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Quantitative Method for the Analysis of Ivacaftor, Hydroxymethyl Ivacaftor, Ivacaftor Carboxylate, Lumacaftor, and Tezacaftor in Plasma and Sputum Using Liquid Chromatography With Tandem Mass Spectrometry and Its Clinical Applicability

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Background: The novel cystic fibrosis transmembrane conductance regulator (CFTR) modulators, ivacaftor, lumacaftor, and tezacaftor, are the first drugs directly targeting the underlying pathophysiological mechanism in cystic fibrosis (CF); however, independent studies describing their pharmacokinetics are lacking. The aim of this study was to develop a quantification method for ivacaftor and its 2 main metabolites, lumacaftor and tezacaftor, in plasma and sputum using liquid chromatography with tandem mass spectrometry.

Methods: The developed method used a small sample volume (20 μL) and simple pretreatment method; protein precipitation solution and internal standard were added in one step to each sample. Liquid chromatography with tandem mass spectrometry was performed for a total run time of 6 minutes. The method was validated by assessing selectivity, carryover, linearity, accuracy and precision, dilution, matrix effects, and stability.

Results: The selectivity was good as no interference from matrices was observed. In the concentration range from 0.01 to 10.0 mg/L, calibration curves were linear with a correlation coefficient >0.9997 for all compounds. The within-run and between-run accuracy were between 99.7% and 116% at the lower limit of quantitation (LLOQ) and between 95.8% and 112.9% for all concentrations above LLOQ for all analytes in plasma and sputum. Within-run and between-run precisions were <12.7% for LLOQ and <6.7% for the higher limit of quantitation. Samples were stable, with no significant degradation at examined temperatures and time points. Clinical applicability was revealed by analyzing samples from 2 patients with CF.

Conclusions: The presented method enables simultaneous quantification of ivacaftor, lumacaftor, and tezacaftor in plasma and sputum and is an improvement over previous methods because it uses smaller sample volumes, a simple pretreatment protocol, and includes tezacaftor. In future studies, it can be applied for examining pharmacokinetics characteristics of new CF transmembrane conductance regulator modulators.

Key Words: lumacaftor, ivacaftor, tezacaftor, cystic fibrosis, LC-MS/MS

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INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disease, with a prevalence of 1:3000 in the Western world.[1] CF is caused by a defect in the CF transmembrane conductance regulator (CFTR) gene, resulting in dysregulation of ion transport, fluid, and pH in tissues.[2,3] The disease affects multiple organ systems, including the liver, lungs, pancreas, and intestine.[4]

Until recently, CF treatment consisted of symptomatic and supportive care.[5] However, novel therapies that target the underlying disease mechanism, modulators of the CFTR, are now being developed. CFTR correctors, such as lumacaftor and tezacaftor, enhance the cellular transport of the chloride channels and thereby increase functional CFTR. Potentiators of CFTR, such as ivacaftor, enhance chloride transport by increasing the number of open channels of the CFTR protein at the cell surface.[6] Current treatments for CF registered by the FDA and EMA include monotherapy with ivacaftor or combination therapies with lumacaftor–ivacaftor and tezacaftor–ivacaftor. Currently, triple therapy with elexacaftor–tezacaftor–-ivacaftor

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has been registered by the FDA but not yet by the EMA and will therefore not be discussed in this study. The chemical structures of ivacaftor, its major metabolites hydroxymethyl ivacaftor (active) and ivacaftor carboxylic acid (nonactive), lumacaftor, and tezacaftor are shown in Figure 1.

Lumacaftor–ivacaftor seems to show modest efficacy, with only 23%–30% responders in homozygous F508del patients.[7] However, a large number of nonresponders or patients experiencing respiratory side effects have been noted, necessitating the discontinuation of treatment. In post-marketing real-life observational studies, the dropout rates are 2–4 times higher when compared with the registration studies.[8–13] The mechanism underlying these respiratory side effects remains unclear. Initially, it was believed that these side effects were linked to the CFTR correction mechanism of lumacaftor; however, the prevalence was lower after administration of the CFTR corrector tezacaftor in combination with ivacaftor.[12,14] Efficacy data for tezacaftor–ivacaftor are similar to lumacaftor–ivacaftor in homozygous F508del patients.[15]

In addition, lumacaftor, a CYP3A4/2C8/2C9/2C19 inducer, and ivacaftor and tezacaftor, both CYP3A4 substrates, are subject to clinically relevant drug–drug interactions (DDIs).[7,15] DDIs between the CFTR modulators

