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Determination of imatinib mesylate and erlotinib hydrochloride in mice plasma using ultrahigh performance liquid chromatography method

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Abstract

This study aimed to develop a rapid, user-friendly, economic and sensitive method for analyzing imatinib mesylate (IMA) and erlotinib hydrocloride (ERLO) in mice plasma using ultrahigh performance liquid chromatography (UPLC). Separation was achieved by isocratic elution using a mobile phase consisting of the mixture of 3mM triethylamine and acetonitrile with the ratio of 60:40 using XTerra® RP8 column (5 μm 4.6x150 mm). The retention time for IMA was 4.9 minutes with a flow rate of 1.5mL/min, while the retention time for ERLO was 5.0 minutes with a flow rate of 0.8mL/min. Injection volume was set to 20μL and the detector wavelength was 320 nm. Detection limits for imatinib mesylate and erlotinib hydrochloride were 95 and 72 ng/mL, respectively. This method was validated in terms of linearity, selectivity, recovery, precision and accuracy. This simple and sensitive method can be successfully used in research and easily adapted for analyses of other types of tyrosine kinases receptor inhibitors.

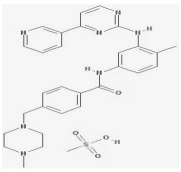
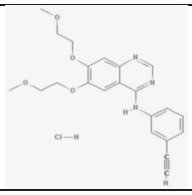
Key words: Erlotinib; imatinib mesylate; plasma; UPLC

1. Introduction

Cancer, a major public health problem, is defined as the uncontrolled growth and spread of abnormal cells. Among cancer treatments such as surgery, radiotherapy, phototherapy, gene therapy, immunotherapy, etc., chemotherapy, particularly nanotechnology-based chemotherapy, appears to be a more promising therapy. It is considered noninvasive, and it has a potential for further development (Gao *et al*., 2014; Payne *et al*., 2008; Degim and Kadioglu, 2013; Ilbasmis and Degim, 2012; Ilbasmis-Tamer *et al*., 2010; Mutlu Agardan *et al*., 2016). The active molecule substance is also important for effective therapy. Tyrosine kinase inhibitors bind to tyrosine kinases so that the cell-cycle is inhibited and thereby tumor growth is hindered. The research results have opened the door to a new era of target-directed therapies (Paul & Mukhopadhyay 2004; Bennasroune *et al*., 2004; Gschwind, Fischer & Ullrich, 2004; Smolle *et al*., 2014). In the human genome, the receptor tyrosine kinase includes 58 receptor types, such as epidermal growth factor receptor (EGFR), platelet derived growth factor receptors, fibroblast growth factor receptors (FGFRs), vascular endothelial growth factor receptors (VGFRs), Met (hepatocyte growth factor/scatter factor [HGF/SF] receptor), the insulin receptor, etc. (Smolle *et al*., 2014; Zwolak *et al*., 2008; Blume-Jensen & Hunter, 2001). Currently, at least 30 tyrosine kinase inhibitors are at various stages of clinical development (Madhusudan & Ganesan, 2004).

In parallel to these improvements, the determination methods and the method validations for these drugs should be required. Therefore, two different orally available drugs categorized as tyrosine kinases receptor inhibitors were chosen (imatinib mesylate and erlotinib hydrochloride) with the goal to determine their efficacy in mice plasma. Imatinib (IMA) has been used for gastrointestinal stromal tumors because of its inhibitor effects on PDGFR. It also can be used for chronic myeloid leukemia via its inhibitor effect on nonreceptor tyrosine kinases. Erlotinib hydrochloride (ERLO) has been used as a selective inhibitor of the EGFR for non-small-cell lung cancer treatment (Hartmann *et al*., 2009; Arora & Scholar, 2005). See Table 1 for the chemical properties of imatinib mesylate and erlotinib hydrochloride.

