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# Liquid chromatography–tandem mass spectrometric assay for the T790M mutant EGFR inhibitor osimertinib (AZD9291) in human plasma

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ABSTRACT

A method for the quantitative analysis by ultra-performance liquid chromatography–tandem mass spectrometry of the highly selective irreversible covalent inhibitor of EGFR-TK, osimertinib in human plasma was developed and validated, using pazopanib as an internal standard. The validation was performed in a range from 1 to 1000 ng/ml, with the lowest level corresponding to the lower limit of quantitation. Gradient elution was performed on a 1.8 μm particle trifunctional bonded C18 column by 1% (v/v) formic acid in water, and acetonitrile as mobile phase. The analyte was detected in the selected reaction monitoring mode of a triple quadrupole mass spectrometer after positive ionization with the heated electrospray interface. Within-day precisions ranged from 3.4 to 10.3%, and between-day precisions from 3.8 to 10.4%, accuracies were 95.5–102.8%. Plasma (either lithium heparin or sodium EDTA) pretreatment was performed by salting-out assisted liquid–liquid extraction using acetonitrile and magnesium sulfate. This method was used to analyze the osimertinib blood plasma levels of five adult patients with metastatic T790M mutated non-small cellular lung carcinoma for therapeutic drug monitoring purposes.

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## 1. Introduction

With the first generation epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI), advanced non-small cell lung carcinoma (NSCLC) patients harboring a sensitizing EGFR mutation (EGFRm(+)) were provided an initial significant improvement in terms of treatment [1]. Unfortunately, most of these patients ultimately develop disease progression due to the acquiring of a second mutation in the EGFR-TK gene. In approximately 60% of these patients a T790M mutation leads to decreased affinity of the current TKIs [2]. Osimertinib (AZD9291, Merelitinib, Tagrisso©; Fig. 1) is a third generation, highly selective, irreversible covalent inhibitor of EGFR-TK, selective for both the EGFRm(+) sensitizing, as well as the T790M, L858R ($IC_{50}$: 11.4 nM), and exon 19 deletion ($IC_{50}$: 12.9 nM) EGFR-TKI resistance mutations, leaving the wild-type EGFR-TK ($IC_{50}$: 493.8 nM) untouched [1,3–5]. Osimertinib was granted accelerated approval by the US-FDA for patients with advanced T790 M mutated tumors, and developed disease progression after treatment with other EGFR-blocking therapy [6]. Osimertinib has a relatively long plasma half-life of 48 h, with the main metabolites circulating at approximately 10% of the parent compounds geometric mean exposure. The main active metabolites are the desmethyl metabolites AZ7550 and AZ5104 [3]. These metabolites could not be included in the assay due to the lack of analytical standards. To the best of our knowledge, one LC–MS/MS method for the determination of osimertinib and metabolite concentrations in biological

*Abbreviations:* TKI, tyrosine kinase inhibitor; EGFR-TK, epidermal growth factor receptor-tyrosine kinase; IS, internal standard; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LLE, liquid–liquid extraction; NSCLC, non-small cell lung carcinoma; PP, protein precipitation; SALLE, salting-out assisted liquid–liquid extraction; SPE, solid-phase extraction.

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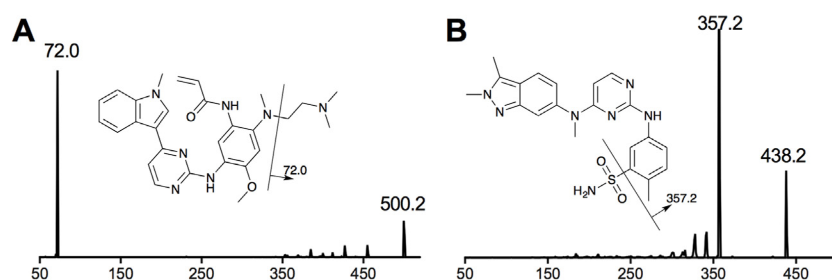


**Fig. 1.** Product spectra, chemical structures and proposed fragmentation patterns of A) osimertinib ($m/z$ 500.2) at −25 V collision energy, and B) the internal standard pazopanib ($m/z$ 438.2) at −30 V.

samples has been published hitherto, giving very little detail about the materials, procedure, and validation [7].

