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RAPID AND SENSITIVE UPLC-MS/MS VALIDATED METHOD FOR ELTROMBOPAG DETERMINATION IN HUMAN PLASMA AND ITS APPLICATION TO BIOEQUIVALENCE STUDY

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ABSTRACT

A rapid, simple, sensitive, reproducible, and reversed-phase UPLC-MS/MS method was established and validated for determining eltrombopag in human plasma. Eltrombopag was extracted from plasma samples by the protein precipitation technique using acetonitrile. Eltrombopag was separated on a C18- column of Acquity UPLC BEH adjusted at 35°C and mass analysed in a positive electrospray ionization mode. A simple mobile phase of formic acid (0.1%) and acetonitrile in a 25:75 (v/v) ratio was utilized at a flow rate of 400 µL min^{-1}. The analysis of eltrombopag was carried out within 2 min over a broad range between 50 and 10000 ng mL^{-1} (mean r^2 = 0.9937). Accuracy ranged from 102.70 to 111.43%, whereas the precision (inter- and intra-assay) was not more than 15%. The results confirmed the eltrombopag stability under various conditions. The proposed and validated method was effectively applied to a bioequivalence study of eltrombopag tablet (50 mg) in healthy Egyptian volunteers (n = 31).

Key Words: Eltrombopag, UPLC-MS/MS, Human plasma, Bioequivalence, Incurred sample reanalysis

INTRODUCTION

Eltrombopag belongs to the thrombopoietin receptor agonist agents' family. Since 2012, eltrombopag has been permitted by the Food and Drug Administration. Eltrombopag has been used for therapeutic purposes, commonly to treat patients who suffer from thrombocytopenia, especially chronic immune (idiopathic) thrombocytopenic purpura **(Department of Health and Ageing 2010 ; Cheng, 2011 and European Medicines Agency 2016)**. Eltrombopag has a large protein-binding character, and it is initially removed by metabolism in the gastrointestinal tract and liver **(Bauman *et al.*, 2011)**.

As the literature discloses, few analytical methods were available to estimate eltrombopag in bulk and tablet dosage forms, including UV

spectrophotometry **(Marakatham *et al.*, 2017)**, HPLC **(Manoharan, 2018 ; Ahir *et al.*, 2020 and Yanagimachi *et al.*, 2021),** UPLC (**Mohan *et al.*, 2018 and Patel *et al.*, 2020)**. Furthermore, very few UPLC-MS/MS methods have been described for eltrombopag determination in human plasma or in other biological samples **(Maddela *et al.*, 2014** and **Basha *et al.*, 2015)**. However, to the best of the authors' knowledge, only the work of **Maddela *et al.*, (2014)** has applied their proposed method to a bioequivalence study, but on a small number of volunteers (n = 8).

Herein, the present study was aimed to develop a rapid, economical, sensitive, and reproducible UPLC-MS/MS method for measuring eltrombopag in human plasma. The benefits of conserving time and columns with the current method were proven by the shorter run time and lower flow rate than the previously published ones. The proposed method was applied to a bioequivalence study of eltrombopag (50 mg) tablets in healthy Egyptian volunteers (n = 31). Moreover, to ensure the method's reproducibility and the authenticity of the measurement of the study data, incurred samples reanalysis was carried out.

EXPERIMENTAL

1. Chemicals and reagents

Eltrombopag olamine (purity: 99.82%) was supplied by Optrix Laboratories (Private Limited, India), whereas the internal standard eltrombopag-^{13}C4 (IS) (purity: 98.80%) was obtained from Toronto Research Chemicals, Canada. The generic test product (Versapenia 50 mg tablets) was granted by Amoun Pharmaceutical Company (Obour City, Egypt), while the innovator reference product (Revolade 50 mg tablets), manufactured by Glaxo Operations UK Limited for Novartis (Switzerland), was obtained from the local market. Formic acid, acetonitrile, and methanol (HPLC grade) were supplied by Merck (Germany). Blank human plasma was supplied by the Shabrawishy hospital blood bank (Giza, Egypt) and stored at -30ºC prior to use. The water system of Millipore Direct-Q® ultrapure (Millipore, France) was employed to obtain the ultrapure water. Every other chemical was of analytical grade, and it was utilized with no further purification.

