

MODERN INSTRUMENTAL METHODS FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF LAPATINIB IN BIOLOGICAL FLUIDS AND DOSAGE FORMS (REVIEW)

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ABSTRACT

Lapatinib is a small molecule, a heterocyclic quinazoline derivative. The drug is used for targeted therapy of patients with breast cancer, in which there is overexpression of the human epidermal growth factor receptors (HER/ErbB). This review is devoted to studying modern instrumental methods of qualitative and quantitative analysis of lapatinib, which can be used both for quality control and standardization (of bulk pharmaceuticals and dosage forms) and pharmacokinetics studies of a drug. Reverse-phase high-performance liquid chromatography (RP-HPLC) is mainly used to identify lapatinib in tablets. Depending on the purpose of the study, various detectors are used (ultraviolet or diode-matrix detector), which makes it possible to determine not only the native compound but also the products of its degradation. Definition of lapatinib in the presence of degraded products is necessary for forced degradation studies to determine drug stability. When a drug is being developed, it is important to define and understand its pharmacokinetics. For such studies, high-performance liquid chromatography (HPLC) coupled with the mass selective detector is often used. It allows determining lapatinib in biological fluids. However, these methods are not applicable for identifying the drug directly in dosage forms and require further development and validation.

Keywords: Lapatinib, HPLC, Fluorimetry, Validation, Simultaneous determination, Pharmacokinetics

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INTRODUCTION

Breast cancer is the most common malignant tumor in women in the world [1]. Overexpression or activation of the HER/ErbB is often observed in breast cancer. Moreover, in 15-20 percent of cases, overexpression of human epidermal growth factor receptor 2 (HER2/neu) is observed [2]. One of the treatment options for such patients is the use of targeted therapy, which is aimed at disrupting specific biological processes that lead to tumor growth [3]. The main strategies of targeted therapy are, on the one hand, the use of monoclonal antibodies (for example, trastuzumab, pertuzumab, etc.), and on the other, tyrosine kinase inhibitors (TKI) (for example, lapatinib, neratinib, etc.) [4].

The advantages of using TKI are the oral administration, a variety of targets, and less cardiotoxicity compared to monoclonal antibodies administered intravenously (i. v.) [5]. Moreover, the use of TKI (in particular, lapatinib) has made it possible to overcome resistance to trastuzumab [6].

Lapatinib (N-[3-Chloro-4-(3-fluorobenzyloxy) phenyl]-6-[5-[[[2-(methylsulfonyl)ethyl] amino] methyl]-2-furyl] quinazolin-4-amine) (fig. 1) is a small molecule, a heterocyclic compound derived from quinazoline. The lapatinib molecule exists in 3 forms: monohydrate, anhydrate, and solvate crystal forms. The active substance is in the form of the monohydrate (fig. 2) and exists as a single polymorphic form [7]. The drug's mechanism of action is as follows: it disrupts the phosphorylation of epidermal growth factor receptor (HER1) and HER2/neu by reversible competitive inhibition of adenosine triphosphate (ATP) binding sites in the intracellular kinase region. This leads to disruption of downstream signalling through RAF kinases, protein kinase B (Akt), extracellular signal-regulated kinase (ERK kinase), and phospholipase C- γ . As a result, the efficiency of inducing apoptosis significantly increases, which limits the development and migration of tumor cells [8, 9].

The drug is freely soluble in dimethyl sulfoxide (DMSO), slightly soluble in methanol, practically insoluble in acetone, alcohol, dichloromethane, and water. Due to its low solubility in water, lapatinib belongs to the II class according to the biopharmaceutical classification system (BCS) [10]. Furthermore, analysis of the thermodynamic properties of the drug indicates that the dissolution process is endothermic [11].