This paper describes the development and validation of an ultra-performance liquid chromatographic tandem mass spectrometric method for the quantitative analysis of osimertinib in human plasma, using pazopanib as an internal standard. Samples were pretreated by an optimized salting-out assisted liquid–liquid extraction (SALLE), using a system of plasma, acetonitrile and magnesium sulfate ($MgSO_4$). This method provides a simple, fast and accurate method for the quantitative analysis of osimertinib. In this paper we describe the osimertinib blood plasma levels of five adult patients who have received 80 mg osimertinib once daily to support treatment with the drug.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Osimertinib (>98% purity) and pazopanib (99.1% purity) were acquired from Sequoia Research Products (Pangbourne, UK). Water (LC–MS grade), and acetonitrile (HPLC-S grade) were purchased from Biosolve (Valkenswaard, The Netherlands). Anhydrous magnesium sulfate was supplied by Sigma Aldrich (St. Louis, MO). Formic acid was of analytical grade and originated from Merck (Darmstadt, Germany). Analytical grade dimethyl sulfoxide (DMSO) was supplied by Acros (Geel, Belgium). Pooled human lithium-heparinized plasma and EDTA plasma was obtained from Sera Laboratories (Haywards Heath, West Sussex, UK). For the evaluation of selectivity and matrix effect, lithium-heparinized plasma and EDTA plasma of six individual human donors was obtained from Innovative Research (Novi, MI, USA). Pure water used for other uses than mobile phase was prepared in house using a Synergy UV apparatus (Merck Millipore, Darmstadt, Germany).

### 2.2. Chromatography and MS/MS method

The UHPLC–MS/MS equipment consisted of an Accela pump and auto-injector, coupled through a diverter valve to a TSQ Quantum Ultra triple quadrupole mass spectrometer with a heated electrospray ionization interface (HESI; all from Thermo Fisher Scientific, San Jose, CA, USA). Partial loop injections of 10 μl were made onto a Waters® Acquity UPLC® BEH300 C18 column (50 mm × 2.1 mm, 1.7 μm, Waters, Milford, USA). The components were eluted by a gradient of A) 1.0% formic acid in water and B) acetonitrile. The 2-min gradient was running linearly from 10% B to 55% B in 1.2 min after injection, after which an 100% organic flush with acetonitrile of 0.3 min was performed. The flush-period was followed by a 0.5 min re-equilibration of the column. The whole eluate was introduced into the HESI source in positive ionization mode, starting at 0.6 min after injection by switching the diverter valve, up to 1.3 min.

For initial tuning of the MS parameters, a 10 μg/ml solution of osimertinib was infused at 5 μl/min, while introducing a 600 μl/min flow mixture of 0.1% (v/v) formic acid in water, and methanol (1:1; v:v). The highest response was obtained with a 5000 V spray voltage, 400° C spray temperature, and a 329° C capillary temperature. The nitrogen sheath, ion sweep and auxiliary gasses were set at respectively 60, 0 and 5 arbitrary units; the skimmer voltage was set off (0 V). The SRM mode with 0.2 s dwell times was used with argon as the collision gas at 1.5 mTorr. The tube lens offset was 114 V for osimertinib and 130 V for pazopanib. Osimertinib was monitored at $m/z$ 500.2 → 72.1 at −31 V collision energy, the internal standard pazopanib at $m/z$ 438.2 → 377.15 at −28 V collision energy. The mass resolutions were set at $m/z$ 0.7 for both separating quadrupoles.

#### 2.2.1. Data processing

For acquiring chromatography–mass spectrometric data, Thermo Fisher Xcalibur software (version 2.0.7 SP1) was used. For further data processing Microsoft Excel (Office 2016, Version 15.11.2) was used. Averages (±SD), and outcomes of the validation were calculated using MS Excel.

### 2.3. Standard solutions and quality controls

For osimertinib, a stock solution was made by weighing an amount of osimertinib, which was dissolved in an appropriate volume of DMSO, giving a final concentration of 500 μg/ml. The same method was used to create a 500 μg/ml stock solution of the internal standard pazopanib, which was then diluted to 25 ng/ml using acetonitrile. Serial dilution of the osimertinib stock standards using blank human plasma was performed to produce calibration standards at 1000, 500, 100, 50, 10, 5, and 1 ng/ml. From a separate stock solution, the QC samples of 800 (high), 200 (medium), 3 (low) and 1 ng/ml (LLoQ) were produced. Calibration and QC samples were aliquoted in 0.5 ml polypropylene reaction tubes, and stored at −30° C until further use.