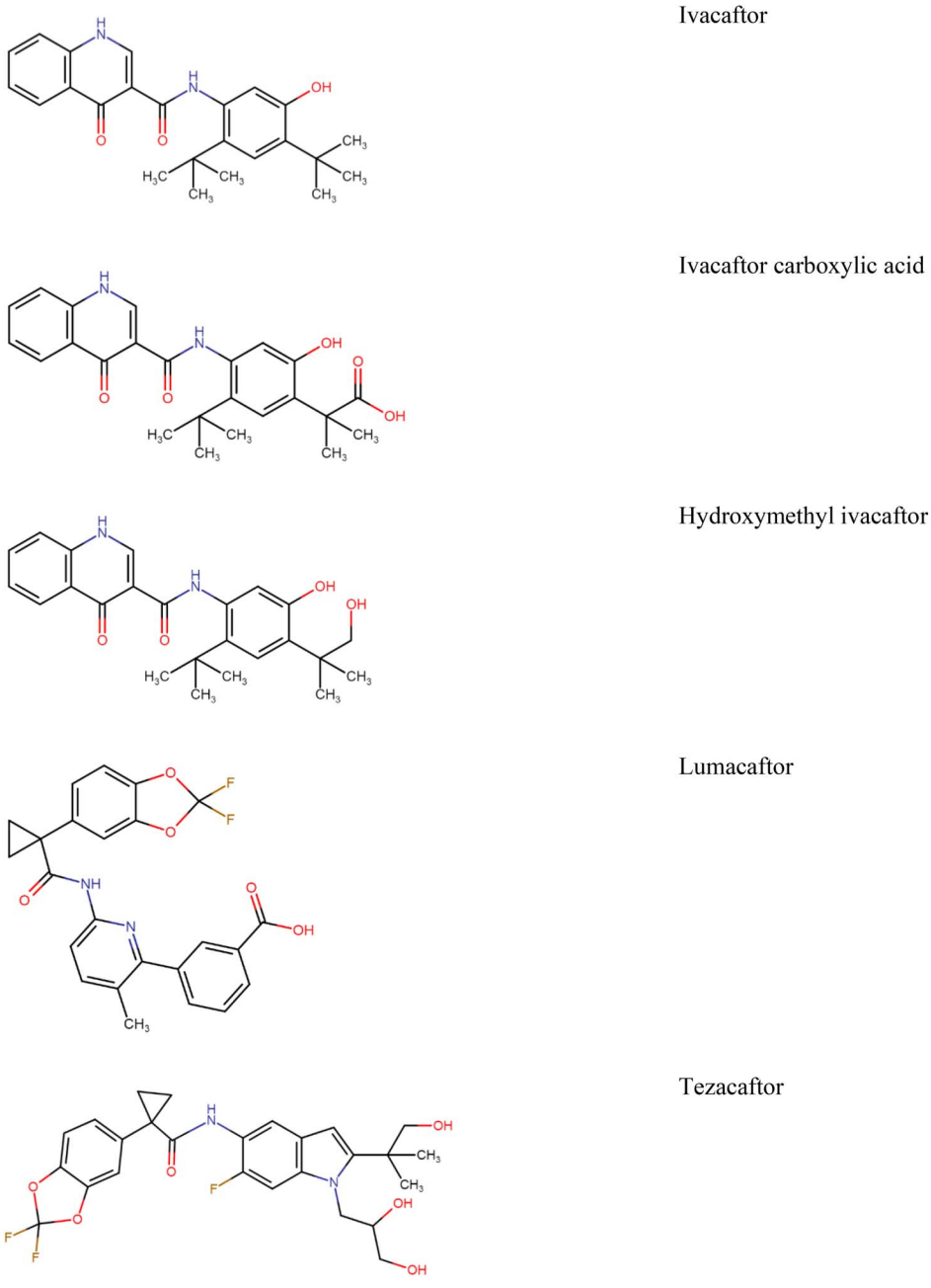

FIGURE 1. Chemical structures of ivacaftor, ivacaftor carboxylic acid, hydroxymethyl ivacaftor, lumacaftor, and tezacaftor.

themselves and other concomitant medications used by patients with CF may result in altered plasma concentrations.

To date, independent studies describing the pharmacokinetics (PK) of these CFTR modulators have not been reported. PK information can only be found in registration documents of the marketing authorization holder. Knowledge regarding PK may provide further insights into the exposure–response relationships and its interpatient variability. This knowledge may lead to an improved understanding of drug efficacy and contribute to the possible development of individualized dosing schemes. Furthermore, assessing the PK of CFTR modulators may help understand and avoid the occurrence of these DDIs and is therefore crucial in the treatment of CF.

In this study, we aimed to validate an additional quantification method in sputum besides the method in blood, as the collection of sputum is fast and noninvasive, and daily routine in CF treatment. Second, measurements in sputum may provide more information regarding the concentration of CFTR modulators at the site of action in the lungs.

Hence, in this study, a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was developed for the quantification of ivacaftor, its major metabolites hydroxymethyl ivacaftor (active) and ivacaftor carboxylic acid (nonactive), lumacaftor, and tezacaftor in plasma and sputum. Furthermore, its applicability was tested in samples obtained from patients with CF using different CFTR modulators.