2. Instrumentation

UPLC-Acquity H-class ultra-performance liquid chromatograph (Waters, USA), connected to a triple quadruple mass spectrometer detector, TQD MS/MS (Waters, USA), and Acquity auto-sampler (Waters, USA), was employed for analysis. The Mass-lynx software (version 4.1) was employed for data acquisition and processing.

3. LC-MS/MS instrumental conditions

Chromatographic separation was achieved using a C18 column of Acquity UPLC BEH (50 x 2.1 mm) with a particle size of 1.7 µm,

adjusted at 35°C, and a mobile phase comprising a combination of 0.1% formic acid and acetonitrile, at a ratio of 25:75 (v/v). An injection volume of two µL was employed, and the mobile phase's flow rate was adjusted to 400 µL min^{-1}. For the detection of eltrombopag and eltrombopag-^{13}C4, multiple reaction monitoring (MRM) was utilized in the positive ion mode employing electrospray ionization (ESI). **Table 1** summarizes the MS/MS instrument's settings.

1.1 Standard solutions and calibrators

A precisely weighed amount of eltrombopag was dissolved in methanol to prepare a standard stock solution (1 mg mL^{-1}). Using methanol, the stock solution was further diluted progressively to prepare different 8 standard wording solutions with concentrations in the range of 50 to 10000 ng mL^{-1} of calibration standards (CCs). Additionally, 0.1 mL of each standard solution was spiked into a final volume of 10 mL of plasma to prepare matrix-based calibrators. A working IS solution (192 ng mL^{-1}) was prepared in acetonitrile. Also, quality control samples (QCs) of 150, 750, 4000, and 7500 ng mL^{-1} were prepared. Until analysis, all CCs and QCs were kept at −80°C.

Table 1: Tandem mass spectroscopic conditions for the analysis of eltrombopag and eltrombopag ^{13}C4

Parameter, unit	Value	
Source temperature, °C	150	
Cone gas, L/H	30	
Collision gas, psi	0.15	
Ion spray voltage, V	3	
Entrance potential, V	32	
External potential, V	2	
Dwell time per transition, ms	0.161	
Desolvation gas, L/H	700	
Desolvation temperature, °C	500	
	Eltrombopag	Eltrombopag-^{13}C4
MRM transition	443.24 → 183.08	447.18 →183.08
Collision energy, V	-46	-50

1.1. Sample extraction

Eltrombopag was extracted from the plasma samples using the protein precipitation method with acetonitrile. The samples were vortexed for 10 s before being spiked. First, the collected plasma (200 µL) was pipetted along with 1000 µL of IS (192 ng mL^{-1}), where it was vortexed for 30 s at 1200 rpm. Then, the mixture was centrifuged at 4000 rpm for 20 min, and the collected supernatant (500 µL) was vortexed (5 s) after being diluted with 1 mL water. Finally, 2 µL of the diluted supernatant was injected into the LC–MS/MS to be analyzed.

1.2.Method validation

The developed bioanalytical method was validated as stated by **European Medicines Agency (2012) ; Food and Drug Administration (2018)**, with respect to the following parameters: system suitability, selectivity, sensitivity, matrix effect, linearity, precision, accuracy, extraction recovery, dilution integrity, carry-over, and stability.

1.3.Application to bioequivalence study and incurred sample reanalysis

The developed method was effectively applied to evaluate the bioequivalence in healthy volunteers using two tablet formulations of eltrombopag: Versapenia (test product) and Revolade (reference product). The study evaluated identical doses of each product (50 mg) administered to healthy Egyptian participants while fasting. The study comprised 32 healthy participants (adult males) with ages ranging from 18 to 45. The Medical Research and Ethics Committee (Ministry of Health, Egypt) authorized the clinical protocol, and the volunteers had signed their informed consent. The present study was carried out in conformity with the Declaration of Helsinki and its amendments' ethical standards for studies involving humans.

A randomized, open-label, 2-period crossover design with a 2-week washout interval was used in this study. The volunteers were taken to a hospital, where they received one dose of the prescribed tablet along with water (240 mL) orally after overnight fasting (12 h). After the medication was administered, there was a four-hour fast.