### 2.4. Sample preparation

Samples were prepared by SALLE. For this method, 20 μl of plasma was transferred to a 1.5 ml polypropylene reaction vial. Subsequently, 20 μl of a 2 M $MgSO_4$ solution and 100 μl of 25 ng/ml pazopanib in acetonitrile was added. The tubes were closed and vortex mixed for approximately 10 s after which they were mixed further by inverting on a Rotamix RM1 mixer (ELMI, Riga, Latvia) at 45 RPM for 15 min. The reaction vials were then centrifuged for 5 min at 15,000 × $g$ at 22° C to aid phase separation. Of the upper organic liquid, 75 μl was transferred to an injection vial with a 200 μl insert. Before closing the vial, 75 μl water was added to ensure compatibility with the chromatographic system.

### 2.5. Analytical method validation

A laboratory scheme based on international guidelines, published by the EMA [8] and FDA [9] was used for the validation procedures.

#### 2.5.1. Calibration and carry-over

The calibration samples were prepared as described in Section 2.3, and were processed for each daily calibration. Least squares linear regression ($1/X^2$ weighted) was used to define the calibration curve, using the analyte/IS peak ratio. Auto injector carry-over was determined by injecting the highest calibration standard, followed by injection of three blank samples. The response of the blanks was then compared to the LLOQ.

#### 2.5.2. Precision and accuracy

Separate stock solutions of 500,000 ng/ml of osimertinib were used to obtain validation samples in human heparin plasma (800, 200, 3, and 1 ng/ml). Precisions and accuracies were determined by sextuple analysis of each QC sample in three analytical runs on three separate days for all QCs (total: n = 18 per QC). Relative standard deviations and accuracies were calculated for both within- and between-day precisions. For EDTA samples precisions and accuracies were determined by sextuple analysis of each QC sample (n = 6 per QC).

#### 2.5.3. Selectivity

Selectivity of the method was determined by comparing the response of six individual human lithium heparin plasma samples and six individual human EDTA plasma samples. The relative and absolute effect was measured by processing samples spiked at LLoQ level (1 ng/ml), and blank samples with (blank) and without (double blank) internal standard.

#### 2.5.4. Recovery and matrix effect

To determine the recovery of osimertinib, processed QC samples (high, medium, and low, n = 4) were compared to blank plasma extracts spiked with reference solutions at the same levels, representing 100% extraction efficiency. For evaluation of the matrix effect, the responses of the spiked reference solutions were compared to matrix-free solutions, at QC levels high, medium, and low. Relative matrix effects for both lithium heparin and EDTA were determined at the high and low QC levels. Matrix-free solutions consisted of acetonitrile with water in the same proportions as the sample to insure the compatibility with the chromatographic system. Additionally, an infusion experiment was performed, where the MS response was monitored while continuously infusing osimertinib and pazopanib (both 5 μg/ml, at 5 μl/min), during which extracted blank plasma samples were injected.

#### 2.5.5. Stability

*2.5.5.1. Benchtop stability.* Quadruplicate stability tests at room temperature were evaluated for heparinized plasma by comparing the response of QC samples at the low, medium, and high levels stored at room temperature for four hours, with fresh samples.

*2.5.5.2. Freeze-thaw stability.* Quadruplicate analysis of human heparin plasma samples from separate tubes was performed after storage at 20° C for 8 h, followed by three freeze-thaw cycles (thawing at 20° C during ca. 2 h and freezing again at −30° C for at least 12 h).

*2.5.5.3. Long term stability.* QC samples at low, medium, and high levels were produced in lithium heparin, and stored at −30° C for an extended period of 75 days. These samples were analyzed, and the relative responses were compared to freshly prepared QC samples.

