromatographic behaviour compared with DAS, IMA and NIL. Final analysis was performed with the mobile phase consisting of acetonitrile and water–methanol (50:50 v/v) and 0.1% formic acid (55:45, v/v). Under these chromatographic conditions, sharp and symmetrical peaks of all of the drugs were obtained (DAS eluted at 0.44 ± 0.02 min, IMA eluted at 0.44 ± 0.02 min, NIL eluted at 0.45 ± 0.03 min and ERL [IS] eluted at 0.51 ± 0.01 min).

3.2 | Sample preparation

Solid-phase extraction was used for sample preparation. A Strata® C_{18}-E (55 µm, 70 Å, 200 mg/3 ml) column was found to be optimum because it provided better and consistent extraction recoveries of the analytes compared with the octyl C_8 (200 mg, 3 ml) using plasma samples spiked with 150 ng/ml DAS or NIL or 400 ng/ml IMA. The obtained clear supernatants after treatment with methanol were

TABLE 2 Extraction efficiency of C_{18} cartridges in the UPLC–MS/MS analysis of standard mixtures of DAS, IMA and NIL with ERL (IS)

	C_{18} SPE		
	Concentration spiked (ng/ml)	Mean recovery (%) ± RSD[a]	E_r (%)[b]
DAS	25	95.33 ± 3.08	−4.67
	250	97.71 ± 1.45	−2.29
	400	96.20 ± 6.25	−3.80
IMA	50	97.97 ± 2.93	−2.03
	500	91.58 ± 4.80	−8.42
	800	100.89 ± 2.19	0.89
NIL	25	96.37 ± 3.13	−3.63
	250	94.27 ± 0.65	−5.73
	400	94.01 ± 2.12	−5.99

[a]Mean recovery (%) ± RSD of six determinations.

[b]Percentage relative error.

further purified using SPE. The cartridges were further evaluated by replicate analysis ($n = 6$) of the plasma samples spiked with DAS, IMA and NIL at the QC levels of 25, 250 and 400 ng/ml DAS and NIL and 50, 500 and 300 ng/ml IMA. C_{18} cartridges provided excellent recovery for DAS (95.33–97.71%), IMA (91.58–100.89%) and NIL (94.01–96.37%) (Table 2).

3.3 | Method validation

The guidelines of the US Food and Drug Administration for bioanalytical methods (US Food and drug Administration, Center for Drug Evaluation and Research, 2001) were employed to validate the UPLC method.