MATERIALS AND METHODS

Standards, Reagents, and Chemicals

The reference standards ivacaftor, hydroxymethyl ivacaftor, ivacaftor carboxylic acid, lumacaftor, and the internal standard (IS) ivacaftor-d9 were purchased from Toronto Research Chemicals (TRC, Toronto, Canada). Tezacaftor was purchased from Chiron (Trondheim, Norway). Ultrapure water was purified and deionized using a Purelab option Q7 from Elga (High Wycombe, United Kingdom). The reagents methanol (MeOH, hypergrade for LC-MS), acetonitrile (ACN, hypergrade for LC-MS), and formic acid were purchased from Merck Chemicals (Amsterdam, the Netherlands). Ammonium formate (grade for MS) was purchased from Sigma-Aldrich (Steinheim, Germany). Drug-free human plasma, used for the preparation of the calibration standards and quality control (QC) samples, was obtained from pooled plasma obtained from healthy volunteers; drug-free sputum was obtained from residual sputum samples from patients with CF not using tezacaftor–lumacaftor–ivacaftor (Amsterdam University Medical Centers, location AMC, the Netherlands). The local medical ethics review committee provided a letter of no objection to perform the study. Hence, no informed consent was required from patients for the use of drug-free plasma and sputum samples.

Apparatus

The LC-MS/MS system used in this study consisted of an LC-30 Nexera (Shimadzu, Kyoto, Japan) system with 2 LC30AD pumps, a CT020AC column oven, a SIL30ACMP autosampler, and a DGU20A5 degasser, which was coupled to a 5500 QTrap mass spectrometer (SCIEX, Concord, Canada). The chromatographic data system Analyst 1.6.3 (SCIEX) was used.

LC-MS/MS Settings

A HyPURITY C18 HPLC (50 × 2.1 mm, 1.9 μm) column (Thermo Scientific, Waltham, MA) was used to separate the 5 analytes at 0.5 mL/min. The injection volume was 2 μL. The mobile phase consisted of 0.1% vol/vol formic acid and 0.05% vol/vol ammonium formate in ultrapure water (eluent A) and 0.1% vol/vol formic acid and 0.05% vol/vol ammonium formate in ACN (eluent B). At t = 0, the gradient was set at 65% A and 35% B. At t = 3.0 minutes, A was set to 25% and B to 75%. At t = 3.01 minutes, the gradient was set to 10% A and 90% B for 1 minute. At t = 4.01 minutes, the gradient was set back to 65% A and 35% B until a total run time of 6 minutes was reached. Changes in the gradient were linear over time. The temperature of the column and autosampler was set to 30°C and 10°C, respectively. The analytes were detected in a positive electrospray ionization mode. The ion spray voltage was set at 5500 V. Mass transitions and collision energies are shown in Table 1.

Preparation of Stock Solutions, Calibration Standards, Internal Standards, and Control Samples

For each analyte, stock solutions were prepared in MeOH at a concentration of 1 mg/mL. Subsequently, the stock solutions were further diluted in MeOH/H_2O (1:1) to reach a concentration of 20 mg/L. Seven calibration standards of each analyte were prepared at 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mg/L in human plasma. No calibration standards were prepared in sputum because sputum QC samples were measured using a plasma calibration line. The stock solution and calibration standards were stored at −80°C until use. Stock solutions of the IS were prepared in MeOH at a concentration of 0.1 mg/mL. The IS working solution was prepared by diluting the IS stock solution in ACN:MeOH (420:80) to reach a concentration of 0.05 mg/L. The IS working solution was stored at −20°C until use. For each analyte, QC samples were prepared at 4 concentration levels in human plasma: the predefined lower limit of quantitation (QC LLOQ) at 0.01 mg/L, 3 times the QC LLOQ (QC LOW) at 0.03 mg/L, a middle concentration level (QC MLQ) at 0.5 mg/L, and a high concentration level (QC HLQ) at 7.5 mg/L. In drug-free sputum, QC samples were prepared at 2 concentration levels for each analyte: QC LOW at 0.03 mg/L and QC HLQ at 7.5 mg/L. The QC samples were stored at −80°C until use.

Pretreatment of Sputum

To render the sputum homogeneous and pipettable, drug-free sputum and sputum from patient samples were sonicated before further use with Vibra Cell (Sonics & Materials, Danbury, CT) for 10 seconds at 40 J. No liquefying agents were used.