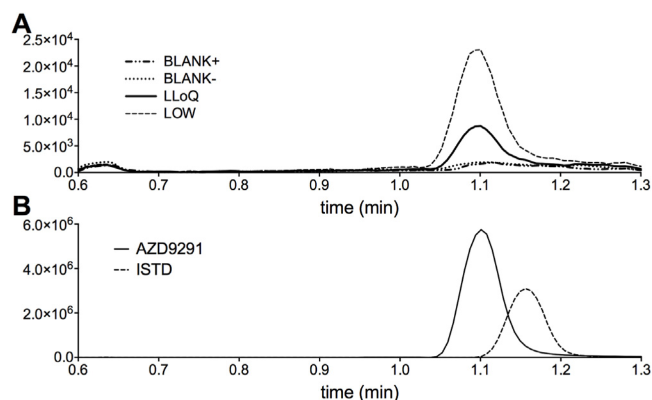


**Fig. 2.** Chromatograms of extracted human plasma, A) with and without analyte and/or IS. B) representative chromatogram, showing the retention times and overlap of osimertinib and the internal standard (pazopanib).

*2.5.5.4. Auto injector stability.* An analytical run of 18 QC samples was re-injected after 24 h. The auto injector cooling was turned off during this period, mimicking device failure.

*2.5.5.5. Stock stability.* The responses of osimertinib from the stock solutions in DMSO, after 2 months at −30° C (n = 2) were compared to fresh stock solutions with LC–MS/MS after appropriate dilution of the samples with 50% (v/v) acetonitrile, and adding internal standards. Of the diluted stock, 500 μl was transferred to a vial, and 500 μl water was added before closing the vial to increase the compatibility with the chromatographic system.

### 2.6. Patient samples

The method was used to determine blood-plasma levels for 34 patients receiving oral osimertinib therapy, 80 mg once daily. Drug analysis was part of a therapeutic drug monitoring service to support therapy. Sodium-EDTA plasma samples were taken after 24 h (trough levels), and were analyzed in duplo. The samples were analyzed in two separate runs to evaluate the incurred sample analysis.

## 3. Results and discussion

### 3.1. Method development

#### 3.1.1. Chromatography and LC–MS/MS method

The settings for ESI–MS/MS were optimized for osimertinib as to obtain maximal sensitivity; a product spectrum of osimertinib is presented in Fig. 1. Formic acid provided the maximum response compared to other MS compatible additives, such as ammonium hydroxide and ammonium acetate. Acetonitrile resulted in narrower, sharper peaks than methanol when used as eluent. Gradient elution was optimized to facilitate the 10 μl injection volume and was therefore started at a relatively low percentage of 10% acetonitrile. Representative chromatograms of extracted human plasma, with and without analyte and/or IS are shown in Fig. 2. Column equilibration for 0.5 min (with an additional between run time of ca. 0.5 min for the autosampler duty cycle) showed sufficient equilibration based on retention time stability (RSD < 0.5%). Osimertinib eluted at 1.09 min, the internal standard pazopanib at 1.14 min.

Incorporation of a high organic flush at the end of each run prevented the build-up of strongly retained plasma-components that could cause long term suppressive effects on ion formation, such as phospholipids. Positive ESI–MS/MS settings were optimized for

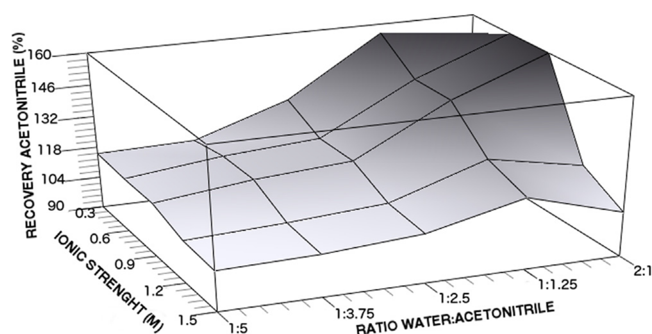


**Fig. 3.** Effect of $MgSO_4$ concentration and water to acetonitrile ratio on partitioning of water acetonitrile mixture. At an ionic strength of 0.3 M and a ratio of 0.2, no phase separation occurred.

protonated osimertinib and the internal standard, pazopanib. The product spectrum of osimertinib is shown in Fig. 1. Because the stable isotope labeled osimertinib was commercially unavailable at the time, pazopanib was chosen as an internal standard because is it not co-administered with osimertinib and because it shows similar retention under the selected conditions.