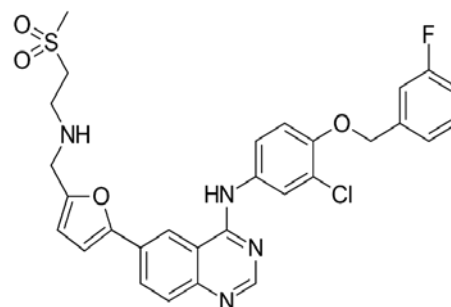


Fig. 1: Lapatinib

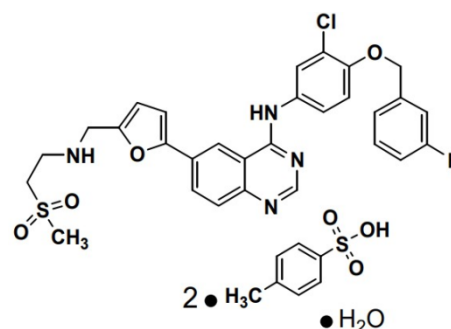


Fig. 2: Lapatinib ditosylate monohydrate

The value of the dissociation constant (pKa) (for the first stage pKa1 = 3.80; for the second stage pKa2 = 7.20) indicates that lapatinib is a strong base [12]. It is an extremely lipophilic molecule (distribution coefficient (logP) 5.4). It permeates membranes by passive diffusion. Due to the ability of lapatinib to cross the blood-brain barrier, the drug can be used to treat breast cancer with brain metastases [5, 13, 14]. Summarized data on the physicochemical characteristics of the drug is given in table 1.

Table 1: Physicochemical characteristics of lapatinib

Characteristic	Value	Reference
Appearance	Non-hygroscopic yellow crystalline solid	[7]
Solubility	Freely soluble in dimethyl sulfoxide (DMSO); slightly soluble in methanol; practically insoluble in acetone, alcohol, dichloromethane, water	[10]
Acid-base properties	Strong base (for the first stage of dissociation pKa1 = 3.80; for the second stage pKa2 = 7.20)	[12]
Lipophilicity	Extremely lipophilic (logP = 5.4)	[12]
Isomers	None	[7]

One of the major disadvantages of lapatinib is limited oral bioavailability, which varies significantly from patient to patient. The main reason for the low bioavailability is its poor solubility, which necessitates very high doses of lapatinib and limits the creation of injectable forms of the drug. In addition, the clinical use of lapatinib in the form of film-coated tablets is limited by its high affinity for albumin (protein binding >99 %), which leads to a decrease in its therapeutic effect [15, 16].

The scientific literature describes methods for the quantitative determination of lapatinib in conjunction with other TKI (e. g., imatinib, nilotinib, sorafenib, erlotinib, dasatinib, etc.). Such studies are used for routine analysis of these drugs in biological fluids (blood plasma, urine) during clinical trials due to the large individual variability of the pharmacokinetics of TKI [17-19]. On the other hand, lapatinib is often used in combination therapy of tumors, for example, together with monoclonal antibodies (trastuzumab), cytostatics (paclitaxel, cisplatin, temozolomide), antineoplastic hormonal agents (letrozole), and antimetabolic drugs (capecitabine) [20-25].

To prepare this review search criterion was the modern instrumental methods of qualitative and quantitative analysis of lapatinib. Literature survey has been done in a range of years 1990-2020 to make the review updated and comprehensive and to show the methods which can be used both for quality control and standardization (of the substance and dosage forms) and for pharmacokinetics studies of the drug. The sources were world-recognized journals. The keywords used were "Lapatinib", "HPLC", "validation", "pharmacokinetics".

Standardization of lapatinib in the dosage form

For the determination of lapatinib in tablets, a simple, selective, fast, accurate, and cost-effective method of reverse-phase high-performance liquid chromatography coupled with an ultraviolet detector (RP-HPLC-UV) using an internal standard (IS) has been developed [26]. Methods that use an IS are most suitable for quality control of drugs because they eliminate errors associated with sample preparation and variability between analyses, increasing the method's accuracy and precision. Moreover, such methods are highly sensitive and specific.

Gemcitabine hydrochloride was used as an IS since it is structurally similar to the test substance, and the peaks obtained in the chromatogram were symmetric and had good resolution.

The chromatogram also showed no pronounced peaks of other compounds except the peak of lapatinib. It indicates that the excipients contained in the tablet did not affect the analysis results, confirming the method's specificity.

The retention time (RT) of lapatinib and gemcitabine hydrochloride were 4.25±0.05 min and 6.10±0.05 min, respectively. The limit of detection (LOD) and limit of quantification (LOQ) of lapatinib were 26.5 and 88.4 µg/l, respectively.

A simple and sensitive method of RP-HPLC-UV was developed to determine lapatinib together with related impurities in the bulk pharmaceuticals and finished tablets [27]. This method has also been validated for routine use in quality control laboratories.

In similar studies [26, 28-30], describing the determination of related impurities of lapatinib by HPLC, the resulting peak shapes of impurities were broad, and there was no clear separation between them. Since impurities can significantly affect the quality, safety, and efficacy of a drug, it is necessary to control its content.

The method described in the study by Ivaturi *et al.* made it possible to obtain a chromatogram with a symmetric peak shape and good resolution between them [27]. For this purpose, the products of forced degradation were introduced into the sample. As a result, it was found that all three known impurities (impurity 1, impurity 2, and impurity 3) were spectrally pure but at the same time had rather wide peak shapes. Analysis using the isocratic elution mode was also time-consuming.

