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Bioanalytical HPLC method with fluorescence detector for determination of Entresto™ when co-administered with ibuprofen and fexofenadine: a pharmacokinetic study†

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Entresto™ (LCZ696) has been approved globally for heart failure management. However, its lifelong use alongside over-the-counter (OTC) drugs like ibuprofen (IBU) and fexofenadine (FEX) necessitates an in-depth investigation of potential pharmacokinetic interactions, as they share the same metabolic and elimination pathways. This study aimed to develop a bioanalytical HPLC method with a fluorescence detector (FLD) to quantify LCZ696 analytes (valsartan, VAL; sacubitril, SAC; and sacubitril active metabolite, LBQ657) in rat plasma. Additionally, an *in vivo* study was performed to investigate the pharmacokinetic interactions of LCZ696 with IBU and FEX. Utilizing HPLC with a gradient-mode mobile phase of acetonitrile and 0.025 M phosphate buffer (pH 3), the study demonstrated a significant increase in the bioavailability of LCZ696 analytes (VAL and LBQ657) when co-administered with IBU (C_{max} 0.23 ± 0.07 and 0.53 ± 0.21 μg mL^{-1}, respectively) compared to the control (0.17 ± 0.03 and 0.33 ± 0.14 μg mL^{-1}). A more significant increase in C_{max} was noticed with FEX (0.38 ± 0.01 and 0.77 ± 0.18 μg mL^{-1}, respectively). Moreover, a decrease in the clearance (Cl/*F*) of VAL and LBQ657 was observed (18.05 ± 1.94 and 12.42 ± 2.97 L h^{-1} kg, respectively) with a more pronounced effect in the case of FEX (30.87 ± 4.29 and 33.14 ± 9.57 L h^{-1} kg, respectively) compared to the control (49.99 ± 7.31 and 51.19 ± 9.12 L h^{-1} kg, respectively). In conclusion, our study underscores the importance of cautious administration and appropriate dose spacing of IBU and FEX in patients treated with LCZ696 to prevent elevated serum concentrations and potential toxicity. The novelty of this work lies in its dual contribution: developing a highly sensitive HPLC-FLD method and comprehensively elucidating significant pharmacokinetic interactions between LCZ696 and common OTC drugs.

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1 Introduction

Entresto™ (LCZ696), shown in Fig. S1,† was the first approved FDA supramolecular complex (1 : 1 molar ratio) of valsartan (VAL) and sacubitril (SAC) for management of heart failure patients in many countries.[1] After being taken orally, LCZ696 is absorbed relatively rapidly in terms of the onset and the rate in all species. Then, LCZ696 dissociates into VAL and the prodrug SAC which is quickly transformed by non-specific esterases into the active metabolite (LBQ657).[2,3] Therefore, Entresto™ possesses a dual-effect regarding the suppression of the angiotensin II receptor and neprilysin respectively. Subsequently, it has valuable effects on endothelial dysfunction.[4,5] It is widely known that enzymes and drug transporters have a crucial role in the drug pharmacokinetics processes. Transporter proteins have a special gatekeeper role in regulating drug access to enzymes that metabolize drugs and excretory pathways since they are localized in the organs in charge of drug biotransformation and excretion.[6] The multidrug resistance-associated protein 2 (MRP2), and especially the P-glycoprotein (P-gp) transporter, have demonstrated their ability to transport LBQ657 (active metabolite) and VAL which aids in intestinal elimination of both compounds.[7] On the other hand, LCZ696 analytes are less likely to contribute to CYP-mediated metabolism and are not extensively metabolized in the liver.[3,8,9]

Fexofenadine (FEX), Fig. S1,† is a specific antagonist of the histamine H1 receptor, used as an antihistaminic OTC drug for curing allergy symptoms. FEX is a substrate of P-gp efflux

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transporters and some influx transporters including organic anion transporting polypeptide (OATP) and MRP2.[10–12] Additionally, ibuprofen (IBU), Fig. S1,† is the most frequently prescribed non-steroidal anti-inflammatory drugs (NSAID) as an antipyretic and analgesic drug.[13] IBU is extensively metabolized in the liver by CYP2C9 and is excreted *via* the renal route as metabolites or their conjugates.[14] Inhibition of enzymes or transporter proteins occurs when two drugs compete for the same receptor site which reduces the metabolism or transport of the competing drug. This may result in higher serum levels of the unmetabolized entity and increasing the risk of toxicity.[15,16] Consequently, co-administration of Entresto™ with either FEX or IBU medications is critical and possible pharmacokinetic (PK) interaction may be expected.

The drug interaction potential between LCZ696 and either IBU or FEX will be evaluated due to their shared metabolic and elimination pathways. FEX is a substrate of P-gp efflux transporters, which facilitate the intestinal elimination of LBQ657 (the active metabolite) and VAL.[7,10,11] On the other hand, IBU inhibits first-pass metabolism of LCZ696 analytes.[14] These interactions can significantly alter the pharmacokinetics of LCZ696, potentially leading different effects on its pharmacological outcomes. It is worth mentioning that there are no existing bioanalytical reports on the simultaneous analysis of FEX or IBU with LCZ696 in plasma. This study is the first to fill this gap by applying a pharmacokinetic study to investigate any possible drug–drug interactions (DDIs) between them, ensuring the safety and efficacy of LCZ696 in patients.

However, many reports for potential DDIs of Entresto™ are shown with statins;[17,18] sildenafil,[19] furosemide,[20] digoxin and warfarin,[21] hydrochlorothiazide, amlodipine and carvedilol,[22] omeprazole, metformin, and ethinyl estradiol[23] and nebivolol, chlorthalidone and esomeprazole.[24] Moreover, a number of prior analytical methodology have been reported for the analysis of SAC and VAL in plasma using LC with UV detector,[25] fluorescence detector[26] and LC-MS/MS.[27] However, these reports[25,26] lack the analysis of the active metabolite of SAC, not validated according to the FDA bioanalytical guidelines and the first report [25] was done in spiked plasma only and showed higher linearity range than the proposed method. Furthermore, few reports analyzed VAL and SAC with the active metabolite of SAC (LBQ657) using LC-MS/MS[28] and UPLC MS/MS.[29]

The current work aims to develop a validated, sensitive, and accurate HPLC coupled with a fluorescence detector (FLD) method that can detect, and quantify simultaneously FEX, IBU and LCZ696 analytes (VAL, SAC, and LBQ657) in rat plasma. Furthermore, the presented bioanalytical method proves its applicability to investigate the possible DDIs and PK profiles of LCZ696 analytes (VAL, SAC, and LBQ657) in the presence of selected OTC drugs, either FEX or IBU. The proposed study adds to the scientific community that lacks recommendations about the cited drugs' safety when co-administered. Furthermore, the study evaluated the PK drug interaction potential in plasma. This is a crucial issue for heart failure patients' dose control, drug therapy effectiveness, and drug safety monitoring.