#### 3.1.2. Sample preparation

Various methods have been used in the bioanalysis of small-molecule tyrosine kinase inhibitors, following either LC–MS/MS or less common, LC-UV [10]. The most commonly used methods are simple crash-dilute-and-shoot protein precipitation or the more labor intensive and more selective liquid–liquid extraction and solid-phase extraction, following evaporation of non LC–MS/MS compatible solvents [11]. Recently, another method emerged in this field: Salting-out Assisted Liquid–Liquid Extraction (SALLE) [12]. The present SALLE method uses three simple steps, and avoids laborious steps, such as evaporation of non-compatible solvents.

SALLE was chosen as the pretreatment method over protein precipitation (PP) and the more laborious regular liquid extraction (LLE) and solid-phase extraction (SPE). Extraction methods provide a cleaner sample compared to PP, and LLE uses organic solvents like TBME, whereas SALLE uses ACN that is more common and often less toxic for personnel and/or environmental impact. For 'wetting', equilibration and elution SPE usually uses up to 1 ml of solvent, whereas for PP and SALLE less than 100 μl is sufficient. The reduced volume, and LC-compatible solvent removes the need for solvent evaporation [10] resulting in an improved handling speed of SALLE over LLE and SPE.

Various salts were tested during optimization (namely $CH_3COONH_4$, $KH_2PO_4$, $K_2HPO_4$, NaCl, $MgCl_2$, $MgSO_4$, $ZnSO_4$), in multiple concentrations. The concentration ranged from 0.3 to 1.5 M final concentration, or a saturated solution when saturation occurred below 3.0 M. $MgSO_4$ provided the optimal signal-to-noise. Although some other salts showed comparable results, the advantage of $MgSO_4$ over other salts is that after centrifugation a layer of precipitated protein is formed between the two liquid layers, effectively sealing the two layers from each other. This layer stabilizes the phase-separation and provides a good visual aid in pipetting off the upper layer. For the SALLE method, the volume ratio of acetonitrile versus water was determined experimentally by varying the amounts of both, as well as the concentration of $MgSO_4$ (Fig. 3). The optimization was focused on minimizing the acetonitrile volume to limit sample dilution by maximizing salt concentration, whilst avoiding precipitation of salts. Based on the volume of the upper phase (mostly acetonitrile), a sample:saline:acetonitrile ratio of 1:1:5; v/v was used for sample pretreatment, with a saline concentration of 2.0 M $MgSO_4$. The selected ratio resulted in a sufficient recovery of osimertinib.

**Table 1**
Within and between day precision, and accuracy of osimertinib in lithium heparin spiked human plasma samples (n = 18).

| Level (ng/ml) | Within-day precision | Between-day precision | Accuracy |
|---|---|---|---|
| 800 | 3.4% | 3.8% | 95.5% |
| 200 | 2.8% | 3.6% | 96.9% |
| 3 | 6.5% | 10.2% | 99.5% |
| 1 | 10.3% | 10.4% | 102.8% |

**Table 2**
Within day precision, and accuracy of osimertinib in spiked human Sodium-EDTA plasma samples (n = 6).

| Level (ng/ml) | Within-day precision | Accuracy |
|---|---|---|
| 800 | 2.6% | 101.5% |
| 200 | 5.3% | 103.7% |
| 3 | 4.5% | 102.2% |
| 1 | 4.4% | 101.2% |

### 3.2. Analytical method validation

For this assay, the upper limit of quantitation was proposed at 1000 ng/ml because initial drug levels were reported at approximately 50–1000 ng/ml [1,4]. A range of 1000 orders of magnitude was deemed suitable for quantifying osimertinib in patients, so the validation was performed at a range from 1 to 1000 ng/ml. The sensitivity reported by Planchard et al. (25 pg/ml) was far below the therapeutic range and was not required for our TDM application [7].

#### 3.2.1. Calibration

The calibration curves (n = 5) were linear over the range examined, with a coefficient of determination ($R^2$) of 0.993 ± 0.004. Since the calibration curves of osimertinib did not show non-linearity (data not shown), a linear-regression model using least squares regression (quadratic weighting, $1/X^2$) was used. The linear functions were $Y = (0.00151 \pm 0.00055) * X + (0.000398 \pm 0.000090)$ for osimertinib. Here, X is the ratio of the analyte response versus the internal standard response, and Y the concentration in ng/ml. Auto injector carry-over from the highest calibration sample was found to be less than 20% of the LLOQ, as required by the EMA and FDA guidelines [8,9].