TABLE 1. Mass Transitions, Declustering Potential, Entrance Potential, Collision Cell Exit Potential, and Collision Energy of Ivacaftor, Ivacaftor Carboxylic Acid, Hydroxymethyl Ivacaftor, Lumacaftor, Tezacaftor, and Ivacaftor-d9

Analyte	Mass Transition		Declustering Potential (V)	Entrance Potential (V)	Collision Cell Exit Potential (V)	Collision Energy (V)
	Precursor (m/z)	Fragment (m/z)				
Ivacaftor	393.20	337.10	131	10	12	19
Ivacaftor carboxylic acid	423.15	367.10	136	10	12	19
Hydroxymethyl ivacaftor	409.15	353.20	111	10	12	19
Lumacaftor	453.05	131.10	101	10	14	49
Tezacaftor	521.10	449.10	181	10	14	29
Ivacaftor-d9	402.20	346.20	81	10	12	19

m/z, mass-to-charge ratio; V, voltage.

Sample Pretreatment

Calibration standards, QC samples, and patient samples were thawed and vortexed before analysis, and 20 μL was pipetted into a 1.5 mL vial. Proteins were precipitated by adding 500 μL of the IS solution to the samples. The samples were vortexed for 1 minute and cooled for 10 minutes at −20°C, vortexed again for 1 minute, and centrifuged for 5 minutes at 4000 rpm (2750x*g*). Then, 2 μL of the supernatant was injected into the LC-MS/MS system. For patient samples that required 10 × dilution, 10 μL of plasma or sputum was pipetted into a vial and 90 μL of drug-free plasma or sputum was added; subsequently, the same sample pretreatment steps were followed.

Calculation of the Concentration

MS response was expressed as an integrated area of the chromatographic peak. For calibration, the concentrations of prepared calibration standards were the known variable (x). For the unknown variable (y), the ratio of analyte MS response was divided by the IS MS response per calibration level. Patient samples were back-calculated using the calibration line with their respective area ratio of analyte/IS MS response. All components fitted a quadratic curve, and for ivacaftor, hydroxymethyl ivacaftor, lumacaftor, and tezacaftor, 1/x was the best weighting. For ivacaftor carboxylic acid, $1/x^2$ was the best weighting. Calculation and reporting of the concentration were automatically performed by Analyst 1.6.3.

Validation Method in Plasma

For the 5 analytes, the following parameters were validated in plasma according to the requirements of the EMA guideline for bioanalytical method validation[16]: selectivity, carryover, linearity, accuracy and precision, dilution, matrix effect, and stability.

Selectivity

To differentiate the analytes of interest and IS from other components in the matrix, the selectivity was determined using 6 individual sources of the appropriate blank human plasma, which were protein precipitated in ACN:MeOH (420:80) without adding the IS. The average area of the 6 blanks on the retention time (RT) of the 5 analytes and IS was compared with the average area of the 6 QCs LLOQ measured for accuracy and precision. The blanks should not contribute to more than 20% of the average LLOQ of the analyte and not more than 5% of the IS.

Carryover Effect

The carryover was measured by alternately injecting the upper LOQ (ULOQ; 10 mg/L), followed by a blank, 6 times. The average area of the 6 blanks on the RT of the 5 analytes and IS was compared with the average area of the 6 QCs LLOQ measured for accuracy and precision. The carryover of the ULOQ on the blank should not exceed 20% of the LLOQ of the analyte and not more than 5% of the IS.

Linearity

For all 5 analytes, 7 different concentrations were measured in 6 different runs to obtain 6 calibration lines. All calibration standards in run 1 were measured in duplicate; in run 2–6, they were measured singularly for a minimum of 2 different days. The used curve parameters for the validation are shown in Table 2. The correlation coefficient R^2 should be > 0.990. The back-calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ, for which it should be within ±20%. At least 75% of the calibration standards, with a minimum of 6 calibration standard levels, must fulfill this criterion.

Accuracy and Precision

The accuracy and precision were calculated by running the QC samples of LLOQ, LOW, MLQ, and HLQ 6 times for each analyte. In the first run, the QC samples were prepared in 6 replicates and were injected singularly. In run 2–6, the QC samples were singularly prepared and injected. Precision is expressed as the coefficient of variation (CV). The between-run precision was calculated from the first measured value from run 1 and the values from run 2–6 and should not exceed the CV value by 20% for QC LLOQ and 15% for QC LOW, MLQ, and HLQ. The within-run precision was calculated from the results of run 1 and should not exceed the same