2 Experimental

2.1. Instrumentation

HPLC (Agilent Technologies, USA) attached to multiple wavelength DAD (Agilent 1200 series) and FLD (Agilent 1260 series) for programmable single excitation and emission wavelength. It was also equipped with quaternary pump, auto-injector and vacuum degasser. Christ rotational vacuum concentrator (RVC 2-18 CD plus, Germany) was used for sample gentle drying.

2.2. Materials and reagents

VAL (99%) was a kind gift from Medizen pharmaceutical industries company, Egypt. SAC (99.5%), Sacubitilat (LBQ657, 99%) and biphenyl methyl pyrrolidinone as an internal standard (IS) were kindly supplied by Abblis chemical company, China. FEX and IBU (98.5) were kindly supplied by European Egyptian Pharmaceutical Industries, Egypt.

HPLC grade acetonitrile (C_2H_3N), methanol (CH_3OH), diethyl ether ($C_4H_{10}O$) and ethyl acetate ($C_4H_8O_2$) (Gliwice, ul. Sowinskiego 11, Poland), analytical grade of orthophosphoric acid (H_3PO_4), hydrochloric acid (HCl) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) (EL-Nasr Chemical Co. Egypt) and deionized water were used.

2.3. Chromatographic conditions

A gradient mobile phase mode consists of HPLC grade acetonitrile and 0.025 M phosphate buffer adjusted to pH 3 with orthophosphoric acid were utilized (Table S1†). It was filtered through 0.45 μm membrane filter and degassed. The injection volume was 20 μL. The FLD was tuned at $\lambda_{ex}/\lambda_{em}$ (nm nm^{-1}): 0 min at 232/310 for FEX; 5.5 min at 250/340 for LCZ696 analytes, then 8.5 min at 232/310 for IBU.

2.4. Preparation of stock and diluted stock solutions

Stock solutions containing 1000 μg mL^{-1} of the five analytes and IS prepared in HPLC grade methanol. These stocks were kept in a refrigerator. Then different diluted working standard solutions for all the analytes were prepared by diluting using HPLC grade methanol from the abovementioned stock solutions. These solutions were employed in the construction of the calibration and quality control (QC) samples to be analyzed using FLD.

2.5. Preparation of calibration and quality control samples

The calibration standards and QC samples were prepared by spiking 100 μL of drug free rat plasma with various volumes of the working standard solutions of the mentioned drugs to finally obtain their concentrations in Table 1. For the IS, the final concentration was 1 μg mL^{-1}. Protein precipitation and extraction of the drugs with 50 μL hydrochloric acid (0.1 M) and 1000 μL acetonitrile (ACN) were done. High speed vortex was used to mix the tubes for two minutes then centrifugation was done for 10 minutes at 5000 g. The organic layer was delicately removed and evaporated until dry using vacuum concentrator. To achieve final concentrations inside the defined range shown

Table 1 Regression and statistical parameters for the determination of LCZ696 analytes, FEX and IBU in spiked rat plasma samples using the proposed HPLC-FLD method

	FEX	LBQ657	VAL	SAC	IBU
Linearity range[a]	2–30	0.02–10	0.025–15	0.02–10	2–30
a	0.05	0.01	−0.01	0.02	0.01
S_a[b]	0.003	0.01	0.01	0.02	0.001
b	0.03	0.51	0.25	0.45	0.01
S_b[c]	0.0002	0.006	0.002	0.004	0.0001
$S_{y/x}$[d]	0.005	0.027	0.02	0.04	0.002
r	0.9994	0.9991	0.9997	0.9995	0.9996
F[e]	8514	7496	21 433	11 621	11 362
Significance F	8.27×10^{-8}	1.60×10^{-10}	6.85×10^{-12}	1.30×10^{-9}	4.60×10^{-8}

[a] The linearity ranges in μg mL^{-1}. [b] Standard deviation of the intercept. [c] Standard deviation of the slope. [d] Standard deviation of the residuals. [e] Variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals).

in Table 1, the residues were reconstituted in 500 μL mobile phase (50% v/v of ACN and phosphate buffer). Single set of standards and QC samples were analyzed using the indicated methodology on each working day.

The concentrations for calibration standards were: 2, 2.5, 5, 10, 15, 20 and 30 μg mL^{-1} for both FEX and IBU, 0.02, 0.05, 0.2, 0.5, 5, 8 and 10 μg mL^{-1} for LBQ657 and SAC, 0.025, 0.06, 0.2, 0.5, 5, 10 and 15 μg mL^{-1} for VAL and 1 μg mL^{-1} IS. The four QC samples were prepared at lower limit of quantitation (LLOQ), low quality control (LQC), medium quality control (MQC) and high quality control (HQC) for each analyte as following: 2, 5, 10, 20 μg mL^{-1} for both FEX and IBU, 0.02, 0.06, 5, 8 μg mL^{-1} for both LBQ657 and SAC, and 0.025, 0.06, 5, 10 μg mL^{-1} for VAL.

2.6. Pharmacokinetic study of the investigated drugs in rat plasma both in presence and absence of FEX and IBU

The main aim of the current investigation is to ascertain the influence of some selected over the counter medications (FEX and IBU) on the PK features of LCZ696 analytes (VAL, SAC and LBQ657) in Wistar male rats. After the ethical committee's consent (Faculty of Pharmacy, Alexandria University, Egypt, ethical approval number AU 062024193214), a pharmacokinetic study was performed on fasting, healthy male Wistar rats (weighing between 250 g and 300 g). Three groups of the rats were randomly selected (each group contains 6 rats) and orally administrated 10 mg kg^{-1} by an oral gavage the following drugs: LCZ696, LCZ696 with IBU and LCZ696 with FEX. From the retro–orbital plexus, blood samples were collected at different intervals: 0, 0.1, 0.25, 0.5, 1, 2, 4 and 6 h. These samples were gathered and placed in polypropylene tubes with K3 EDTA solution to prevent clotting. Plasma was isolated after centrifuging the blood at 5000 g for 10 minutes. The samples were frozen at −20 °C and thawed at room temperature on the day of the analysis, and then vortexed for two minutes. The samples were examined utilizing the same method outlined in Section 2.5.