#### 3.2.2. Precision and accuracy

In Tables 1 and 2, the accuracy and precision of the method in human lithium heparin and EDTA plasma are shown. The precisions and accuracies for three analytical runs were within the ±15% for high, medium, and low QC's, and ±20% for the LLOQ QC's, as required [8,9]. For the spiked EDTA plasma samples the within day accuracy and precision were similar to heparin plasma samples.

#### 3.2.3. Selectivity

The analysis of six independent blank human plasma samples of both heparin and EDTA showed no interfering peaks in the SRM traces for osimertinib or the internal standard (data not shown). Blank osimertinib responses were all <20% of the LLOQ response (9.8% ± 3.6%) as required [8]. Blank IS responses were below 1% (0.11% ± 0.05%) of the normal response. At the LLOQ of 1 ng/ml (n = 6) a mean concentration of 0.96 ± 0.07 ng/ml for osimertinib in heparinized plasma (95.7 ± 7.6%) was found, and 1.05 ± 0.10 ng/ml for EDTA plasma (105.3 ± 9.9%) with one value >20% (120.2%), justifying the investigated LLOQ level in human plasma [9]. The results of six different spiked plasma samples (lithium heparin and sodium EDTA) are shown in Table 3.

**Table 3**
Selectivity for six spiked plasma samples at LLoQ level (1 ng/ml).

| | Lithium Heparin | | Sodium EDTA | |
|---|---|---|---|---|
| Replicate | Calculated Amount (ng/ml) | Diff. Spec. Amount. (%) | Calculated Amount (ng/ml) | Diff. Spec. Amount. (%) |
| 1 | 0.90 | 89.8% | 1.05 | 105.4% |
| 2 | 1.03 | 102.8% | 1.04 | 103.9% |
| 3 | 1.01 | 100.5% | 1.20 | 120.2% |
| 4 | 0.92 | 91.7% | 0.88 | 88.4% |
| 5 | 1.03 | 103.0% | 1.03 | 103.0% |
| 6 | 0.86 | 86.5% | 1.11 | 110.7% |
| Average | 0.96 | 95.7% | 1.05 | 105.3% |
| %VC | 7.6% | 7.6% | 9.9% | 9.9% |

**Table 4**
Recovery and matrix effect for osimertinib in human plasma in lithium heparin (mean ± SD; n = 4).

| Level (ng/ml) | Extraction Recovery | | | Matrix Effect | | |
|---|---|---|---|---|---|---|
| High (800) | 93.9% | ± | 5.5% | 88.8% | ± | 9.2% |
| Med (200) | 98.9% | ± | 2.7% | 88.5% | ± | 1.7% |
| Low (3) | 103.7% | ± | 9.8% | 87.2% | ± | 13.7% |

**Table 5**
Relative matrix effect at high (800 ng/ml) and low (3 ng/ml) QC levels for six spiked individual lithium heparin and sodium EDTA samples.

| | Heparin | | EDTA | |
|---|---|---|---|---|
| Indiv. | High | Low | High | Low |
| 1 | 92.3% | 115.5% | 94.1% | 110.8% |
| 2 | 96.9% | 102.0% | 92.3% | 97.5% |
| 3 | 93.8% | 100.4% | 96.3% | 105.5% |
| 4 | 90.7% | 96.8% | 97.8% | 106.3% |
| 5 | 96.0% | 101.1% | 88.2% | 95.0% |
| 6 | 95.3% | 95.8% | 98.1% | 101.2% |
| Average | 94.2% | 101.9% | 94.5% | 102.7% |
| RSD | 2.5% | 7.0% | 4.0% | 5.7% |

### 3.2.4. *Recovery and matrix effect*

By performing infusion experiments, it was shown that along with chromatographic separation, any remaining endogenous substance does not cause any effect on ionization of osimertinib and internal standard. As shown in Table 4, the extraction recoveries showed no losses for both target compound and IS and ranged from 94% to 104% in lithium heparin. No substantial matrix effects were observed, and recovery ranged from 87 to 89% for osimertinib at the investigated levels. Relative matrix effects for both matrices were determined (Table 5), and were found to be within the limits set in the international guidelines for analytical method validation. The high extraction recovery and low matrix effects aided the successful validation of the method. The low matrix effect can be attributed to the efficient sample pre-treatment procedure.