Table 1. Chemical Properties of Imatinib Mesylate and

	IMATINIB MESYLATE [21,22]	ERLOTINIB HCl [23,24,25]
Chemical Structure		
Chemical name	4-(4-methyl-piperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]-benzamide)	N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine
Molecular formula	$C_{29}H_{31}N_7O.CH_4SO_3$	$C_{22}H_{23}N_3O_4.HCl$
Molecular weight	589.7	429.90
pKa	7.8	5.42
Solubility in water	>100 g/L (at pH 4.2) 49 mg/L (at pH 7.4)	very slightly dependent on pH (max. solubility at pH:2)
Solubility in solvent	soluble in aqueous buffers ≤ pH 5.5 very soluble in methanol, and ethanol insoluble in n-octanol, acetone, and acetonitrile.	slightly soluble in methanol insoluble in acetonitrile, acetone, ethyl acetate and hexane

(Manley *et al*., 2010; PubChem Open Chemistry Database 2016; Hiltona *et al*., 2013; PubChem Open Chemistry Database 2016).

Different analytical techniques spectrofluorimetry, UV Spectrophotometric, High Performance Liquid Chromatographic (HPLC), spectroscopic, extractive colorimetric methods have been performed to determine the amount of IMA and ERLO in pharmaceutical dosage forms (Latha *et al*., 2013; Nageswaria, Reddy and Mukkanti, 2012; Kumar *et al*., 2010; Annapurna, Venkatesh & Chaitanya, 2014). Different analytical techniques have been performed to determine IMA and ERLO amounts in pharmaceutical dosage forms. This techniques include liquid chromatography-mass spectrometry, HPLC methods or ultrahigh performance liquid chromatography (UPLC) tandem mass spectrometry, their analyses in biological samples are still considered challenging (Kralj *et al*., 2012; Golabchifar *et al*., 2011; Neville *et al*., 2004). On the other hand, UPLC serves many advantages beyond the standard HPLC methods because they are able to resist very high pressures (15,000 psi). With these high pressures, system separating power and sensitivity increases, while the run time decreases (Marczylo, Steward & Gescher, 2009).

The aim of the study was to develop a suitable method for analyzing imatinib and erlotinib which could be applicable to the determination of several other tyrozine kinases receptor inhibitors. The development of a simple, cost-effective, selective and validated UPLC method is reported here. The method includes the PDA detector for the quantitation of two different tyrozine kinase receptor inhibitors in mice plasma.

2. Experimental

2.1. Materials and reagents

Imatinib mesylate (IMA) and erlotinib HCl (ERLO) obtained from Biotang Inc. Triethylamine, acetonitrile and ethanol with analytical grade obtained from Sigma Aldrich.

2.2. Instrumentation

A UPLC system (Waters Inc., Bedford, MA, USA) consisted of an Acquity UPLC binary solvent manager equipped with an Acquity automatic sample manager. A photodiode array detector (PDA) was used for the chromatographic determination of analytes.

2.3. Chromatographic conditions

Separation was achieved by isocratic elution using a mobile phase consisting of a mixture of 3mM triethylamine and acetonitrile with the ratio of 60:40. The column was XTerra® RP8 (5 μm 4.6 x 150 mm). The freshly prepared mobile phase was filtered through 0.20 μm PTFE filter and degassed continuously. The column temperature was kept at 40° C and the flow rate was fixed to 1.5 ml/min or 0.8 ml/min for imatinib mesylate or erlotinib hydrochloride, respectively. The run time was 8 min for imatinib mesylate and 7 min for erlotinib hydrochloride. The detection was achieved at 320 nm, and the injection volume was 20 μL.

2.4. Preparation of plasma samples for analysis

Imatinib mesylate aqueous solution was prepared with the concentration of 0.34 mM. Erlotinib hydrochloride (0.47 mM) was dissolved in 20% ethanol. These stock solutions were diluted to the desired concentration. Plasma samples of 200 μL were added to 200 μL of drug-containing solutions. A set of samples was prepared in 6 replicates under the same conditions. Samples were vortexed for a few seconds. Finally, 600 μL of acetonitrile were added and then the mixture was vortexed for more 30 seconds. All samples were centrifuged (15,000 rpm) for 10 minutes at 4° C. Supernatants were collected, filtered and analyzed.