2.7. Statistical analysis

The PK parameters were calculated using the concentration data sets obtained for VAL and LBQ657 in both the presence and absence of FEX and IBU. The best fit values for the area under the concentration–time curve (AUC) where found by fitting the plasma concentration *vs.* time data into the non-compartmental extravascular model using PK Solver software add in program in Microsoft Excel.[30] All collected data in absence and presence of the two drugs (FEX and IBU) were calculated as the mean ± standard deviation (SD) and were estimated for statistical significance at $p < 0.05$ by one-way analysis of variance (ANOVA), followed by Tukey's post hoc analysis (Prism 8.0.1; GraphPad, San Diego, CA, USA).

3 Results and discussion

3.1. Optimization of the analytical method

3.1.1. Chromatographic procedure. Various organic modifiers have been investigated using Zorbax Eclipse plus-C18. It was noticed that all the examined drugs' retention times were significantly influenced by the type of organic modifier. Acetonitrile was the best option since it permitted analytes elution at reasonable time with good resolution. Conversely, methanol significantly slowed down the elusion and strongly retained the studied analytes.

Gradient elution was more effective than isocratic elution in separating the five analytes from one another and from plasma interferences that eluted early. This assisted in reducing the matrix interference.

The pH selection is a crucial step and should be away from the pK_a values of the studied analytes. The pKas values are 8.76 (tertiary nitrogen) and 4.28 (carboxylic acid) for FEX, 3.9 (carboxylic group), and 4.7 (tetrazole-NH group) for VAL, 4.6 for SAC and 5.2 for IBU. As a result, phosphate buffer at pH values (3 and 6.5) with acetonitrile at a gradient mode was tried. At pH 6.5, broadening of VAL peak with low number of theoretical plates ($N = 1239$) and coelution of SAC and IBU were obtained. Therefore, using pH 3 for the current study is the best option and this was consistent with the authors' earlier LCZ696 analytical reports.[31,32]

Using various phosphate buffer ionic strength values (0.025, 0.05 and 0.1 M) did not significantly alter the retention time or peak symmetry of the drugs under study. Therefore, 0.025 M was chosen for our current investigation.

In order to maintain good accuracy, precision as well as robustness of the bioanalytical procedure, an internal standard (IS) was used. Several IS were injected into HPLC and biphenyl methyl pyrrolidinone was chosen. The selected IS eluted between the cited drugs' peaks, well separated from them and it showed reasonable response upon injected under the selected chromatographic conditions. Moreover, it was extracted with good recovery using the adjusted extraction procedure.

With the intention of quantifying the six studied compounds with high selectivity and sensitivity, FLD detector was set at $\lambda_{ex}/\lambda_{em}$ (nm nm^{-1}): 0 min at 232/310 for FEX; 5.5 min at 250/340 for LCZ696 analytes, then 8.5 min at 232/310 for IBU.

Under the optimized chromatographic condition, the cited analytes with the IS where eluted within reasonable retention time with good system suitability parameters with no interference from endogenous plasma components (Fig. 1).

3.1.2. Sample extraction procedure. In the initial preliminary trials, liquid–liquid extraction was evaluated for determining the best extraction solvent and setting up the best extraction conditions by utilizing several solvents such as ethyl acetate and diethyl ether (Fig. S2†). The studied analytes showed poor recovery. Then a protein-precipitation method was carried out using acetonitrile to disrupt the protein–bound drug complex and extract the analytes. Though, the outcomes using acetonitrile in protein precipitation was more promising than liquid–liquid extraction but still did not yield the best extraction recovery (48–66%). The ionization state of the compound can significantly change its extraction characteristics, making pH modulation important. Since the investigated analytes are acidic in nature, reducing the pH of the sample to below the compounds' p*K*a will make the analytes unionized, boosting their extraction efficiency. As a result, an acidic condition was performed, which helped to increase the extraction recoveries. Acetonitrile was supplemented with acetic acid, hydrochloric acid, and formic acid (0.1 M). Acidification improved the extraction efficiency (>88%), but no difference was observed between the different types of acids and consequently, 0.1 M hydrochloric acid was chosen to continue the study. Different