TABLE 2. Overview of Validation Parameters in Plasma and Sputum

		Analytes				
Parameter	**Level**	**Ivacaftor**	**Ivacaftor Carboxylic Acid**	**Hydroxymethyl Ivacaftor**	**Lumacaftor**	**Tezacaftor**
Plasma						
Selectivity blank matrix (%) (n = 6)		2.0	0.8	1.2	5.7	1.0
Selectivity IS (%) (n = 6)		0.0	0.0	0.0	0.0	0.0
Carryover (%) (n = 6)		3.3	0.8	1.1	7.8	1.2
Linearity (mean R^2) (n = 6)		0.9999	0.9997	0.9997	0.9997	0.9999
Within-run accuracy (%) (n = 6)	LLOQ	109.5	99.7	99.7	99.8	113.0
	LOW	104.9	99.7	106.1	102.7	108.3
	MLQ	106.7	108.3	112.9	108.0	113.0
	HLQ	107.2	101.2	106.0	104.3	105.5
Between-run accuracy (%) (n = 6)	LLOQ	111.3	112.4	100.3	102.3	116.0
	LOW	99.3	95.8	98.4	97.3	100.5
	MLQ	98.7	98.8	104.5	101.6	101.0
	HLQ	97.8	96.5	97.9	99.1	99.6
Within-run precision CV (%) (n = 6)	LLOQ	6.4	6.3	3.3	11.9	5.8
	LOW	3.3	3.4	1.6	2.8	1.5
	MLQ	1.6	2.5	1.1	1.6	1.7
	HLQ	2.1	2.6	2.6	1.2	2.4
Between-run precision CV (%) (n = 6)	LLOQ	12.7	8.0	11.6	8.8	2.2
	LOW	5.8	5.1	5.0	5.1	6.5
	MLQ	5.5	6.5	4.6	5.0	7.7
	HLQ	5.9	4.0	6.7	4.0	5.7
Dilution, 10 times (%) (n = 6)	HLQ	96.4	94.0	96.2	95.7	95.1
	5x ULOQ	97.7	91.5	96.8	102.1	97.2
Matrix factor*	LOW	0.945 ± 4.2	0.937 ± 3.5	1.005 ± 4.9	1.029 ± 7.4	1.014 ± 2.6
	HLQ	0.945 ± 3.7	0.881 ± 4.6	0.952 ± 4.0	0.961 ± 5.1	0.951 ± 4.0
Freeze–thaw 3× stability†	LOW	3.4 ± 3.7	−6.3 ± 5.3	1.0 ± 5.5	4.0 ± 2.5	−1.4 ± 3.5
	HLQ	2.1 ± 1.8	−4.1 ± 1.8	0.6 ± 0.9	0.7 ± 1.1	−0.4 ± 3.6
Freezer (−80°C) 9 days stability†	LOW	3.9 ± 2.3	−8.2 ± 6.9	−7.1 ± 4.5	−1.6 ± 3.8	1.3 ± 3.7
	HLQ	−4.2 ± 1.3	−12.2 ± 1.2	−5.0 ± 1.0	−5.2 ± 2.2	−2.6 ± 1.7
Room temp. 72 h stability†	LOW	2.8 ± 6.3	0.1 ± 5.4	−0.1 ± 11.2	0.9 ± 6.9	1.9 ± 4.3
	HLQ	−4.3 ± 0.4	−6.8 ± 1.2	−5.1 ± 1.9	−6.4 ± 0.9	−4.6 ± 0.3
Autosampler (10°C) 72 h stability†	LOW	1.7 ± 3.1	−7.1 ± 1.5	−5.7 ± 3.1	−12.1 ± 3.7	2.3 ± 5.4
	HLQ	−2.6 ± 2.1	−4.6 ± 2.7	−3.3 ± 2.2	−4.4 ± 0.9	−1.6 ± 2.2
Sputum						
Accuracy (%) (n = 3)	LOW	112	112	111	99	114
	HLQ	101	99	105	98	113

*Data are presented as mean ± CV (%) (n = 6).
†Data are presented as a percentage deviation of the measured concentration versus the nominal concentration ± CV (%) (n = 3).
QC levels: LLOQ, lower limit of quantitation; LOW, 3 times the LLOQ; HLQ, high concentration level.

levels for the QC levels as the between-run precision. The between-run accuracy was calculated from the first measured value from run 1 and values of run 2–6; the mean concentration should be between 80% and 120% for QC LLOQ and between 85% and 115% for QC LOW, MLQ, and HLQ. The within-run accuracy was calculated from the results of run 1, and the mean concentration should not exceed the same levels for the QC levels as the between-run accuracy.

Dilution

If a measured sample was greater than the ULOQ, the sample should be diluted. The dilution of the sample must not affect the accuracy and precision, and therefore, the dilution was validated. QC HLQ and 5 × ULOQ were diluted by 10 times with blank human plasma. Six replicates per dilution were run. The mean accuracy and imprecision should be between 85% and 115%.

Matrix Effect

In MS, the signal of the analyte may be influenced by the presence of other substances in samples. This influence is expressed as the matrix effect and was obtained by calculating the ratio of the peak area in the presence of matrix (analyte spiked in the matrix) to the peak area in the absence of matrix [analyte spiked in MeOH/H_2O (1:1)]. In addition, the IS normalized matrix factor should be calculated by dividing the matrix factor of the analyte by the matrix factor of the IS and must be around 1. The CV of the 6 IS-corrected matrix factor should not exceed 15%.