To reduce the analysis time, its conditions were optimized. The elution mode was changed to the gradient. Mobile phase A was a ten mmol ammonium formate solution (pH 4.5), and mobile phase B was acetonitrile. As a result, the total analysis time was reduced to 35 min. The LOD and LOQ of lapatinib were 1 and 4 µg/l, respectively [27].

The method of reverse-phase high-performance liquid chromatography using a diode array detector (RP-HPLC-DAD) was used to detect lapatinib in the bulk pharmaceuticals in the presence of its degradation products [28]. The RT of lapatinib was 4 min. The LOD and LOQ are 1 and 5 µg/l, respectively.

Identification using a diode array is explained by the need to control the purity of the peaks. In addition, degradation products were analyzed by mass spectrometry to understand the degradation pathways better. The purpose of this study was not to identify degradation products of lapatinib but to study the purity of its peaks in the chromatogram.

This method allows to separate lapatinib and its degradation products; therefore, it can be used for analysis in forced degradation studies to determine the stability of the formulation. These tests showed that lapatinib is stable when exposed to high temperatures. Moreover, it is stable in a neutral environment and less stable in acidic and alkaline environments.

Some researchers point to the efficacy of the combined use of lapatinib and paclitaxel in patients with squamous cell carcinoma of the esophagus and HER2-positive breast cancer [31, 32]. Therefore, a reliable and fast RP-HPLC-DAD method was developed to determine these two drugs simultaneously [33]. This method was also used to assess the release profile of paclitaxel and lapatinib from polymeric micelle formulation.

Chromatographic conditions were similar to those in the study [31]. The differences were in the slower flow rate (0.5 ml/min), the volume of injected samples (25 µl), and the intervals between injections of the sample (every 30 min). The isocratic elution mode was also chosen for the analysis since it is simple and involves fewer variables that can potentially affect the method optimization process. The RT was 9 and 17 min for paclitaxel and lapatinib, respectively. The LOQ was 5 µg/l for both drugs.

Research by S. Biswal and S. Mondal aimed to develop and validate an ultra-high-performance liquid chromatography method specific to evaluating lapatinib in tablets in the presence of its degradation products [34].

The conditions for the analysis were optimized: the mobile phase was a mixture of 0.1 % OPA buffer and acetonitrile (30:70 by volume). The flow rate was 0.25 ml/min, and the volume of the injected sample was 5 µl. In addition, a matrix photodiode detector was chosen for detection.

The maximum absorption of lapatinib was observed at a wavelength of 309 nm (fig. 3). The total analysis time was 3 min. The LOD was 0.06 µg/l and the LOQ was 0.18 µg/l.

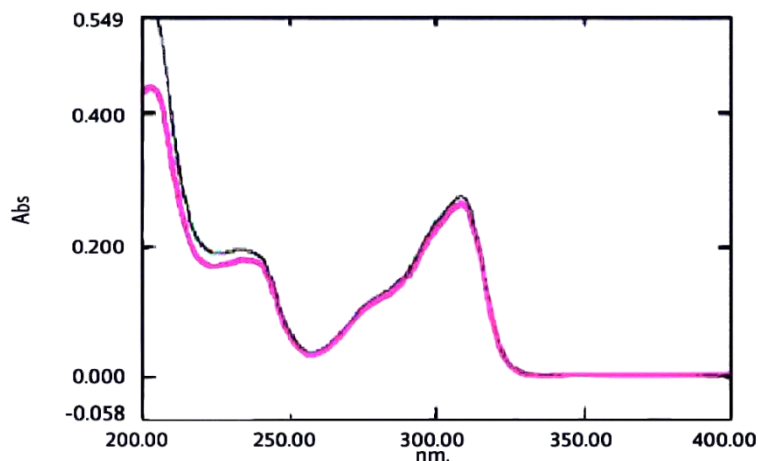


Fig. 3: Ultraviolet spectrum of lapatinib (with maximum absorption at a wavelength of 309 nm) [31]

Based on the results of analysis of samples of forced degradation using the described method, it can be concluded that there is no intersection of their peaks with the major peak of lapatinib. This indicates that the developed method is specific for evaluating lapatinib in the presence of its degradation products. In addition, this method has excellent sensitivity, accuracy, and reproducibility.