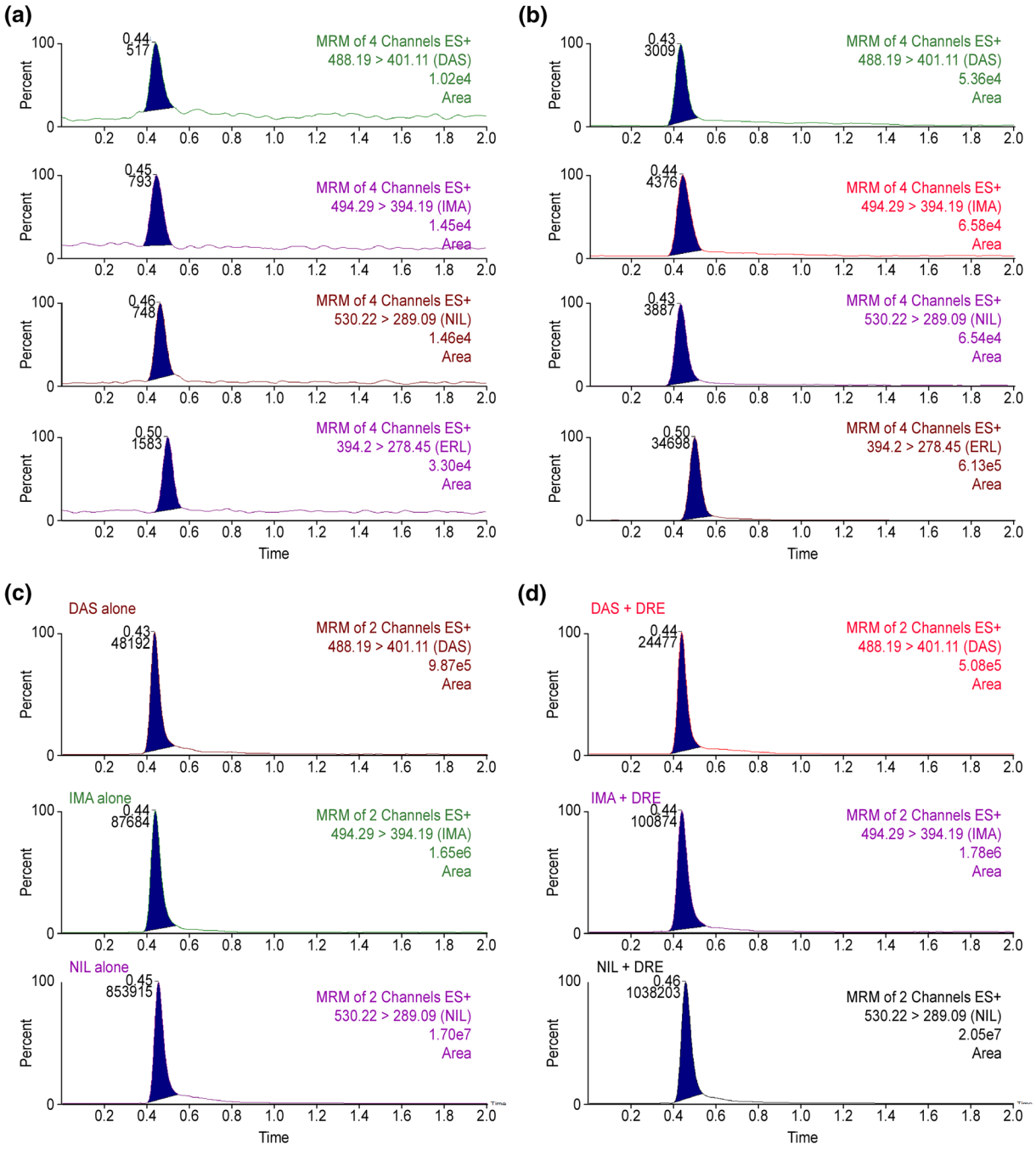

FIGURE 2 Multiple reaction monitoring (MRM) of (a) blank plasma, (b) plasma sample spiked with a standard mixture of DAS, IMA and NIL at their LLOQ level with ERL (IS), (c) plasma sample of treated rats collected 1 h after the oral administration of DAS, IMA and NIL (25 mg/kg) and (d) plasma sample of treated rats collected 1 h after the oral administration of DAS, IMA and NIL (25 mg/kg) combined with dandelion root extract (DRE); 500 mg/ml)

3.3.1 | Specificity

The specificity of the method was evaluated by comparing the chromatograms of plasma extracts taken from six different rats with those spiked with the LLOQ of DAS, IMA and NIL along with ERL (IS; Figure 2). These results demonstrate the specificity of the method. There was no interference with the retention times of the analytes and the IS.

3.3.2 | Linearity

Linearity was assessed by analysing rat plasma samples (50 µl) spiked with eight different concentrations of the three drugs over the concentration range (25–500 ng/mlfor DAS and NIL and 50–1000 ng/mlfor IMA). The peak area ratios of each drug to ERL (IS) were used to construct the matrix-based calibration, and regression equations were derived for DAS, IMA and NIL. The regression and statistical parameters are presented in Table 3. The *r*-value was >0.9996, indicating a high degree of linearity of the proposed method. A low degree of scatter in the experimental data points around the regression line was indicated by the high *F*-values and low values of $S_{y/x}$ (Miller & Miller, 2000).

3.3.3 | Lower limit of detection and lower limit of quantification

The lower limit of detection (LLOD) and LLOQ of DAS, IMA and NIL were calculated based on the analyte concentrations having analytical signals of at least three times or five times that of the blank signals, respectively. Additionally, the signals at the LLOQ should be identifiable and have at least ±20% error and deviation values. Representative MRM chromatograms of the drug-free plasma and the plasma samples spiked with DAS, IMA and NIL at their LLOQ are shown in Figure 2. The LLOQ and LLOD were 25 and 15 ng/ml for DAS, 50 and 25 ng/ml for IMA, and 25 and 15 ng/ml for NIL, respectively (Table 3).