Five mL of blood samples (venous) were gathered into labelled EDTA tubes, at the following time intervals: instantly before eltrombopag administration and at 0.50, 1.00, 1.50, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.50, 6.00, 7.00, 8.00, 10.00, 12.00, 24.00, 48.00, and 72.00 h after dose intake. After centrifuging the blood samples for 8 min at 4000 rpm, the obtained plasma was kept at −80ºC until analysis. The bioequivalence study assessment was relevant to the main pharmacokinetic (PK) parameters, including C_{max}, T_{max}, $t_{1/2}$, MRT, $AUC_{0-\infty}$, and AUC_{0-t}. The calculations were carried out by the non-compartmental model with the WinNonlin program (version 8.1), and the statistical evaluation was performed using SAS® software (version 9.1.3, USA). The two eltrombopag formulations can be deemed bioequivalent, when the geometric mean ratios' 90% confidence interval (CI) is in the range of 80-125%. On the other hand, the incurred sample reanalysis (ISR) was conducted on 31 dosed volunteers (two samples from each) in compliance with the recommendations of FDA. For the study, > 10% of the total samples was chosen. The per cent change in the data between the previously obtained values and ISR values should be < ± 20%.

RESULTS AND DISCUSSION

1. LC-MS/MS method development

A LC-MS/MS method for measuring eltrombopag in human plasma has been developed and optimized. **Table 1** summarizes the optimized experimental conditions. Product ion mass spectra of eltrombopag and IS were recorded, and the transition *m/z* 443.24 → 183.08 was chosen for quantitation of eltrombopag, whereas the transition *m/z* 447.18 → 183.08 was chosen for IS. The selection of mobile phase, the type of column, flow rate, and injection volume were considered throughout the method development. The optimization of chromatographic conditions was to accomplish good peak symmetry, high resolution, and a short retention time for eltrombopag and IS, as shown in Fig (1). The effects of various volume ratios of formic acid and acetonitrile were examined. The most effective combination of the mobile phase was found to be a combination of formic acid (0.1%) and acetonitrile at a ratio of 25:75 (v/v), which gave the optimum peak shape, sensitivity, and efficiency. It was also found that the C18-column of Acquity UPLC BEH (50 x 2.1 mm) with a particle size of 1.7 µm provided satisfactory peak shape and response for the eltrombopag and IS even at the lowest concentration level. The mobile phase flow rate was set to 400 µL min^{-1}. For eltrombopag and IS, the retention times were 1.10 and 1.10 min, correspondingly, with a short run time of 2.0 min.

In this study, the sample preparation was carried out using the simple protein precipitation technique, which provided high-level recoveries of eltrombopag and IS. Moreover, eltrombopag had a stronger protein-binding character and was easily precipitated using acetonitrile. Eltrombopag-$^{13}C4$ (stable labelled isotope of eltrombopag) was utilized as the IS in order to improve assay precision and reduce the variability in eltrombopag and IS recovery.

2. Method validation

2.1. System suitability

System suitability was conducted prior to validation, and at the beginning of every analytical batch. System selectivity, carry-over, and precision parameters were evaluated to indicate system suitability. Blank, zero, the lower limit of quantitation (LLOQ), low-QC (LQC), medium-QC (MQC1 and MQC2), and high-QC (HQC) samples of eltrombopag were analyzed (6 replicates), and the CV% was calculated. The system suitability can be deemed satisfactory when the eltrombopag peak area in the blank sample is not > 20% of the LLOQ peak area, and the IS peak area in the blank sample is not > 5% of the original peak area. In addition, the CV% of the spiked concentrations is less than 15%. **Table 2** displays the results of the system suitability.