Stability

Freeze and thaw, autosampler, short-term, and long-term stability of the plasma and sputum samples were evaluated in triplicate using QC LOW and QC HLQ levels. They were analyzed immediately after preparation and after the applied storage conditions. For freeze/thaw stability, samples were frozen (for a minimum of 12 hours), thawed (for a minimum of 12 hours), and frozen again with 3 freeze/thaw cycles in total. Autosampler stability (10°C) was determined by comparing the concentration of processed samples stored for 72 hours with their initial concentration. Room temperature (15–25°C) and fridge (2–8°C) stability were determined after 72 hours, and freezer (−80°C) stability after 9 days. The QC samples were analyzed against a calibration curve, obtained from freshly spiked calibration standards. The mean concentration at each level should be within ±15% of the nominal concentration.

Validation Method in Sputum

For validation in sputum, the accuracy and precision were measured by running the QC LOW and HLQ samples 3 times for each analyte on one day. The QC samples in sputum were measured on a plasma calibration line, and the same requirements were set as for the plasma QC samples; this eliminates any matrix effect that is present in the sputum. The mean accuracy was calculated from the complete run 1–3 and should be between 85% and 115% for QC LOW and HLQ. If the mean accuracy fulfilled the predefined requirements, no additional parameters were validated for the method in sputum.

Patient Samples

To present the applicability of the method, concentrations of 5 analytes were measured in plasma and sputum samples of 2 patients with CF. Patients had been treated with lumacaftor–ivacaftor 400/250 mg twice daily or with tezacaftor–ivacaftor 100/150 mg once daily in combination with 150 mg ivacaftor once daily. Plasma and sputum samples were stored at −80°C until analysis. The patients provided informed consent. The local medical ethics review committee gave official approval for the lumacaftor–ivacaftor patient samples and confirmed that the Medical Research Involving Human Subjects Act does not apply for the use of the residual plasma samples for tezacaftor–ivacaftor quantification.

RESULTS

Chromatography

RTs for lumacaftor, ivacaftor, ivacaftor carboxylic acid, tezacaftor, hydroxymethyl ivacaftor, and ivacaftor-d9 were 2.56, 2.64, 1.42, 1.88, 1.54, and 2.62 minutes, respectively. Figure 2 presents the ion chromatograms after the injection of blank human plasma and MLQ of the 5 analytes and the IS.

Validation in Plasma

An overview of all plasma validation results is shown in Table 2.

The selectivity of the method was good as the mean percentage of the 6 blank human plasma samples did not contribute to more than 20% to the average LLOQ and 5% of the ISs of the analytes. No carryover effect was observed as the results met the preset requirements. This implies that a sample with a low concentration can be measured after a sample with a high concentration without problems. The linearity of all calibration curves showed mean correlation coefficients (R^2) greater than 0.9997.

The within-run and between-run accuracy as well as within-run and between-run precision, met the preset requirements for all analytes and did not exceed 20% for QC LLOQ and 15% for QC LOW, MLQ, and HLQ. For all analytes, a 10 times dilution had no significant effect on the concentration, which means that samples that are greater than the ULOQ can be diluted and quantitatively measured. The matrix factor was around 1 for all compounds at LOW and HLQ levels.

The CV of the 6 IS-corrected matrix factor did not exceed 15%, and hence, there will be no value changes owing to the presence of substances other than the analyte in the sample. For all analytes, the concentration ranges from 0.01 to 10.0 mg/L were validated.

Samples showed no significant degradation for freeze and thaw, short-term (72 hours at room temperature), long-term (9 days at −80°C), and autosampler stability (72 hours at 10°C) for any of the components. All measured concentrations did not exceed ±15% of the nominal concentration.

Validation in Sputum

The validation results in sputum are shown in Table 2.

The accuracy met the preset requirements for all analytes and did not exceed 15% for QC LOW and HLQ levels.

Pretreatment of Sputum Samples

During sonication of sputum samples, a drug-free sputum sample was sonicated after every 10 samples. The drug-free sputum samples were analyzed to exclude the carryover of components by the sonication needle between samples. In all drug-free sputum samples, none of the components were detected, indicating the absence of contamination between samples.

Patient Samples

All analytes were successfully analyzed in the samples of patients with CF. Table 3 presents the results of the plasma