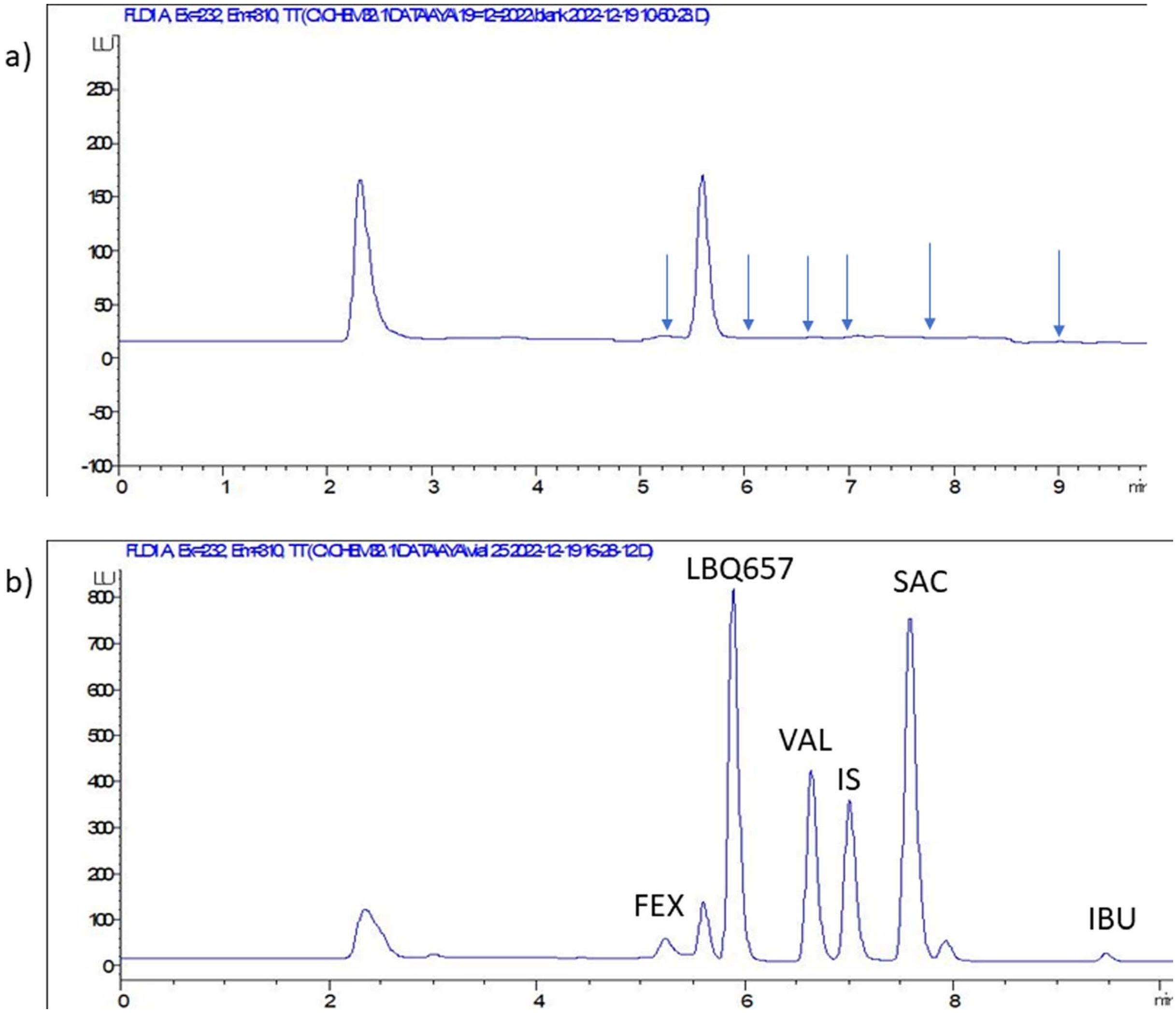

Fig. 1 HPLC chromatograms of (a) blank plasma, (b) blank plasma spiked with 5 μg mL^{-1} of FEX, LBQ657, VAL, SAC and IBU and IS at 1 μg mL^{-1} using FLD with programmed wavelength (arrows in the blank chromatogram indicate no interference from endogenous plasma components at the same sites of the analytes peaks).

volumes of acetonitrile (0.5–2 mL) were tried and 1 mL showed good recovery for all the analytes. Hence, the best outcomes were attained when 50 μL of 0.1 M hydrochloric acid and 1 mL of acetonitrile as an extraction solvent were added to plasma samples spiked with analytes and IS solutions.

3.2. Validation of the proposed methods

The FDA's 2018 Industry Guidance on Bioanalytical Method Validation will be used to validate the suggested analytical methods.[33–35]

3.2.1. Linearity. Calibration curves for the investigated analytes were performed in spiked rat plasma samples covering the complete range, including LLOQ. The calibration curves were linear over the concentration ranges 2–30 μg mL^{-1}, 0.02–10 μg mL^{-1}, 0.025–15 μg mL^{-1}, 0.02–10 μg mL^{-1} and 2–30 μg mL^{-1} for FEX, LBQ657, VAL, SAC and IBU respectively. Table 1 illustrates the statistical parameters and performance data. Higher than 0.9991 correlation coefficient values imply good linearity and high *F* values serve as additional confirmation of the good linearity (higher than 7496). LLOQ samples demonstrated appropriate sensitivity by showing accuracy between 94.32 and 102.85%, besides RSD% was less than 8.14% for the six LLOQs.

3.2.2. Accuracy and precision. QC samples ($n = 6$) at four levels (LLOQ, LQC, MQC and HQC) were spiked with different concentrations of the studied drugs (including its LLOQ) and analyzed. Table S2† summarizes the accuracy and both the intra and inter-day precision results. The intra-day precision (RSD%) at four levels does not exceed 8.14%, while the inter-day precision does not exceed 6.21%.