Table 2: System suitability results

Parameter	Value
System selectivity	
LLOQ peak area	**399**
IS peak area in zero sample	**11462**
Analyte% in blank	**0**
Analyte% in zero sample	**0**
IS% in blank	**0**
System carry-over	
Analyte peak area in blank	**0**
IS peak area in blank	**0**
System precision (MQC1)	
Mean (n = 6)	**5221.33**
SD	**269.51**
CV%	**5.16**

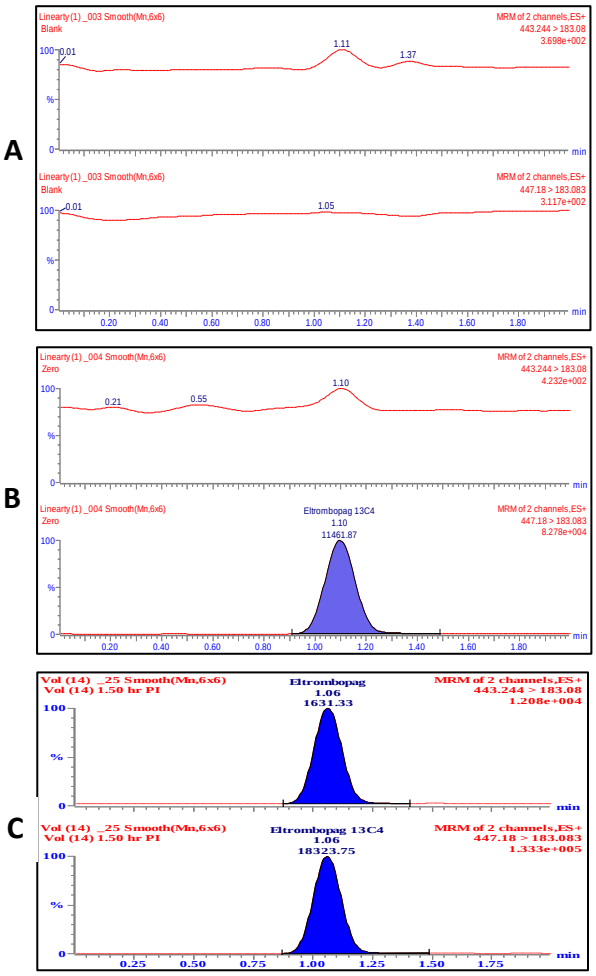

Fig 1: MRM chromatograms of the analysis of blank plasma (A), blank plasma spiked with eltrombopag (50 ng mL^{-1}) and IS (192 ng mL^{-1}) (B), and a participant plasma (1.5 hours after an administration of a tablet containing 50 mg eltrombopag in the fasting state) (C)

2.2.Selectivity and sensitivity

The proposed method's selectivity was assessed via the assay of six diverse sources of human plasma (normal and hemolysed), each of which was independently analyzed and assessed for plasma constituents' interference with eltrombopag and IS. The interferents' peak areas should be < 20% of the LLOQ peak area and 5% of the IS peak area. Fig 2 illustrates the chromatograms of blank plasma (not including eltrombopag and IS), a zero sample with IS, eltrombopag at LLOQ level (50 ng mL^{-1}) with IS, participant plasma sample at 3.0 hours after one dose of eltrombopag (50 mg tablet). As shown, no interference from the endogenous constituents was detected at the retention times of eltrombopag and IS. LLOQ concentration was employed to express the method sensitivity that was measured with adequate accuracy and precision, as indicated in **Table 3.** As noted, the values of CV% and recovery % of LLOQ were within acceptable limits.