3.3.4 | Recovery and matrix effect

The extraction recovery of DAS, IMA and NIL from plasma samples was determined at the four QC levels previously mentioned in the Experimental section. The recovery was calculated by comparing the peak area obtained from the spiked plasma samples with those

TABLE 4 Evaluation of the intraday and interday accuracy and precision for the determination of DAS, IMA and NIL in rat plasma by the proposed UPLC–MS/MS method

		Intraday (*n* = 6)		Interday (*n* = 18)	
	Concentration added (ng/ml)	Mean recovery (%) ± RSD	E_r (%)[a]	Mean recovery (%) ± RSD	E_r (%)[a]
DAS	25	97.89 ± 1.43	−2.11	99.62 ± 0.20	−0.38
	100	97.11 ± 3.08	−2.89	100.50 ± 2.86	0.50
	250	98.16 ± 1.57	−1.84	96.15 ± 2.62	−3.85
	400	99.40 ± 4.69	−0.60	100.10± 6.87	0.10
IMA	50	95.18 ± 4.39	−4.82	96.08 ± 3.70	−3.92
	200	101.32 ± 2.89	1.32	98.85 ± 1.77	−1.15
	500	98.93 ± 1.53	−1.07	95.02 ± 2.30	−4.98
	800	98.38 ± 2.61	−1.62	101.38 ± 3.91	1.38
NIL	25	98.50 ± 1.25	−1.50	97.25 ± 1.49	−2.75
	100	100.49 ± 1.79	0.49	99.71 ± 0.86	−0.29
	250	95.95 ± 3.02	−4.05	96.10± 4.34	−3.90
	400	99.45 ± 3.77	−0.55	99.44 ± 3.27	−0.56

[a]Percentage relative error.

TABLE 3 Regression and statistical parameters for the determination of DAS, IMA and NIL rat plasma by the proposed UPLC–MS/MS method

Parameter	DAS	IMA	NIL
Linearity range (ng/ml)	25–500	50–1000	25–500
LLOQ[a] (ng/ml)	25	50	25
LLOD[b] (ng/ml)	15	25	15
Intercept (*a*)	0.0088	−0.0020	0.0367
Slope (*b*)	0.0455	0.0073	0.0843
Correlation coefficient (*r*)	0.9997	0.9998	0.9998
S_a[b]	0.00781	0.00285	0.01170
S_b[d]	0.00039	0.00005	0.00060
$S_{y/x}$[c]	0.01202	0.00452	0.01801
F[d]	13,360.74	20,567.42	20,366.22
Significance, *F*	3.3595×10^{-8}	3.127×10^{-10}	1.4461×10^{-8}

[a]LLOQ, Lower limit of quantification. [b]LLOD, lower limit of detection.

[b]S_a, Standard deviation of intercept. [d] S_b, standard deviation of slope.

[c]$S_{y/x}$, Standard deviation of residuals.

[d] *F*, Variance ratio, equals the mean ofsquares owing to regression divided by the mean of squares about regression (owing to residuals).

obtained from standard drug solutions of the same nominal concentration (n = 6).

Recovery values at the four QC concentration levels were ≥94.43% (DAS), 92.98% (IMA) and 94.71% (NIL). In addition, ERL (IS) provided an acceptable recovery of 97.52%. The recovery results summarized in Table 2 revealed that the proposed method for sample treatment provided a high degree of extraction efficiency for DAS, IMA and NIL from the plasma samples.

The same procedure as per evaluation of extraction recovery was used for the "matrix effect", with the exception that the processed standard samples (without plasma) were used as a reference. We did not detect any apparent matrix effect in the determination of DAS, IMA and NIL in plasma samples. The matrix effect at the different concentrations for DAS, NIL and IMA was ≤5.57% for DAS, 7.02% for IMA, and 5.29% for NIL (Table 4). Additionally, the matrix effect for ERL (IS) at the specified concentration level was 6.78%.

TABLE 5 Evaluation of the matrix effect for the determination of DAS, IMA and NIL in rat plasma by the proposed UPLC–MS/MS method

	Concentration added (ng/ml)	Mean recovery (%) ± RSD[a]	E_r (%)[b]
DAS	25	98.24 ± 2.08	−1.76
	100	97.29 ± 2.71	−2.71
	250	94.43 ± 3.69	−5.57
	400	94.84 ± 6.84	−5.16
IMA	50	95.32 ± 5.73	−4.68
	200	92.98 ± 1.11	−7.02
	500	95.26 ± 3.39	−4.74
	800	96.69 ± 6.56	−3.31
NIL	25	98.07 ± 2.54	−1.93
	100	94.71 ± 4.92	−5.29
	250	96.50 ± 1.76	−3.50
	400	100.21 ± 1.90	0.21

[a]Mean recovery (%) ± RSD of six determinations.