In the study by Darwish *et al.* the conditions of fluorometric analysis of lapatinib in tablets and urine and in the presence of RH40

colliphore to enhance fluorescence were described for the first time [35]. The spectra (fig. 4) were recorded on a JASCO FP-8200 fluorescence spectrometer (JASCO Corporation, Japan) with a 150 W xenon lamp, quartz cuvettes 1 cm long, and a monochromator slit of 5.0 nm. An 8-vial automatic dissolution tester (Abbott Corporation, US) was used to study drug release *in vitro*. The measurement was carried out at an analytical wavelength of 420 nm and an excitation wavelength of 292 nm. The LOD was 27.31 $\mu\text{g/l}$, and the method accuracy was $\geq 99.82\%$.

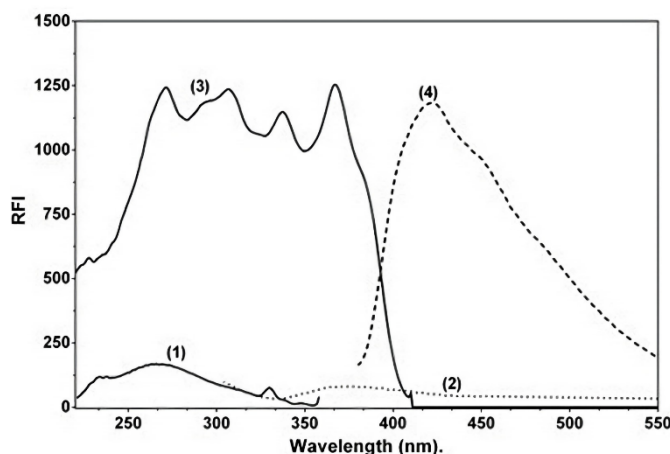


Fig. 4: Fluorescence spectra of lapatinib (1000 ng/ml) and a mixture of lapatinib-colliphore RH40 (2%) [32], (1) and (2)-excitation and emission (respectively) of an aqueous solution of lapatinib in the absence of an RH colliphore; (3) and (4)-excitation and emission (respectively) of an aqueous solution of lapatinib containing RH colliphore

This method has been used successfully to determine lapatinib in tablets, perform dissolution tests and determine content uniformity. The scope of the technique was expanded to determine the drug in urine samples with an accuracy of $(99.82 \pm 3.45)\%$. The fluorimetric analysis is an environmentally friendly and efficient alternative to existing analytical methods for lapatinib determination.

Pharmacokinetics studies

When a drug is developed, it is important to define and understand its pharmacokinetics, including its absorption, distribution, metabolism, and excretion [36].

Most of the assays described in the literature have been developed to detect and quantify lapatinib after high doses of the drug in daily clinical practice [30, 37-39]. The purpose of Musijowski J. *et al.* study was to develop and validate a sensitive method of liquid chromatography in combination with a single quadrupole mass

analyzer to determine lapatinib in human blood plasma [40]. This method is applicable for pharmacokinetics studies after a single oral dose of 0.25 g of lapatinib.

This method is characterized by high sensitivity: LOQ was 5 $\mu\text{g/l}$. The total analysis time was 11 min, the lapatinib RT was 3.5 min.

For economic reasons, triple quadrupole mass spectrometry detectors are not as widely available as single quadrupole ones. Thus, this method expands the instrumental capabilities for the analysis of the drug.

Pharmacokinetics preclinical studies

Patients with metastatic breast cancer often suffer from pain at different stages of the disease. Paracetamol is one of the most common antipyretic and pain-relieving drugs. The most serious adverse reaction associated with paracetamol intake is the

hepatotoxic effect of one of its metabolites, N-acetyl-p-benzoquinone imine (NAPQI) [41]. Hepatotoxicity can be observed with insufficient amounts of glutathione [42]. Paracetamol and lapatinib will likely be used at the same time. Therefore, possible interactions between drugs can affect their pharmacokinetics. Consequently, it is important to know the potential effect of lapatinib on paracetamol metabolism *in vivo*.

Karbownik A. *et al.* study aimed to explore the effect of lapatinib oral administration on the pharmacokinetics of paracetamol, its glucuronidation, and sulfation in rats [43]. In addition, they analyzed the changes of lapatinib pharmacokinetics parameters after concomitant administration with paracetamol.

The study consisted of two parts. First, the concentrations of paracetamol, its glucuronide, and sulfate were determined using HPLC coupled with an ultraviolet detector (HPLC-UV) method. Second, quantitative determination of lapatinib in blood plasma samples was performed by HPLC in combination with a three-quadrupole mass spectrometric detector (HPLC-MS/MS). The LOQ was 0.25 µg/l.