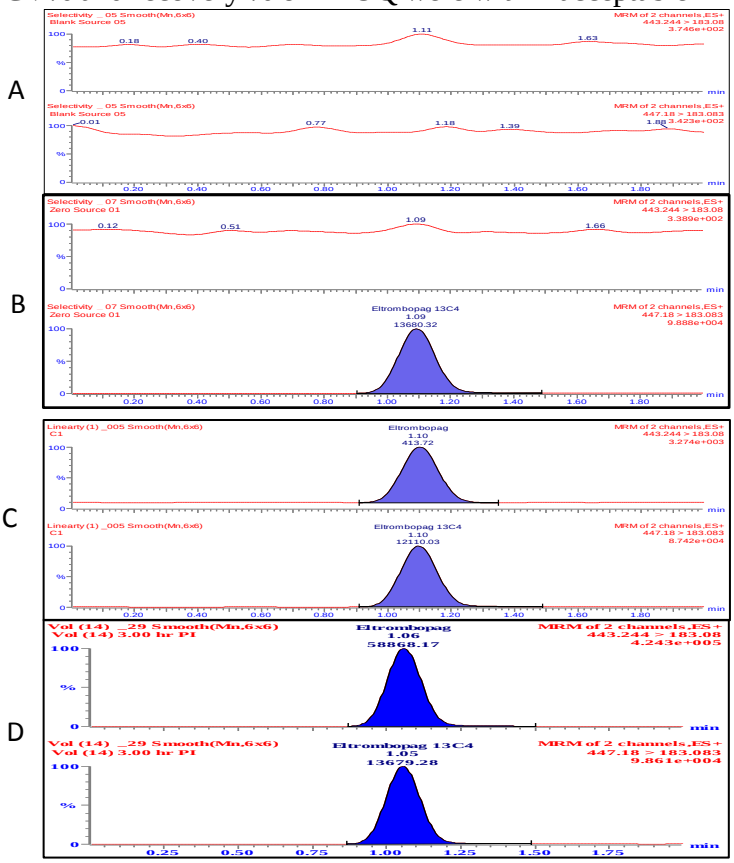

Fig 2: Representative chromatograms of blank plasma (A), human plasma spiked with eltrombopag-^{13}C4 (B), a LLOQ sample with eltrombopag-^{13}C4 (C), and volunteer plasma sample; 3.00 h following administration of an eltrombopag tablet (50 mg) (D)

Table 3: Precision and accuracy results

QC level	Spiked conc. (ng mL^{-1})	Conc. found (ng mL^{-1})	CV%	Recovery %
Intra-day (n = 6)				
LLOQ	**50**	**55.716**	**2.54**	**111.43**
LQC	**150**	**150.527**	**3.09**	**100.35**
MQC1	**750**	**772.392**	**1.40**	**102.99**
MQC2	**4000**	**4474.975**	**0.94**	**111.87**
HQC	**7500**	**8465.127**	**0.85**	**112.87**
Inter-day (n = 18)				
LLOQ	**50**	**51.351**	**2.87**	**102.70**
LQC	**150**	**146.222**	**0.87**	**97.48**
MQC1	**750**	**764.759**	**0.69**	**101.97**
MQC2	**4000**	**4493.734**	**0.93**	**112.34**
HQC	**7500**	**8279.405**	**3.76**	**110.39**

2.3.Matrix effect

To figure out the probable improvement or suppression of ionization, the matrix effect was assessed via the comparison of the peak area ratios of eltrombopag and IS at two concentration levels (LQC and HQC) for 12 diverse sources of blank plasma with the response of the eltrombopag standard at the same concentrations.

The quantification of matrix factor (MF) was carried out by determining the IS-normalized MF for predicting the matrix effects' variability in samples from the individual participants, as demonstrated by **Loh *et al.*, (2021) and Xu *et al.*, (2022)**. The IS-normalized MFs of eltrombopag were determined to be 0.0754 and 3.3829 in quality control samples of 150 and 7500 ng mL^{-1}, correspondingly. Also, the precision of MF (CV%) should be < 15%. Accordingly, the matrix effect can be deemed negligible since the CV% values were found to be < 3.86% for the six different matrices at each concentration.

2.3.Linearity, precision, and accuracy

The method's linearity was determined by the analysis of six calibration curves. Each calibration curve was obtained via the assay of blank plasma samples that were spiked with the eltrombopag standard. Then, peak areas' ratios of eltrombopag/IS were plotted versus eltrombopag concentrations (50–10000 ng mL^{-1}) to construct the calibration curves. A weighted linear regression ($1/x^2$) analysis was applied to assess the validity of the calibration curve. The samples of 50, 150, 750, 4000, and 7500 ng mL^{-1} were analyzed six times to evaluate the proposed method's precision and accuracy.

At each concentration level, the accuracy should be within 100 ± 15% of the actual concentration, whereas the calculated precision (CV%) should be < 15%. On the other hand, at the LLOQ level, the precision

and accuracy should be < 20%. The linearity of the six calibration curves between 50 and m 10000 ng mL^{-1} exhibited good results with a mean regression coefficient (r^2) of 0.9937. The calibration equation was y = (0.00047785 ± 0.00000372) x + (0.010621 ± 0.002006), where the peak area ratio of the analyte/IS is y, and the analyte concentration is x. Table 3 shows the quantified eltrombopag concentration in each QC sample and the precision and accuracy data. As shown, the CV% values were all < 15%, while they were < 20% at the LLOQ level. Furthermore, the method's accuracy was in the range 97.48 - 112.87%.