2.5. Validation

2.5.1. Linearity

To evaluate the linearity of this method, stock solutions of the aforementioned drugs were diluted, and various concentrations of the drug solutions were obtained, ranging from 40 μg/ml – 0.5 μg/ml. Samples were analyzed using UPLC. The peak areas of each analytes were calculated. Calibration curves were obtained using the peak areas of samples and concentrations of analytes.

2.5.2. Selectivity

Method selectivity was assessed comparing the chromatograms of drug samples and drug-free plasma samples. Drug-free plasma samples were prepared using only an addition of acetonitrile.

2.5.3. Recovery

The recovery of samples was evaluated by analyzing three different quantities (low, medium and high) of the drugs. The results were expressed as percentages. Analyses were replicated six times. Mean, standard deviation (SD) and relative standard deviation (RSD) were also calculated.

2.5.4. Precision and accuracy

Intra-day and inter-day accuracy and precision were evaluated by performing ten replicates of the same sample (μg/ml) in the same day (intra-day precision and accuracy) and three replicate determinations of the drug at three different concentrations (20, 5, 1 μg/ml) within the three following days (inter-day precision and accuracy). The peak area was measured and the concentration of samples were calculated with mean, standard deviation and relative standard deviation.

2.5.5. Sensitivity (limit of detection (LOD) and of quantification (LOQ))

The limit of detection (LOD) was calculated as the concentration of samples which had three times higher than the background signal. The limit of quantification (LOQ) is the lowest concentration that can be determined. LOD and LOQ were calculated using the equations:

LOD: 3.3x σ /S

LOQ: 10x σ /S

where σ is the standard deviation of the lowest concentration measured and S is the slope of calibration line.

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by a Tukey-Kramer post hoc test using Instat2 (Graph Pad Inc., San Diego, CA). Differences were not considered significant if the p value was equal or higher than 0.05.

2.7. Results and discussion

2.7.1. Chromatographic conditions

The chromatographic conditions were optimized by considering the compositions of the solvents, mobile phase flow rate, and detector wavelength. These factors can affect the peak shape, retention time, height and the area of peaks. In this study, it is concluded that the most appropriate mobile phase was the mixture of 3mM triethylamine and acetonitrile (at a ratio of 60:40), considering smooth peak shape, explicit separation, and eliminated peak tailing. The flow rate of the mobile phase was selected by considering the separation of the analytes from the plasma peak. Therefore, the flow rate was fixed (1.5 ml/min) for IMA. However, the higher flow rates resulted in shorter retention times, but the plasma peak neared the peak of ERLO. For this reason, the flow rate was decreased, and the desired separation was obtained with the flow rate of 0.8 ml/min. As for the detection wavelength, after getting full spectrum, the maximum absorption wavelength was observed at 320 nm.

2.8. Method validation

2.8.1. Linearity

Linearity was determined for both IMA and ERLO and evaluated by analyzing seven different concentrations of samples at six times in UPLC. The peak areas with means and standard deviations were calculated for each analytes. Peak area versus concentration is depicted in Figure 1. The correlation coefficients, as an indicator of linearity, were 0.999 for IMA and ERLO.

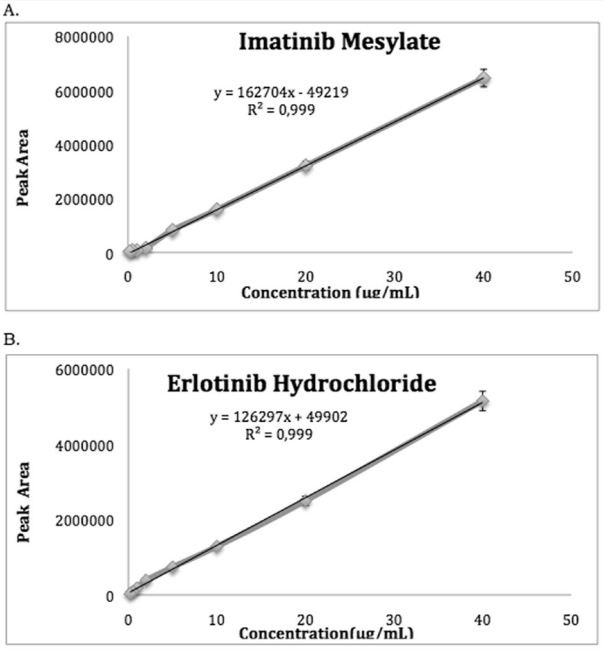


Figure 1. Linearity Graphs of Imatinib Mesylate (A) and Erlotinib Hydrochloride (B) (n=6).