[b]Percentage relative error.

3.3.5 | Precision and accuracy

Precision and accuracy were evaluated by analysing the four QC samples. The samples were treated and then analysed six times on the same day for the intraday levels or on three consecutive days for the interday levels. The actual drug concentration in each sample was calculated from the corresponding regression equations. Accuracy in terms of percentage relative error (E_r %) and precision in terms of percent relative standard deviation (RSD) were assessed. The calculated relative errors were in the ranges of −4.82–1.32 and −4.98–1.38% for intraday and interday errors, respectively, while the calculated RSD values were in the ranges of 1.25–4.69 and 0.20–6.87% for intraday and interday deviations, respectively. All of the results are presented in Table 5.

3.3.6 | Dilution integrity

Dilution integrity was assessed by using plasma samples spiked with high concentrations of DAS, IMA and NIL (1000 ng/ml for DAS and NIL and 2000 ng/ml for IMA) after being diluted with blank rat plasma (1:2 and 1:5). In each case, the found concentrations were related to the nominal values, and the recovery (±RSD) was calculated. The calculated recoveries (±RSD) obtained for DAS, IMA and NIL following

TABLE 7 Evaluation of the dilution integrity of DAS, IMA and NIL in rat plasma

Concentration spiked (ng/ml)	Dilution fold	Mean recovery (%) ± RSD[a]	E_r (%)[b]
DAS	1:2	96.63 ± 3.33	−3.37
	1:5	97.53 ± 1.94	−2.47
IMA	1:2	100.65 ± 2.65	0.65
	1:5	98.89 ± 3.31	−1.11
NIL	1:2	97.69 ± 1.61	−2.31
	1:5	98.58 ± 1.12	−1.42

[a]Mean recovery (%) ± RSD of six determinations.

[b]Percentage relative error.

TABLE 6 Evaluation of the stability of DAS, IMA and NIL in rat plasma

Stability	Concentration added (ng/ml)	Mean recovery (%) ±RSD[a]		
		DAS	IMA	NIL
Auto-sampler stability (10°C, 56 h)	50	98.45 ± 3.35	99.05 ± 0.46	95.92 ± 7.35
	200	100.09 ± 0.69	98.03 ± 0.91	98.74 ± 4.66
Short-term stability (25°C, 6 h)	50	97.23 ± 2.28	96.78 ± 4.60	99.04 ± 2.24
	200	99.06 ± 1.04	95.01 ± 7.83	94.38 ± 1.26
Long-term stability (−30°C, 30 days)	50	92.98 ± 2.01	94.65 ± 2.72	97.84 ± 4.60
	200	94.98 ± 2.67	93.64 ± 3.58	90.42 ± 3.08
Freeze–thaw stability (−30°C, three cycles)	50	99.74 ± 0.41	97.77 ± 1.42	99.35 ± 0.90
	200	101.99 ± 4.17	98.69 ± 0.40	97.73 ± 2.26
Refrigerator (4°C, 3 months)	50	97.46 ± 2.94	96.58 ± 1.06	98.99 ± 1.44
	200	97.93 ± 3.83	98.91 ± 2.47	98.55 ± 1.34

[a]Mean recovery (%) ± RSD of six determinations.

the dilution of the plasma samples are shown in Table 6. The results did not exceed the ±15% acceptance limits, indicating the integrity of the analytes.

3.3.7 | Stability studies

The stability of DAS, IMA and NIL in rat plasma was assessed by analysing QC samples at two concentration levels, low (50 ng/ml) and high (200 ng/ml) (n = 6). Plasma samples were subjected to different conditions to assess autosampler stability, short-term (bench-top) stability, long-term stability and stability to freeze–thaw cycles. In each case, the recoveries were calculated by relating the found concentration of the drugs to that of the nominal concentration. To evaluate the post-preparation stability, the extracted samples were left in the autosampler at 10°C for 56 h prior to injection. Short-term and long-term stability were assessed using samples left at room temperature (25°C) for 6 h or at −30°C for 30 days, respectively. Finally, the stability of the drugs following three freeze–thaw cycles (freezing at approximately −30°C and then thawing at room temperature) was evaluated. The drugs were found to be stable (the calculated recoveries did not exceed ±15%) at 4°C for 3 months and at room temperature for 6 h. The results of the stability studies are summarized in Table 7.