Recovery

A recovery test was carried out by adding a known concentration of eltrombopag to the plasma prior to extraction, and then the obtained chromatographic peak areas of eltrombopag and IS were compared to those produced when the eltrombopag was added after extraction. The mean recoveries (n = 6) of eltrombopag at the levels of 150, 4000, and 7500 ng mL^{-1} were 84.50, 79.90, and 78.16%, respectively, and the IS recovery (192 ng mL^{-1}) was 74.51%.

2.4.Dilution integrity

A dilution integrity assessment was conducted to ensure that a dilution can be conducted by the proposed method at higher eltrombopag concentrations above the ULOQ with satisfactory precision and accuracy. This test can be acceptable when the CV% is less than 15% and the accuracy is within ± 15% of the nominal concentrations for the diluted samples. The eltrombopag ULOQ can be increased up to 16786.59 ng mL^{-1} using double and 4-times dilution. The values at 1/2 and 1/4 dilution for CV% were found to be 0.91 and 0.62%, while for accuracy, they were 111.91 and 112.05%, respectively.

2.5.Carry-over

In order to ensure that the proposed method's precision and accuracy were not jeopardized, a carry-over test was carried out. Carry-over was assessed through the assay of three replicates of blank samples and eltrombopag at LLOQ and ULOQ levels. The carry-over can be deemed acceptable if the eltrombopag peak area is < 20% of the LLOQ and that of the IS is less than 5% of the LLOQ. The results shown in **Table 4** confirmed that no carry-over was observed.

Table 4: Carry-over results

LLOQ peak area = 414 **IS peak area = 12110**				
Blank samples after injection of ULOQ	**Drug area in Blank**	**IS area in Blank**	**Carry-over for IS (%)**	**Carry-over for drug (%)**
1	**0**	**0**	**0**	**0**
2	**0**	**0**	**0**	**0**
3	**0**	**0**	**0**	**0**

2.6.Stability study

Eltrombopag stability was estimated at two levels (LQC and HQC) with three replicates. The solution stability was evaluated by comparing the peak area response of the analyte (stability samples) with the QC samples' responses that were freshly prepared. The study of short-term stability was carried out by keeping the spiked plasma samples for 24 h (at 25ºC), while for the study of long-term stability, the samples were kept in the freezer for 107 days (-80 ± 10ºC). In the case of the study of auto-sampler stability, the samples were placed in the auto-sampler (at 15 ± 3°C) over 48 h. On the other hand, for the stability study of freeze-thaw cycles, the stability was determined at 0, first, third, and fifth cycles. The stability of the stock solutions of eltrombopag and IS was also carried out, with their solutions kept for 24 h (at 25ºC) and in the freezer for 107 days (at -80°C). The results presented in **Table 5** indicated that the QC samples of eltrombopag stayed stable for up to 24 h on a benchtop and for five freeze-thaw cycles (-80°C). Also, the results of the post-preparative stability of the spiked QC samples showed that they were stable for 48 h when kept in the auto-sampler (15°C). The results of the long-term stability study revealed the stability of eltrombopag in plasma for up to 107 days, whereas the stock solutions of eltrombopag were also stable for 107 days. These results demonstrated the stability of eltrombopag under various conditions.

Table 5: Stability results of eltrombopag and IS (n = 3)

Stability test (Storage conditions, duration)	Nominal conc. (ng mL^{-1})	Calculated conc. (ng mL^{-1})	CV%	Recovery % (Stability)
Short-term plasma stability (25 ± 4ºC, 24 h)				
LQC	**148.947**	**160.622**	**2.11**	**107.84**
HQC	**8428.124**	**8414.992**	**1.05**	**99.84**
Long-term plasma stability (-80 ± 10ºC, 107 days)				
LQC	**147.465**	**139.146**	**3.79**	**94.36**
HQC	**8421.776**	**7655.912**	**0.20**	**90.91**
Post-preparative stability in auto-sampler (15 ± 3ºC, 48 h)				
LQC	**151.576**	**159.574**	**3.24**	**105.28**
HQC	**7995,024**	**7990.227**	**0.74**	**99.94**
Freeze-thaw cycle stability (-80 ± 10ºC, 5 cycles)				
LQC	**147.595**	**155.7104**	**3.30**	**105.498**
HQC	**8291.638**	**7836.426**	**0.92**	**94.51**
Short-term stock solutions stability (25 ± 4ºC, 24 h)				
LQC	**149.061**	**150.631**	**6.25**	**101.053**
HQC	**8139.330**	**8091.896**	**1.27**	**99.42**
Long-term stock solutions stability (-80 ± 10ºC, 107 days)				
LQC	**139.261**	**137.377**	**3.38**	**98.65**
HQC	**8242.501**	**8219.202**	**1.22**	**99.71**