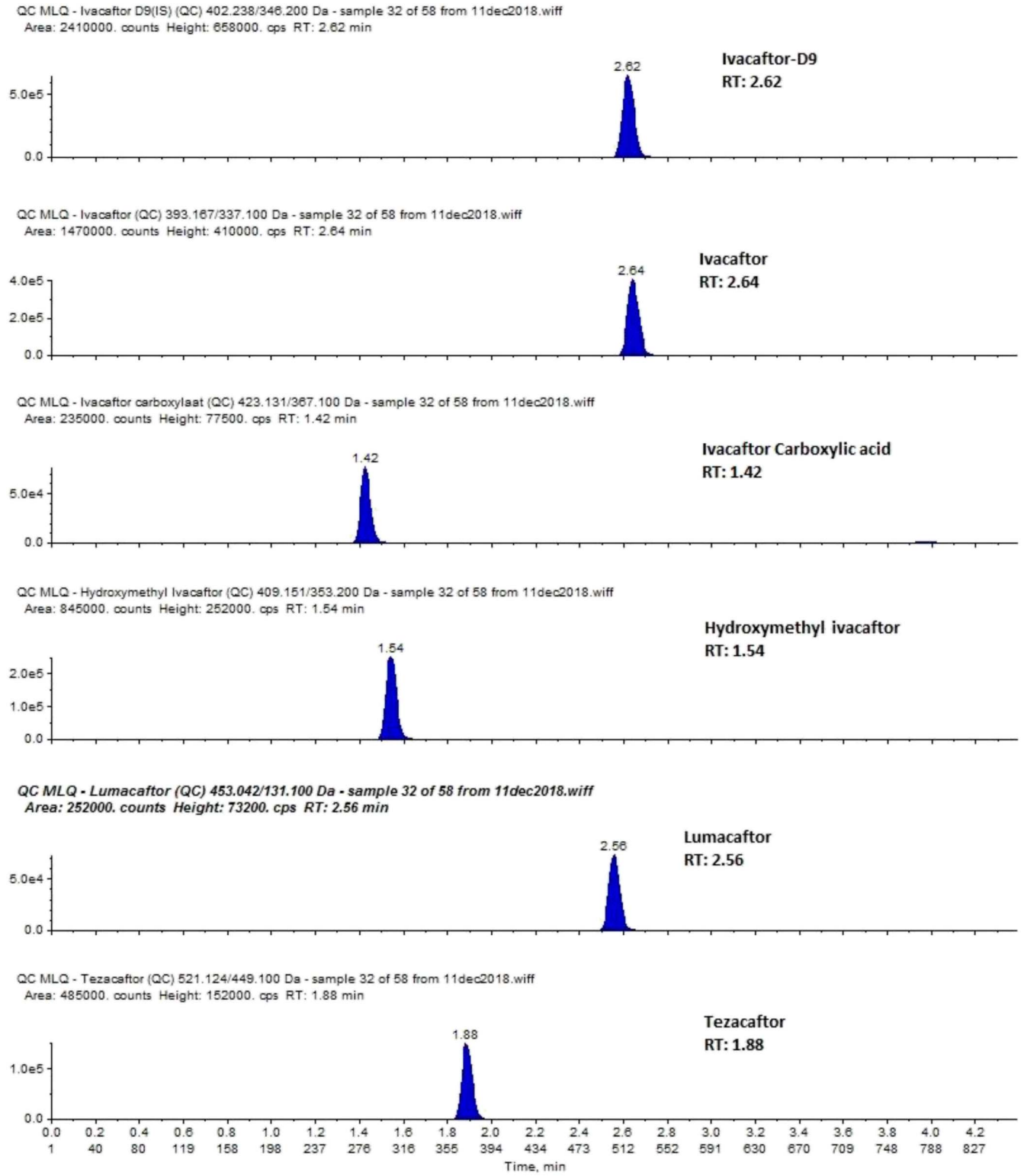

FIGURE 2. Chromatograms of the 5 analytes: Ivacaftor, ivacaftor carboxylic acid, hydroxymethyl ivacaftor, lumacaftor, tezacaftor, and the IS ivacaftor-d9.

and sputum concentrations of analytes measured in a patient using lumacaftor–ivacaftor, as well as the results in the plasma of another patient using tezacaftor–ivacaftor. The sample of patient 1 required dilution to fall within the ULOQ of lumacaftor. Overall, the concentrations of ivacaftor and its 2 metabolites were extremely low when compared with the lumacaftor and tezacaftor concentrations.

DISCUSSION

In this study, an LC-MS/MS method was developed for the measurement of lumacaftor, ivacaftor, its 2 main metabolites, and tezacaftor concentrations in the plasma and sputum of patients with CF. This method can be used in clinical studies examining the PK and pharmacodynamics of new CFTR modulators.