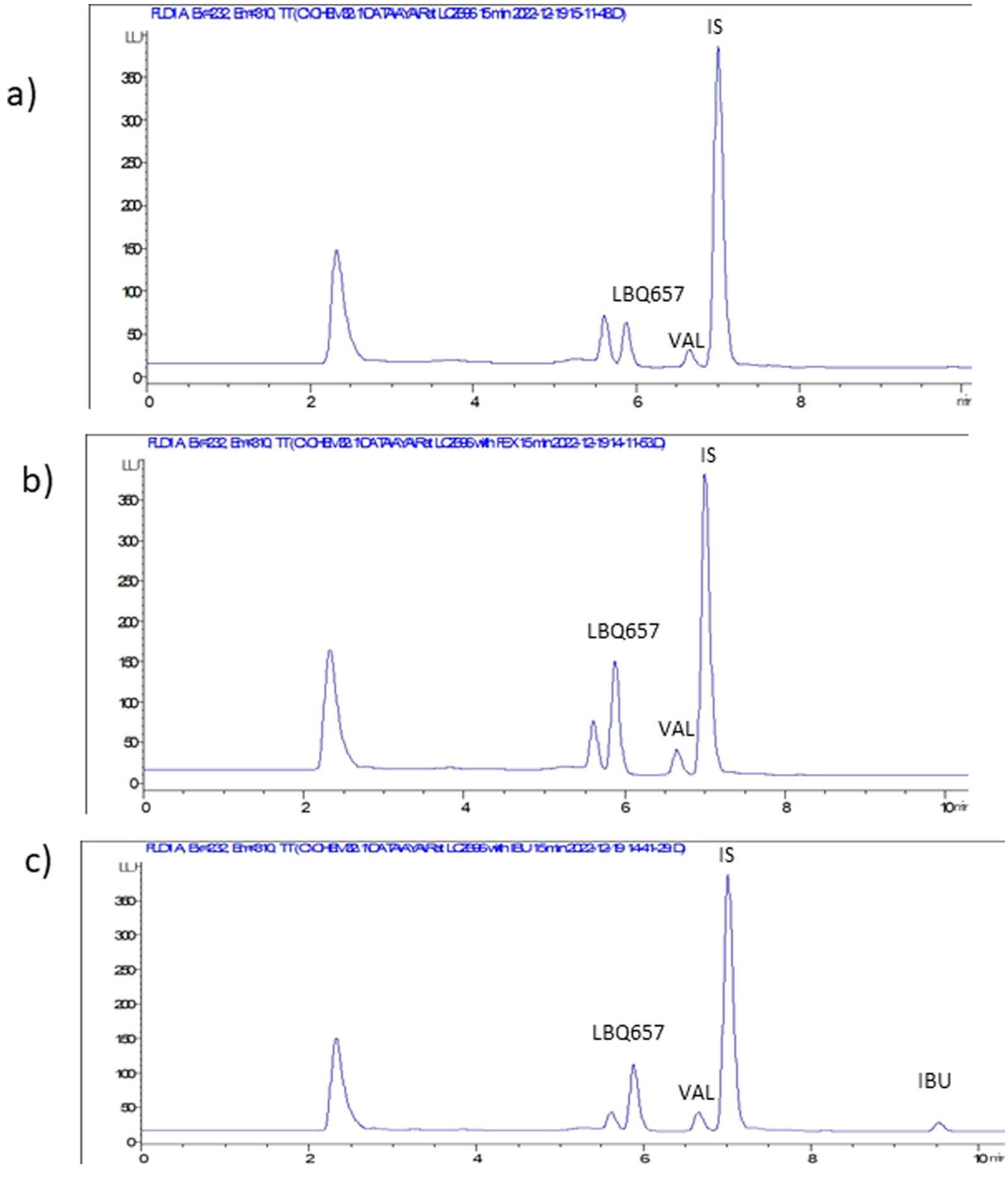

Fig. 2 HPLC chromatograms of (a) real rat plasma sample obtained after 15 min of oral administration of LCZ696, (b) real rat plasma sample obtained after 15 min of oral administration of LCZ696 when co administered with FEX, and (c) real rat plasma sample obtained after 15 min of oral administration of LCZ696 when co-administered with IBU.

3.2.3. Matrix effect and selectivity. To verify the method's selectivity, six blank rat plasma samples were prepared and analyzed using the same protein precipitation method to ensure that endogenous components did not affect the analytes peaks (Fig. 1). Additionally, the purity of the FEX, LBQ657, VAL, SAC and IBU peaks were confirmed by the overlaying of the spectra that were extracted by DAD (Fig. S3†). Peak purity test findings showed that there was no interference from endogenous plasma components or drug metabolites and the purity factor was within the threshold limit, demonstrating the high degree of reliability of the suggested procedure.

3.2.4. Dilution integrity test. A dilution integrity test was carried out to deal with the possibility that a few samples could go over the maximum calibrated concentration. Consequently, rat plasma samples were spiked at higher concentrations 40 μg mL^{-1} over upper limit of quantitation (ULOQ) for the five analytes. After that, 4-fold dilution with blank plasma samples was done. This process was carried out five times and then analyzed using FLD. The RSD and percent error do not exceed 6.72% and 8.23%, respectively for the five analytes.

3.2.5. Extraction recovery. The extraction recovery is determined by comparing the cited analytes responses in spiked plasma that is then processed with the response in a blank plasma sample that is processed first and then spiked with the analytes. The obtained recovery values (>85%) revealed that the proposed methods have acceptable extraction efficiency for the analysis of the studied analytes in rat plasma.

3.2.6. Carryover. Carryover was evaluated by injecting blank plasma samples following the ULOQ to check the leftover analytes in the analytical instrument. The carryover in the blank samples does not exceed 20% of LLOQ of the five analytes.

3.2.7. Incurred sample reanalysis. Incurred sample reanalysis was done on a subset of treated rat plasma samples from various runs during the study. Nine samples were reanalyzed (representing 10% of the total samples) for LBQ657 and VAL. The average recoveries ±SD were 95.03 ± 5.22, and 107.97 ± 7.05 of plasma samples, respectively. It complied with the pre-established regulatory sample reanalysis acceptance criteria (±20% for at least 70% of the reanalysis samples).

3.2.8. Stability studies. Stability tests were carried out to investigate the stability of the studied drugs in rat plasma samples (LQC and HQC, $n = 6$) when stored under different conditions as shown in Table S3.† The recovery values were within 88.44–109.76% and RSD% values were less than 7.84, demonstrating that the cited analytes are regarded as stable in rat plasma under the optimized conditions.

3.3. Application to PK study of LCZ696 analytes in the absence and presence of FEX and IBU in rat real plasma

Although the HPLC method was validated for analyzing the LCZ696 analytes, FEX and IBU using the two detectors, DAD and FLD, only FLD was capable of tracing LCZ696 analytes at all the time intervals. On other hand, the DAD could only quantify the LCZ696 analytes at their $T_{\max}$ and provide their peak purity plots and profiles which confirmed the absence of any interference from endogenous plasma components or drugs' metabolites (Fig. S4†). As a result, the HPLC-FLD was the method of choice to be used successfully to *in vivo* PK drug interaction study in which LCZ696 analytes were measured in the plasma of rats given an oral dosage of LCZ696 with either FEX or IBU.

In rats the conversion of SAC to LBQ657 is fast, high and complete.[2,3] As a result, only the active metabolite LBQ657 is quantified in all rat plasma samples, Fig. 2. On the other hand, VAL was quantified as an intact drug in all rat plasma samples.[3,8,9] These two analytes, LBQ657 and VAL, were trace quantified either alone or with FEX or IBU in order to study their PK parameters in presence and absence of the selected drugs. The calculated PK parameters using non compartmental analysis module in PK Solver software were utilized for the assessment of the DDI.