3. Application and incurred sample reanalysis (ISR)

A total of 31 volunteers completed the study, and the main PK parameters of eltrombopag in these volunteers were estimated and tabulated in **Table 6&7**. Also, the curves of the plasma concentration-time of eltrombopag are displayed in Fig 3. As shown, the main PK parameters, such as C_{max}, T_{max}, $t_{1/2}$, MRT, AUC_{0-72}, and $AUC_{0-\infty}$ were observed to be similar for the two eltrombopag formulations (test and reference), suggesting that the plasma profiles created by the test product were analogous to those produced by the reference product. Furthermore, the estimated values of 90% CI for the main PK parameters of the two formulations were also within the permitted range (80-125%) set by the FDA. These results led to the conclusion that both eltrombopag products under investigation (test and reference) were found to be bioequivalent regarding the absorption degree and rate as well.

The data of ISR study was employed to estimate the reproducibility of the current LC-MS/MS validated method. The obtained results demonstrated that 87.90% of the samples that have been reanalyzed were within ± 20% of the initial values, proving the proposed method's reproducibility.

Table 6: PK parameters of eltrombopag in 31 volunteers

Parameter	Test	Reference
	Mean ± SD	Mean ± SD
C_{max}, ng mL $^{-1}$	6119.823 ± 1444.618	6398.128 ± 1555.454
T_{max}, h	3.924 ± 0.901	3.892 ± 1.044
$t_{1/2}$, h	17.340 ± 5.945	17.216 ± 3.581
MRT	20.876 ± 4.773	20.846 ± 4.090
AUC_{0-72}, ng h mL $^{-1}$	80768.612 ± 26311.893	80214.813 ± 26672.346
$AUC_{0-\infty}$, ng h mL $^{-1}$	86276.187 ± 29818.940	85210.097 ± 29153.098

Table 7: Point estimate and CI limits for mean PK parameters

Parameter	Point estimate %	90% CI		Bioequivalence limit		Conclusion
		Lower %	Upper %	Lower %	Upper%	
C_{max}	95.904	86.55	106.26	80.00	125.00	Bioequivalent
AUC_{0-72}	101.669	92.06	112.28	80.00	125.00	Bioequivalent
$AUC_{0-\infty}$	102.212	92.43	113.02	80.00	125.00	Bioequivalent