Previously, Schneider et al[17] have published 2 reports describing a method for the quantification of ivacaftor, ivacaftor carboxylic acid, hydroxymethyl ivacaftor, and lumacaftor in the plasma and sputum of patients with CF using lumacaftor–ivacaftor.[17,18] In these studies, a respective LLOQ and ULOQ of 0.01 mg/L and 10 mg/L were reported, which is similar to the compound range quantified in our assay. In our assay, tezacaftor was added and a similar LLOQ–ULOQ was used as a maximum plasma concentration (C_{max}) of 6.52 mg/L has been reported after the ingestion of 100 mg once daily in registration documents.[19] In our method, the run time was only 6 minutes, which is similar to the optimized method described by Scheider et al.[17] A short run time is beneficial because it allows a high sample turnover rate. The addition of tezacaftor is an improvement as it recently entered the European market, initiating patient administration. Furthermore, tezacaftor is included in the triple therapy (along with elexacaftor and ivacaftor) currently approved by the FDA. In our method, only 20 μL of patient sample volume, plasma or sputum, was needed for successful analysis, compared with 100 μL and 200 μL described in previous assays.[17,18] The small sample volume is an advantage when this method will be used in more vulnerable patients, for example, children. Another advantage of our approach, compared with the previously described methods, was the simple sample pretreatment combined with the addition of the IS. In our method, proteins in plasma and sputum samples were precipitated in one step by adding the precipitation solution containing the IS, whereas in the method of Schneider et al the IS was not included in the precipitation solution and was only added to the blank samples. Additional partial validation of the new method was successfully performed in sputum. The method seems appropriate to perform analysis in sputum, providing an estimate of concentrations; however, for confirmation, a full validation must be performed. Moreover, further research is warranted to examine any correlation between drug concentrations measured in blood and sputum, and sputum can be used as an alternative sampling method.

Analysis of drugs in sputum may be beneficial for patients with CF because sputum collection is a noninvasive and daily routine in adult CF therapy. In addition, in the younger age groups, where coughing up sputum is not always daily routine, sputum collection is less invasive than a venipuncture. Second, measurements in sputum may provide further information regarding the concentration of CFTR modulators at the site of action in the lungs, the main organ affected by the defect in the CFTR protein. As the CFTR protein is located in the airway epithelial cells, it is expected that the drugs effectively distribute throughout the lungs. It should be noted that sputum CFTR modulator concentrations may not comprehensively reflect the concentrations present in the lung because sputum is nonhomogeneous and can be contaminated with saliva. However, sputum collection is less invasive than other methods, such as bronchoalveolar lavage. The described method will be useful to investigate whether sputum is a proper vehicle to investigate the PK of CFTR modulators.

However, without pretreatment, the sputum obtained from patients with CF is extremely viscous, heterogeneous, and cannot be easily processed. This sputum contains large polymers, such as DNA, filamentous actin, proteoglycans, and biofilms, along with bacteria and inflammatory cells.[20] There are several methods described to homogenize and liquefy sputum, either by chemical or mechanical methods.[21–25] In this study, sonication, a mechanical method, was chosen without the addition of chemicals to prevent possible interactions with the components to be measured.

After the oral administration of lumacaftor–ivacaftor 400/250 mg twice daily and tezacaftor–ivacaftor 100/150 mg once daily in combination with ivacaftor 150 mg once daily, the concentrations in plasma and sputum were

TABLE 3. Concentrations of Ivacaftor, Ivacaftor Carboxylic Acid, Hydroxymethyl Ivacaftor, Lumacaftor, and Tezacaftor in Plasma and Sputum Samples of a Patient Using Lumacaftor–Ivacaftor, and Concentrations in Plasma of a Patient Using Tezacaftor–Ivacaftor

Patient	Dose (mg per day)	Time After Dosing (h)	Matrix	Concentration (mg/L)				
				Ivacaftor	Ivacaftor Carboxylic Acid	Hydroxymethyl Ivacaftor	Lumacaftor	Tezacaftor
1	800 LUM, 500 IVA	2,5	Plasma	0.554	3.24	2.51	29.3	—
		2,5	Sputum	0.0645	0.0577	0.0774	0.229	—
2	100 TEZ, 300 IVA	2	Plasma	0.924	0.631	1.10	—	4.54

LUM, lumacaftor; IVA, ivacaftor; TEZ, tezacaftor.

successfully analyzed for 2 patients. Remarkably, the sputum:plasma ratios of ivacaftor and lumacaftor were extremely small (<<1). A possible explanation for this is the protein binding properties and low solubility of the drugs. Both ivacaftor and lumacaftor are highly bound to plasma proteins, with binding values of ~99%, and the drugs are hydrophobic with low solubility.[7] Owing to the high plasma protein binding properties of the drugs, distribution of the free drug concentration throughout the body seems to be limited to the lung tissue and sputum. However, the metabolites of ivacaftor are more hydrophilic, which makes membrane passage to the lung tissue and sputum more difficult; this could explain the lower sputum:plasma ratios for the metabolites. Both patients were administered CFTR modulator combinations for more than 2 weeks, and hence, steady-state concentrations were achieved.

CONCLUSION

In conclusion, a sensitive and fast LC-MS/MS method for the simultaneous quantification of ivacaftor, ivacaftor carboxylic acid, hydroxymethyl ivacaftor, lumacaftor, and tezacaftor in plasma and sputum was established. This method can be used in future clinical studies to investigate the PK and pharmacodynamics of CFTR modulators in CF. It is important to gain comprehensive knowledge regarding the PK characteristics of new CFTR modulators as this can provide further insights into the relationship between dose and efficacy or toxicity and its interpatient variability. This could improve our understanding of the drug efficacies and contribute to the development of individualized dosing schemes.

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