The pharmacokinetic results (Table 2) revealed that co-administration of either FEX or IBU with LCZ696 increased the extent of absorption of VAL and LBQ657 without change in the rate of absorption $T_{\max} = 0.25$ h. Such findings came in agreement with that reported regarding the $T_{\max}$ for SAC,

Table 2 Pharmacokinetic parameters of VAL and LBQ657 in rats ($n = 6$ per group) in presence and absence of FEX and IBU (over the counter medications)[abc]

Drug	PK parameters	Control	+FEX	+IBU	*P* Value for FEX	*P* Value for IBU
VAL	$T_{\max}$ (h)	0.25	0.25	0.25		
	$C_{\max}$ (μg mL^{-1})	0.17 ± 0.03	0.38 ± 0.01	0.23 ± 0.07	0.0002	0.1904
	AUC$_{0-\infty}$(μg mL^{-1} h)	0.41 ± 0.06	1.12 ± 0.17	0.66 ± 0.10	<0.0001	0.0379
	$t_{1/2}$ (h)	3.27 ± 1.99	2.18 ± 0.81	2.02 ± 0.48	0.4755	0.3853
	Cl/F	49.99 ± 7.31	18.05 ± 1.94	30.87 ± 4.29	<0.0001	0.0011
LBQ657	$T_{\max}$ (h)	0.25	0.25	0.25		
	$C_{\max}$ (μg mL^{-1})	0.33 ± 0.14	0.77 ± 0.18	0.53 ± 0.21	0.0173	0.3023
	AUC$_{0-\infty}$ (μg mL^{-1} h)	0.40 ± 0.08	1.71 ± 0.58	0.66 ± 0.27	0.0020	0.6023
	$t_{1/2}$ (h)	2.66 ± 1.48	1.96 ± 1.11	1.62 ± 0.73	0.6760	0.4397
	Cl/F	51.19 ± 9.12	12.42 ± 2.97	33.14 ± 9.57	0.0002	0.0240

[a] Mean ± standard deviation of the mean. [b] $t_{1/2}$ = half life time, $T_{\max}$ = time at $C_{\max}$, $C_{\max}$ = maximum plasma concentrations, AUC$_{0-\infty}$ = area under the curve from time 0 to infinity, CL/F = oral clearance. [c] Statistical significance at $p < 0.05$ by one-way analysis of variance (ANOVA), followed by Tukey's post hoc analysis.

LBQ657, and VAL drug-related radioactivity (ranging from 0.25 to 2 hours).[2,3] Fig. 3 showed higher VAL/LBQ657 availability that was evidenced by the increase in AUC$_{(0-\infty)}$of VAL by almost 1.6-fold and 2.8-fold and $C_{\max}$ by 1.4-folds and 2.73-fold for IBU and FEX respectively. Similarly, an increase in AUC$_{(0-\infty)}$ of LBQ657 by almost 1.6-fold and 4.2-fold and $C_{\max}$ by 1.6-fold and 2.3-fold for IBU and FEX, respectively. Regarding the elimination phase (elimination half-life $t_{1/2}$), our results were in good agreement with those previously reported by Ayalasomayajula, S. *et al.*,[36] VAL/LBQ656 showed lower clearance and hence higher availability in circulation when co-administered with IBU and FEX. Regarding IBU, it lowered VAL and LBQ657 elimination (CL/F) by 1.6-fold and 1.5-fold, respectively. Such findings may be attributed to the effect of IBU in the avoidance of hepatic elimination (inhibition of CYP 2C9), which is supported by R. Bushra *et al.* [13,37,38] On the other hand, FEX significantly decreased their elimination by 2.7-fold and 4.1-fold, respectively. This may be due to that, FEX possesses the ability to inhibit intestinal efflux elimination *via* blockage of P-gp transporters, which is in harmony with that previously reported by Li. F *et al.*[10,39,40] It is worth mentioning that, the effect of FEX in lowering VAL/LBQ657 clearance and increasing plasma availability was more pronounced than that of IBU. Such results emphasized that intestinal efflux elimination has a key role in the clearance of LCZ696 analytes than hepatic elimination. These findings are consistent with the fact that the LCZ696