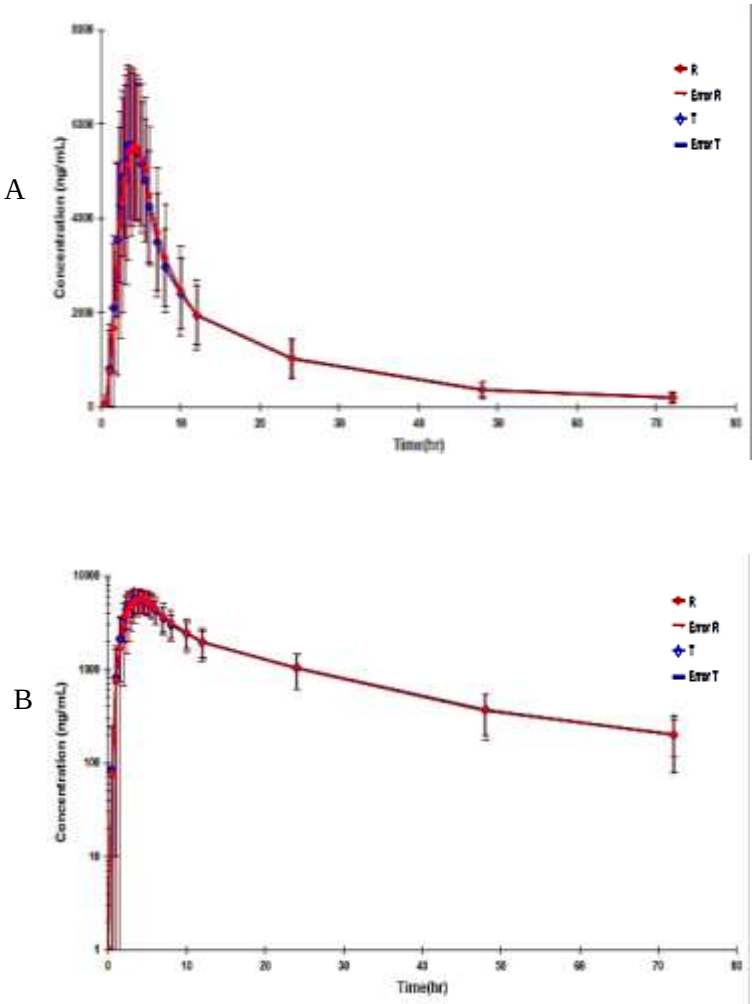

Fig 3: Average plasma eltrombopag concentration vs. time profile (A) and semi-log concentration vs. time profile (B) of the test (T) or reference (R) products (50 mg eltrombopag tablets)

CONCLUSIONS

A rapid, simple, sensitive, and reproducible UPLC-MS/MS method for eltrombopag evaluation in human plasma was successfully developed, validated, and applied to a bioequivalence study. The proposed method demonstrated high sensitivity and selectivity for eltrombopag estimation in human plasma. Furthermore, the results of the ISR study revealed the method reproducibility. The obtained results revealed that the proposed method is appropriate for human clinical investigations with the required precision and accuracy.

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Declarations

Competing interests

The authors declare that there are no competing interests for this study.

Availability of data and materials

Raw data are available on reasonable request.

Ethical conduct of research

The authors have got enough institutional medical board (Cod: FORM05/SOP:QA-004(IRBB) authorization and have established the standards given by the Helsinki Declaration and the International Center for Bioavailability, Pharmaceutical, and Clinical Research. Additionally, the written consent was obtained by all of the volunteers.

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تطبيق طريقه سريعه وعاليه الحساسيه بإستخدام تقنيه الفصل الكروماتوجرافي فائق الكفاءه والذات صله بمطيافيه الكتله لتعيين الالترومبوباج في بلازما الانسان بالاضافه الي تطبيقها في دراسه التكافؤ الحيوي

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يتضمن هذا البحث تطبيق والتحقق من صحة طريقة UPLC–MS/MS السريعة ، والبسيطة، وعالية الحساسية والقابلة للتكرار لتعيين الالترومبوباج في بلازما الانسان. تم تطبيق تقنية ترسيب البروتين بإستخدم الاسيتونيتريل لاستخراج الالترومبوباج من البلازما المستخدمة. وتم فصل وتحليل الالترومبوباج باستخدم عمود الفصل UPLC BEH C18 من Acquity المضبوط عند درجة حرارة 35°م. كما تم تحديد كتلة الالترومبوباج بتقنية التأين بالرش الكهربائي الموجبي . وكانت الطور السائل المستخدم في الفصل عبارة عن مزيج من الاسيتونيتريل وحمض الفورميك بنسبة 75 الي 25 وباستخدام معدل سريان قدره 0.4 ملليليتر لكل دقيقة مع تزامن كلى قدره دقيقتين. تتراوح المعادله الخطية لطريقة تعيين الالترومبوباج من 50 الي 10.000 نانوجرام لكل ملليليتر. واظهرت النتائج أن صحة الطريقة المستخدمة تتراوح من 102.70 الي 111.43 نسبة مئوية علاوة علي أن دقة الطريقة لا تزيد عن 15 بالمئة ومن الناحية الاخري فقد اكدت النتائج ثبات الالترومبوباج تحت الظروف المختلفة.

تم تطبيق الطريقة المقترحة والمتحقق منها في دراسة التكافؤ الحيوي لمستحضر الالترومبوباج بتركيز 50 ملليجرام علي مشاركين اصحاء مصريين.