3.4 | Application to pharmacokinetic studies

Figure 3 shows the mean drug plasma concentration time profiles reported for each combination of DAS, IMA, or NIL with DRE after oral administration compared with the single administration. Different PK parameters were calculated for the drugs, as shown in Table 8.

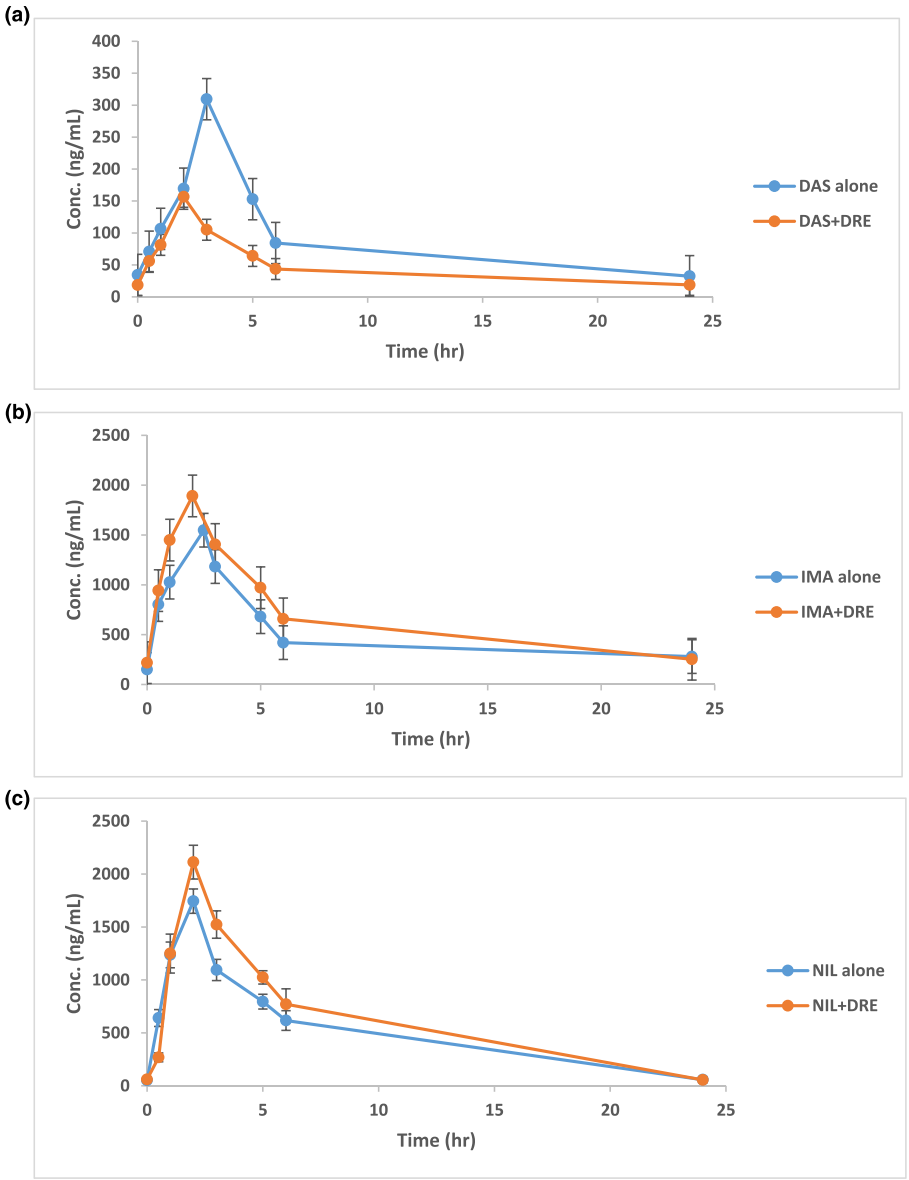

FIGURE 3 Plasma concentration–time profile of DAS, IMA and NIL in rats after an oral administration of a combination of DAS, IMA and NIL (25 mg/kg) along with DRE. Suitable dilutions of prepared plasma samples were made before analysis