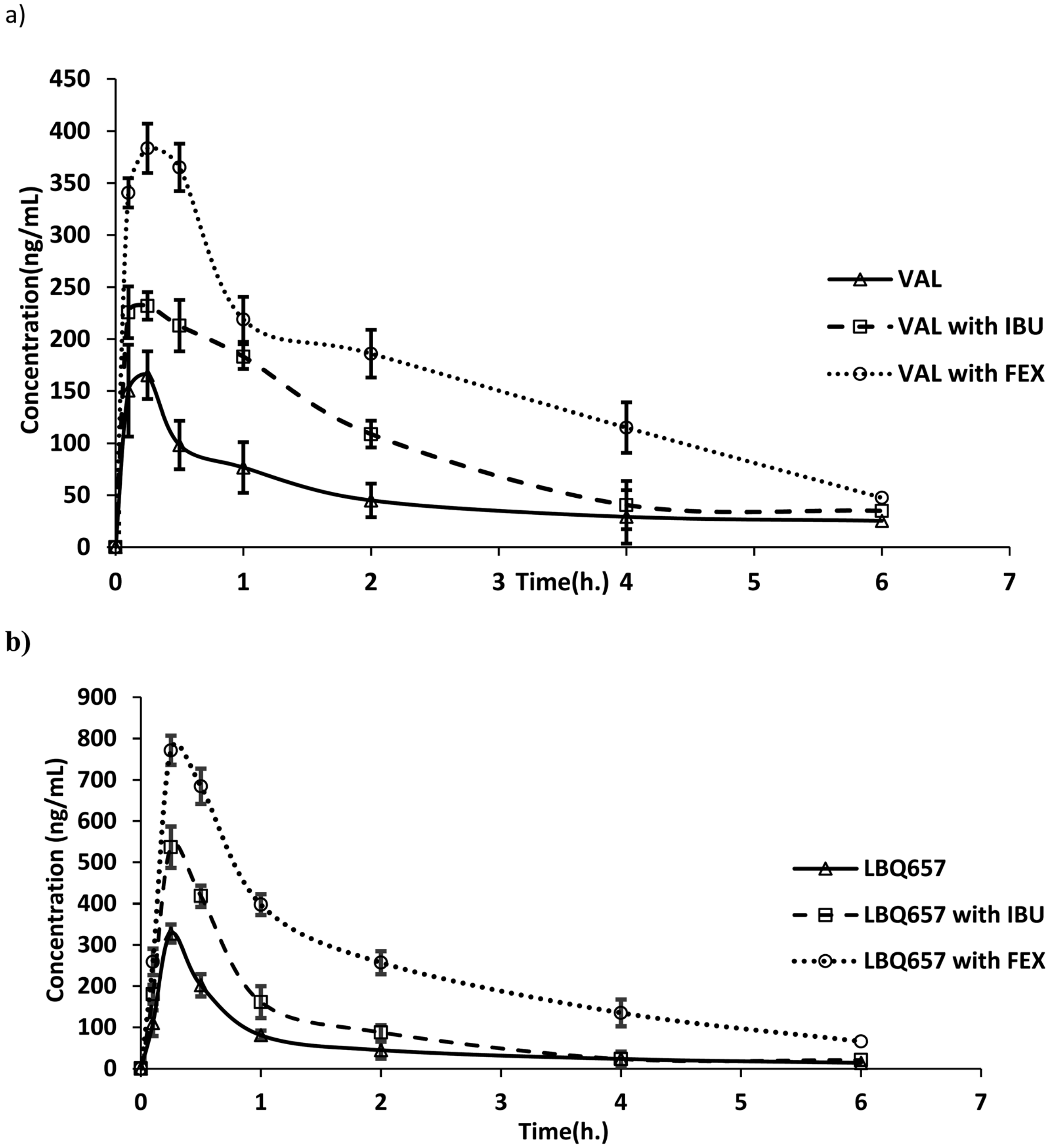

Fig. 3 Mean plasma concentration–time curves for (a) VAL and (b) LBQ657 following individual (LCZ696) and combined oral administration of 10 mg kg^{-1} LCZ696 and 10 mg kg^{-1} FEX or IBU from *in vivo* study in 6 rats' plasma.

analytes are not extensively metabolized by CYP 2C9 isozymes in the liver.[20,41] Consistent with the pharmacokinetic results, concomitant administration of LCZ696 with either IBU or FEX possess a notable impact on the key pharmacokinetic parameters of LCZ696 analytes.

4 Conclusion

In conclusion, this study represents the first comprehensive investigation into the pharmacokinetic interactions between the dual-acting heart failure medication LCZ696 and the commonly used OTC drugs, ibuprofen (IBU) and fexofenadine (FEX). A simple, FDA-validated, sensitive, and accurate HPLC method coupled with fluorescence detection was successfully developed for the simultaneous analysis of LCZ696 analytes (valsartan, sacubitril, and its active metabolite LBQ657) in rat plasma, achieving LLOQs of 25, 20, and 20 ng mL^{-1}, respectively. The *in vivo* pharmacokinetic study revealed that IBU and FEX significantly enhanced the bioavailability of LCZ696 analytes by inhibiting hepatic first-pass metabolism and intestinal efflux elimination, respectively. Hence, a significant DDI must be considered in patients treated with Entresto™ tablets in conjunction with IBU and FEX. As a result, dose monitoring and medical supervision are recommended to avoid possible interactions and hence unwanted side effects. This novel insight fills a crucial gap in understanding drug–drug interactions involving LCZ696 and supports safer clinical practices. Future research should focus on human clinical trials to confirm the pharmacokinetic interactions of LCZ696 with OTC drugs like IBU and FEX, establishing precise dosing guidelines. Additionally, evaluating interactions with other OTC medications will provide a comprehensive safety profile for heart failure patients.

Abbreviations

AUC	Area under curve
$C_{\max}$	Maximum plasma concentrations
DAD	Diode array detector
DDI	Drug–drug interaction
FLD	Fluorescence detector
IS	Internal standard
OTC	Over the counter
PK	Pharmacokinetics
QC	Quality control
$T_{\max}$	Time at $C_{\max}$
$t_{1/2}$	Half life time

Ethical statement

All animal studies in this work including their welfare adhered to the guidelines of the ethical committee (Faculty of Pharmacy, Alexandria University, Egypt) and its consent was obtained (ethical approval number AU 062024193214). The study was performed on fasting, healthy male Wistar rats (weighing between 250 g and 300 g). The biological fluid was obtained from rats in the animal house (Faculty of Pharmacy, Alexandria University, Egypt).

Author contributions

Conception and design of study: Aya R. Ahmed, Shereen M. Galal, Mohamed A. Korany, Manal A Elsheikh, Asser F. Bedair & Marwa A. A. Ragab acquisition of data: Aya R. Ahmed analysis and/or interpretation of data: Aya R. Ahmed, Manal A Elsheikh, Asser F. Bedair & Marwa A. A. Ragab drafting the manuscript: Aya R. Ahmed, Manal A Elsheikh & Marwa A. A. Ragab revising the manuscript critically for important intellectual content: Aya R. Ahmed, Shereen M. Galal, Mohamed A. Korany, Manal A Elsheikh, Asser F. Bedair & Marwa A. A. Ragab.

Conflicts of interest

The authors declare no conflict of interest that is related directly or indirectly to the submitted work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1 P. Khalil, G. Kabbach, S. Said and D. Mukherjee, *Cardiovasc. Hematol. Agents Med. Chem.*, 2018, **16**, 5–11.
2 ENTRESTO (sacubitril/valsartan) Product Monograph, **https://www.novartis.com/ca-en/sites/novartis_ca/files/entresto_scrip_e.pdf**.
3 FDA center for drug evaluation and research: pharmacology/toxicology review and evaluation of Entresto, **https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/207620Orig1s000PharmR.pdf**.
4 H. Zhang, G. Liu, W. Zhou, W. Zhang, K. Wang and J. Zhang, *Sci. Rep.*, 2019, **9**, 6509.
5 M. Saydam and S. Takka, *FABAD J. Pharm. Sci.*, 2007, **32**, 185–196.
6 S. Shugarts and L. Z. Benet, *Pharm. Res.*, 2009, **26**, 2039–2054.
7 I. Hanna, N. Alexander, M. H. Crouthamel, J. Davis, A. Natrillo, P. Tran, A. Vapurcuyan and B. Zhu, *Xenobiotica*, 2018, **48**, 300–313.
8 P. Marques da Silva and C. Aguiar, *Rev. Port. Cardiol.*, 2017, **36**, 655–668.
9 European medicines Agency: Entresto Assessment Report, **https://www.ema.europa.eu/en/documents/assessment-report/entresto-epar-public-assessment-report_en.pdf**.
10 F. Li, K. D. Howard and M. J. Myers, *J. Pharm. Pharmacol.*, 2017, **69**, 274–284.
11 M. Shimizu, K. Fuse, K. Okudaira, R. Nishigaki, K. Maeda, H. Kusuhara and Y. Sugiyama, *Drug Metab. Dispos.*, 2005, **33**, 1477–1481.
12 M. Cvetkovic, B. Leake, M. F. Fromm, G. R. Wilkinson and R. B. Kim, *Drug Metab. Dispos.*, 1999, **27**, 866–871.
13 R. Bushra and N. Aslam, *Oman Med. J.*, 2010, **25**, 155–1661.
14 J. Kindla, F. Müller, M. Mieth, M. F. Fromm and J. König, *Drug Metab. Dispos.*, 2011, **39**, 1047–1053.
15 C. C. Ogu and J. L. Maxa, *Proc. – Bayl. Univ. Med. Cent.*, 2000, **13**, 421–423.
16 Y. Peng, L. Chen, S. Ye, Y. Kang, J. Liu, S. Zeng and L. Yu, *Asian J. Pharm. Sci.*, 2020, **15**, 220–236.