Lapatinib is a substrate and inhibitor of P-glycoprotein (Pgp). Inhibition of Pgp may increase the concentration of drugs that are its substrates [44]. Therefore, the risk of adverse reactions increases significantly when using lapatinib in combination with cytochrome P450 3A4 (CYP3A4) inhibitors such as clarithromycin, a widely used macrolide antibiotic.

Karbownik A. *et al.* investigated the effect of clarithromycin on the pharmacokinetics of lapatinib in rats [45]. For this, drugs were sequentially administered to rats: 0.025 g/kg clarithromycin, then 0.100 g/kg lapatinib.

Quantitative determination of lapatinib in blood plasma samples was carried out by HPLC-MS/MS. The method also assumed the use of erlotinib as an IS since it is a structural analogue of lapatinib. The selected IS ensured the high accuracy and precision of the method. The LOQ was 0.25 µg/l.

In 1997, a group of scientists demonstrated the possibility of molecular imaging of peptides on biological tissue sections using matrix laser desorption ionization in combination with mass spectrometry (MALDI-MSI) [46]. This method has many advantages that make it most suitable for mapping pharmaceuticals in tissues: it is a high-sensitivity method capable of detecting small amounts of the analyte [47]. In addition, detection by mass spectrometry allows simultaneous imaging of several species and distinguishing between the distribution of the parent drug and its metabolites. An additional advantage is that mass spectroscopy does not require the use of radiolabeled standards, so such studies are most cost-effective in the early stages of the drug development process.

Barry J. A. *et al.* detected lapatinib and several metabolites in liver tissue by mass spectrometric imaging using infrared array laser desorption with electrospray ionization (IR-MALDESI). The method was combined with high-resolution mass spectrometers and ion cyclotron Fourier Transform Resonance (FT-ICR). IR-MALDESI is the first method with atmospheric pressure ionization. Although the MALDESI process was first described for ultraviolet desorption, it is independent of the laser wavelength. Hence any laser wavelength can be used, provided the appropriate absorbing matrix (endogenous or exogenous) is selected [48].

Summarized information on the described methods of lapatinib analysis is given in table 2.

Table 2: Methods of lapatinib analysis

Analysis method	Analyzed compound	Analysis object	Purpose of the study	Reference
RP-HPLC-UV	Lapatinib	Tablets	Standardization, quality control	[26]
RP-HPLC-UV	Lapatinib in the presence of degradation products	Tablets	Standardization, quality control	[27]
RP-HPLC-DAD	Lapatinib together with paclitaxel	Polymeric micelles	Standardization, establishment of release profiles from a new dosage form	[28]
RP-HPLC-DAD	Lapatinib in the presence of degradation products	Tablets	Standardization, quality control	[33]
UHPLC	Lapatinib	Tablets	Standardization	[34]
Fluorimetry	Lapatinib	Tablets, urine	Standardization, dissolution test, content uniformity	[35]
HPLC-MS	Lapatinib (in low concentrations)	Blood plasma	Pharmacokinetics studies	[40]
HPLC-MS/MS	Lapatinib in the presence of paracetamol	Blood plasma	Pharmacokinetics preclinical studies	[43]
HPLC-MS/MS	Lapatinib together with clarithromycin	Blood plasma	Pharmacokinetics preclinical studies	[45]
IR-MALDESI	Lapatinib	Liver tissue	Pharmacokinetics preclinical studies	[48]

HPLC is one of the most widely used methods for the analysis of pharmaceuticals [49]. Table 3 shows the main characteristics of

the conditions for the chromatographic determination of lapatinib.

Table 3: Characterization of conditions for chromatographic determination of lapatinib

Analysis method	IS	Elution mode	RT, min	LOD, µg/l	LOQ, µg/l	Reference
RP-HPLC-UV	Gemcitabine	Isocratic	4.25±0.05	26.5	88.4	[26]
RP-HPLC-UV	None	Gradient	15.21	1	4	[27]
RP-HPLC-DAD	None	Isocratic	4	1	5	[28]
RP-HPLC-DAD	None	Isocratic	17	1	5	[33]
UHPLC	None	Isocratic	0.516	0.06	0.18	[34]
HPLC-MS	Isotope labeled lapatinib	Isocratic	3.5	-	5	[40]
HPLC-MS/MS	Erlotinib	Gradient	-	-	0.25	[43]
HPLC-MS/MS	Erlotinib	Gradient	-	-	0.25	[45]

CONCLUSION

From the described methods, it is preferable to use RP-HPLC, using an IS for the quantitative determination of lapatinib in dosage forms.

Several new methods for the quantitative determination of lapatinib using HPLC in combination with various types of detectors in biological fluids have been described. However, these methods are not applicable for determining the drug directly in dosage forms and require further development and validation.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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