2.8.2. Selectivity

No minimal interferences were observed when comparing the chromatograms of drug-free plasma samples with drug-containing plasma samples. Retention times of drug-free plasma samples were approximately 3 minutes for IMA and 1.6 minutes for ERLO. The retention times of drugs were 5.032 minutes for IMA and 4.926 minutes for ERLO. The chromatograms were given in Figure 2.

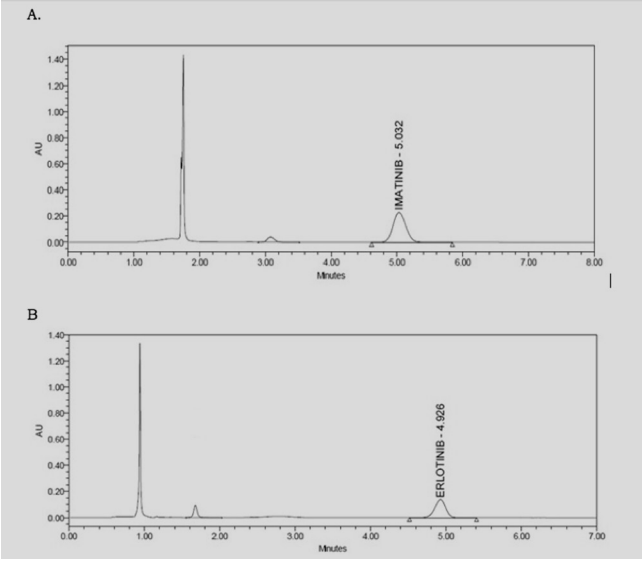


Figure 2. Chromatograms of IMA (A) and ERLO (B) in mice plasma

In the chromatograms, the first peak belongs to the plasma. These chromatograms obviously show that by adjusting the flow rate for the same column, the separation of other tyrosine kinase inhibitors may be possible. Moreover, peaks were found to be clear, smooth and symmetric. No peak-tailing or interference were observed.

2.8.3. Recovery

The recovery of the developed UPLC method for IMA and ERLO was investigated using three different concentrations (80%, 100% and 120%). All data with means, standard deviations and relative standard deviations are given in Table 2. The mean recoveries were found to be similar for both IMA and ERLO. The values of standard deviations for all the measurements were quite low. Furthermore, the values of RSD were lower than 2 for all concentrations.

2.8.4. Precision and accuracy

Precision and accuracy were carried out in terms of intra-day (reproducibility) and inter-day. Intra-day measurements and reproducibility were performed following 10 injections of 10mg/mL of each drug to the column. This method shows high reproducibility with the means of 9.829 and 9.738 and relative standard deviations of 1.572 and 1.976 for imatinib mesylate and erlotinib hydrochloride, respectively. Data are presented in Table 3.

Table 2. Evaluation of recovery for the determination of IMA and ERLO

Concentration of IMA (µg/ml)	Mean Recovery ± SD (µg/ml)	RSD
12	11,921 ± 0,118	1,008
10	9,898 ± 0,098	0,991
8	8,121 ± 0,106	1,310
Concentration of ERLO (µg/ml)	Mean Recovery ± SD (µg/ml)	RSD
12	12,035 ± 0,212	1,692
10	9,903 ± 0,190	1,921
8	8,124 ± 0,142	1,745

Table 3. The results of precision and accuracy for IMA and ERLO

	Conc. (µg/mL)	Recovery of IMA	Recovery of ERLO
1	10	10,058	9,448
2	10	9,910	9,955
3	10	9,801	9,487
4	10	9,591	9,886
5	10	9,962	9,651
6	10	9,734	9,539
7	10	9,816	9,777
8	10	9,878	9,914
9	10	9,594	9,802
10	10	9,947	9,921
Mean	10	9,829	9,738
SD	0	0,155	0,192
RSD	0	1,572	1,976

In addition, inter-day measurements were analyzed on samples with the concentrations 20, 5, 1 μg/mL over three consecutive days.. Six replicate determinations were performed for every single sample. Accuracy was also calculated as percentage (see Table 4) and by confidence interval. Statistical significance was also assayed in an attempt to determine whether possible differences which occurred in the method were significant or negligible. This was done by comparing the measurements over three

successive days. Data show that there was no statistically distinct discrepancy as p values were below 0.05.