17 S. Ayalasomayajula, W. Pan, Y. Han, F. Yang, T. Langenickel, P. Pal, W. Zhou, Y. Yuan, I. Rajman and G. Sunkara, *Eur. J. Drug Metab. Pharmacokinet.*, 2017, **42**, 309–318.

18 B. A. Moussa, H. M. A. Hashem, M. A. Mahrouse and S. T. Mahmoud, *Microchem. J.*, 2018, **143**, 31–38.

19 H. L. Hsiao, T. H. Langenickel, J. Petruck, K. Kode, S. Ayalasomayajula, U. Schuehly, M. Greeley, P. Pal, W. Zhou, M. F. Prescott, G. Sunkara and I. Rajman, *Clin. Pharmacol. Ther.*, 2018, **103**, 468–476.

20 S. Ayalasomayajula, U. Schuehly, P. Pal, F. Chen, W. Zhou, G. Sunkara and T. H. Langenickel, *Br. J. Clin. Pharmacol.*, 2018, **84**, 926–936.

21 S. Ayalasomayajula, P. Jordaan and P. Pal, *Clin. Pharmacol. Biopharm.*, 2015, **4**(4), 1000147.

22 H. L. Hsiao, T. H. Langenickel, M. Greeley, J. Roberts, W. Zhou, P. Pal, S. Rebello, I. Rajman and G. Sunkara, *Clin. Pharmacol. Drug Dev.*, 2015, **4**, 407–417.

23 L. Gan, X. Jiang, A. Mendonza, T. Swan, C. Reynolds, J. Nguyen, P. Pal, S. Neelakantham, M. Dahlke, T. Langenickel, I. Rajman, M. Akahori, W. Zhou, S. Rebello and G. Sunkara, *Clin. Pharmacol. Drug Dev.*, 2016, **5**, 27–39.

24 B. A. Moussa, H. M. A. Hashem, M. A. Mahrouse and S. T. Mahmoud, *Biomed. Chromatogr.*, 2021, **35**, e5203.

25 N. Anjaneyulu, R. N. Kishore, M. R. Kumar and G. Sneha, *J. Pharm. Pharm. Sci.*, 2018, **6**, 105–110.

26 M. Attimarad, S. H. Nagaraja, A. B. Nair, B. E. Aldhubaib and V. N. Katharigatta, *J. Liq. Chromatogr. Relat. Technol.*, 2018, **41**, 246–252.

27 R. H. Chunduri and G. S. Dannana, *Biomed. Chromatogr.*, 2016, **30**, 1467–1475.

28 Y. Ni, Y. Zhang, C. Zou and L. Ding, *J. Braz. Chem. Soc.*, 2021, **32**, 1–7.

29 Y. Jin, Y. He, X. Di, L. Fu, X. Qi, R. Liu, L. Zheng, Y. Wang, H. Zhong and Z. Wang, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2022, **1208**, 123402.

30 Y. Zhang, M. Huo, J. Zhou and S. Xie, *Comput. Methods Progr. Biomed.*, 2010, **99**, 306–314.

31 M. A. A. Ragab, M. A. Korany, S. M. Galal and A. R. Ahmed, *J. Liq. Chromatogr. Relat. Technol.*, 2018, **41**, 33–42.

32 M. A. A. Ragab, S. M. Galal, M. A. Korany and A. R. Ahmed, *J. Chromatogr. Sci.*, 2018, **56**, 498–509.

33 The FDA 2018 Guidance for Industry on Bioanalytical Method Validation, https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf.

34 M. A. Ragab, M. A. Korany, S. M. Galal and A. R. Ahmed, *Bioanalysis*, 2019, **11**, 73–84.

35 M. A. Ragab, M. A. Korany, S. M. Galal and A. R. Ahmed, *Chem. Zvesti*, 2019, **73**, 1209–1219.

36 S. Ayalasomayajula, T. Langenickel, P. Pal, S. Boggarapu and G. Sunkara, *Clin. Pharmacokinet.*, 2017, **56**, 1461–1478.

37 C. K. Yeung, P. Zhao, D. D. Shen and K. E. Thummel, in *Enzyme- and Transporter-Based Drug-Drug Interactions: Progress and Future Challenges*, ed. K. S. Pang, A. D. Rodrigues and R. M. Peter, Springer New York, New York, NY, 2010, pp. 415–435, DOI: 10.1007/978-1-4419-0840-7_17.

38 L. L. Mazaleuskaya, K. N. Theken, L. Gong, C. F. Thorn, G. A. FitzGerald, R. B. Altman and T. E. Klein, *Pharmacogenet. Genomics*, 2015, **25**, 96–106.

39 M. Elmeliegy, M. Vourvahis, C. Guo and D. D. Wang, *Clin. Pharmacokinet.*, 2020, **59**, 699–714.

40 M. Lund, T. S. Petersen and K. P. Dalhoff, *Drugs*, 2017, **77**, 859–883.

41 S. Ayalasomayajula, T. Langenickel, P. Pal, S. Boggarapu and G. Sunkara, *Clin. Pharmacokinet.*, 2018, **57**, 105–123.