### 3.2.5. *Stability*

Quadruplicate stability tests at room temperature was (22° C) for six hours, compared with fresh samples showed minimal degradation of osimertinib. Analysis of samples after three freeze-thaw cycles (thawing at room temperature (ca 22° C), during ca. 4 h and freezing again at −30° C for at least 12 h) resulted in less than 15% degradation. Based on these findings we concluded that osimertinib could undergo multiple freeze-thaw cycles. The final results are noted in Table 6. For long term stability QC samples (low, medium, and high levels) were stored at −30° C for an extended period of 75 days. These samples were analyzed, and the relative response was compared to freshly prepared QC samples. This experiment indicated that osimertinib is stable in human plasma at −30° C. The complete results are shown in Table 6.

*3.2.5.1. Auto injector stability.* An analytical run of 18 QC samples was re-injected after 24 h at room temperature, showing similar results for osimertinib, with no QC exceeding ±15% (data not shown). Osimertinib and the internal standards proved stable in the auto-sampler under the tested conditions.

*3.2.5.2. Stock stability.* The responses of osimertinib from the stock solutions in DMSO, after 2 months at −30° C (n = 2) compared to fresh stock solutions resulted in unchanged concentrations (99.3% recovery) proving the stability of osimertinib under the tested condition.

### 3.3. *Patient samples*

Plasma levels were analyzed for all 34 patients (66.1 ± 11.5 y) receiving oral osimertinib therapy (80 mg once daily) to support drug treatment. The average plasma-levels were 301.6 ± 164.1 ng/ml with none of the samples showing a concentration outside the set range of 1–1,000 ng/ml. These findings are in line with previous literature [1,4], and a plot of the measured plasma concentrations of the 34 plasma-samples are shown in Fig. 4B. The available plasma samples were collected using EDTA as an anticoagulant, opposed to heparin. To show the validity of the change in anticoagulant, additional experiments were performed, including precision and accuracy, selectivity, and relative matrix effect. Incurred sample analysis of these initial samples showed that the method can reliably report the subject sample analyte

**Table 6**
Stability of osimertinib at 4 h at room temperature, after three freeze thaw cycles, and −30° C for 75 days (mean ± SD, n = 4).

| Level (ng/ml) | Short term stability (%) | Freeze-thaw stability (%) | Long term stability (%) |
|---|---|---|---|
| High (800) | 88.6 ± 1.9 | 95.7 ± 2.4 | 92.5 ± 4.7 |
| Med (200) | 92.6 ± 10.1 | 91.8 ± 1.8 | 97.7 ± 9.8 |
| Low (3) | 89.8 ± 6.5 | 89.1 ± 10.0 | 89.1 ± 2.5 |

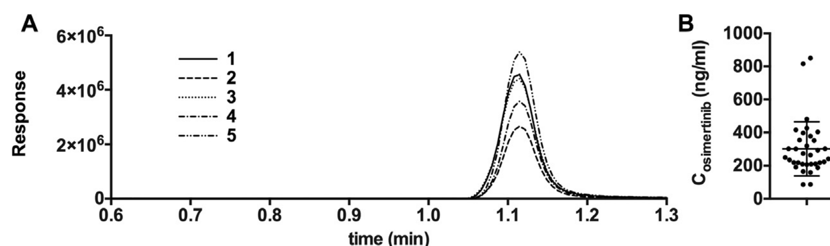


**Fig. 4.** A) Chromatograms from five initial patient samples. B) Average plasma-concentrations of osimertinib in 34 patients (two replicates per sample).

concentrations with less than 20% difference (4.6 ± 3.3% difference) [8].

## 4. Conclusions

An LC–ESI–MS/MS method has been developed and validated for the rapid and precise quantitation of osimertinib in human plasma. The method can be used for both lithium heparin samples, as well as sodium EDTA samples. The method used was successfully validated, was shown adequate in the analysis of osimertinib in samples from patients with a T790 EFGR mutated form of NSCLC, and is the first published validated assay for this drug. Accuracy and precision met the limits as they are described in international guidelines. Long-term, bench-top, freeze-thaw and auto-sampler stability of the samples met these criteria as well. In the analysis of osimertinib in human plasma we found no samples out of the validated range showing that the chosen range is adequate.

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