TABLE 8 Main pharmacokinetic parameters (mean ± SD) after oral administration of DAS (25 mg/kg), IMA (25 mg/kg) and NIL (25 mg/kg), with or without DRE (500 mg/kg) to rats (n = 5)

	Group I DAS	Group II DAS + DRE	Group III IMA	Group IV IMA + DRE	Group V NIL	Group VI NIL + DRE
C_{max} (ng/ml)	309.79 ± 24.50	145.33 ± 33.52*	1552.23 ± 289.34	1893.53 ± 370.94	1741.33 ± 195.97	2120.93 ± 714.79
T_{max} (h)	3.14 ± 0.86	2.12 ± 0.93	2.50 ± 0.77	2.12 ± 0.51	2.15 ± 0.96	1.97 ± 0.94
$t_{1/2}$ (h)	8.07 ± 0.98	9.47 ± 0.96	13.75 ± 2.22	9.32 ± 0.98	6.06 ± 084	4.91 ± 0.29
AUC_{0-t} (ng h/ml)	2092.28 ± 327.17	1088.02 ± 80.85*	12,027.32 ± 1102.28	15,310.79 ± 4076.71	12,470.24 ± 617.91	14,883.2 ± 3085.65
$AUC_{0-\infty}$ (ng h/ml)	2481.82 ± 165.31	1353.08 ± 162.51*	17,395.64 ± 3591.4	19,105.66 ± 5256.61	13,310.20 ± 727.66	14,868.12 ± 4309.35
MRT_{0-t} (h)	7.80 ± 0.91	7.49 ± 0.78	8.52 ± 0.93	7.80 ± 0.89	6.07 ± 094	5.87 ± 0.81
$MRT_{0-\infty}$ (h)	12.16 ± 1.33	12.88 ± 1.16	20.40 ± 1.12	13.17 ± 2.05	7.08 ± 0.78	6.66 ± 0.68
CL (ml/h/kg)	0.0103 ± 0.0012	0.0180 ± 0.0073*	0.0014 ± 0.0004	0.0014 ± 0.0003	0.0019 ± 0.0001	0.0016 ± 0.0009
V_d (ml/kg)	0.1052 ± 0.0088	0.2638 ± 0.0625	0.0284 ± 0.0076	0.0204 ± 0.0088	0.0146 ± 0.0004	0.0107 ± 0.0030

*Indicates significant difference at $P < 0.05$.

C_{max}, Peak concentration; T_{max}, time required to reach C_{max}; $t_{1/2}$, elimination half-life; AUC, area under the curve; MRT, mean residence time; CL, clearance; V_d, volume of distribution.

The results indicated that, compared with single DAS administration, the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of DAS were significantly lowered by almost 50%. In addition, the CL of DAS nearly doubled following DRE administration. Similar to other TKIs, DAS is a substrate for both CYP3A4 and P-gp, and DRE has an inhibitory effect on CYP3A4 and P-gp (Maher et al., 2016; Maher et al., 2016). DAS bioavailability decreased with the co-administration of DRE. This unexpected finding was previously reported with green tea (GT) administration with other TKIs, namely, erlotinib and lapatinib (Maher et al., 2017a). This result could be attributed to a reduction in the fraction of the absorbed drug in the stomach as a result of DRE co-administration. However, no significant changes were observed in any of the calculated PK parameters of either IMA or NIL following DRE administration compared with their single oral administration. This study provides evidence for a possible PK interaction between DRE and some TKIs. Further studies should be performed to give a clear explanation of the mechanism of the interaction between TKIs and DRE. In addition, the study should be extended to clinical observations because the PK profiles in rats may be different from those in humans.

4 | CONCLUSION

In summary, future research should focus on the role and benefits of TDM in TKI therapy because TKIs are now being considered as chronic medications taken in an outpatient setting and because of the large interpatient variability in PK of these TKIs. The findings of the present study suggest that DRE alters the pharmacokinetics of TKIs in rats. Further studies are needed to clarify the *in vivo* effects of DRE on the PK of TKIs.

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DECLARATIONS OF INTEREST

The authors declare that there are no conflicts of interest.

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