Table 4. The results of inter-day measurements for IMA and ERLO

	Recovery of IMA		
Conc. (µg/mL)	20	5	1
1st day	20,155	5,187	0,990
2nd day	20,103	5,094	0,992
3rd day	19,775	5,099	0,991
Mean	20,011	5,127	0,991
SD	0,206	0,052	0,001
RSD	1,029	1,023	0,143
Accuracy	100,1	102,5	99,1
	Recovery of ERLO		
Conc. (µg/mL)	20	5	1
1st day	19,419	5,238	0,969
2nd day	19,839	5,252	0,945
3rd day	19,851	5,303	0,952
Mean	19,703	5,264	0,955
SD	0,246	0,034	0,012
RSD	1,249	0,645	1,344
Accuracy	98,5	105,3	95,5
Inter-day measurements were not statistically significant among the same concentrations (p>0,05			

2.8.5. Sensitivity (limit of detection (LOD) and of quantification (LOQ))

LOD and LOQ were calculated using the equation given in 2.5.5. The LOD in this method was 0.095 for IMA and 0.072 and ERLO. The lowest concentrations which could be accurately measured were 0.289 μg/mL for IMA and 0.218 μg/mL for ERLO.

2.9. Conclusions

This study explained a new method to determine two different tyrosine kinases inhibitors, imatinib mesylate and erlotinib hydrocloride, in mice plasma using very simple techniques. This detection technique was based on chromatography and can be used individually or in co-operative systems with spectrometry among the chromatography techniques. However, UPLC presents more advantages. The method discussed was developed and validated. It is rapid, user-friendly, economical, and beneficial since it is a sensitive method for imatinib mesylate (IMA) and erlotinib hydrocloride (ERLO) in mice plasma. This method provides a higher resolution in shorter time. Further study may reveal that this method may also be applicable for other types of tyrosine kinase inhibitors.

Conflicts of Interest

The authors declare no conflict of interest.

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تحديد Imatinib Mesylate و Erlotinib Hydrocloride في بلازما الفئران باستخدام الاستشراب السائل فائق الأداء

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الملخص

الغرض من هذه الدراسة هو تطوير طريقة سريعة وسهلة الاستخدام، اقتصادية وحساسة لتحليل erlotinib hydrocloride (ERLO) وimatinib mesylate (IMA) في بلازما الفئران باستخدام الاستشراب السائل فائق الأداء (UPLC). تمت عملية الفصل بواسطة الإمرار المتماثل باستخدام الطور المتحرك المُكون من 3 ملي من triethylamine و acetonitrile بنسبة 60:40 باستخدام عمود (5 XTerra® RP8 ميكرومتر 4،6 × 150 مم). كان زمن احتباس IMA هو 4،9 دقائق بمعدل تدفق قدره 1،5 ملليتر / دقيقة وكان زمن احتباس ERLO هو 5 دقائق بمعدل تدفق قدره 0،8 ملليتر / دقيقة. تم تحديد حجم الحقن إلى 20 ميكرو ليتر وكان الطول الموجي للكاشف هو 320 نانومتر. وكانت حدود الكواشف بالنسبة لـ (IMA) و (ERLO) هي 95 و72 نانوجرام / مل، على التوالي. تم التحقق من صحة هذه الطريقة من حيث الخطية والانتقائية والعناية والدقة. ويمكن استخدام هذه الطريقة البسيطة والحساسة في البحث بنجاح ويمكن تكييفها بسهولة لتحليل أنواع أخرى من مثبطات مستقبلات tyrozine kinases.