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Development and application of a multimatrix LC–MS/MS method for quantifying elexacaftor–tezacaftor–ivacaftor: Expanding therapeutic drug monitoring in cystic fibrosis from systemic circulation to airways and sweat

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ABSTRACT

Background: Therapeutic drug monitoring (TDM) is becoming increasingly essential in cystic fibrosis (CF), as a growing number of children and adults are now eligible for elexacaftor/tezacaftor/ivacaftor (ETI) CF Transmembrane conductance Regulator (CFTR) modulator therapies. Although plasma remains the benchmark, its invasive nature limits practical use. Analytical approaches based on dried matrices currently available still rely on venipuncture and intensive workflows. To overcome these limitations, we developed an advanced, multidimensional TDM framework for ETI quantification that incorporates truly venipuncture-free, self-collected, and quantitative dried blood spot (DBS) sampling, along with non-invasive nasal airway swabs (NAS) and sweat. We also evaluated ETI in airway and sweat fluids, offering insight into drug distribution and activity at key target tissues.

Methods: Selectivity, specificity, linearity, accuracy, precision, and inter/intraday stability of the methods applied to plasma, NAS, and sweat specimens were investigated according to ICH M10 guidelines and IATDMCT for DBS.

Results: Analytical performance of the methods in all tested matrices were demonstrated and met the acceptance criteria of ICH M10 guidelines for bioanalytical-method validation, with DBS additionally fulfilling the IATDMCT DBS-specific recommendations. DBS measurements were statistically equivalent to plasma concentrations, supporting their utility as a minimally invasive surrogate for TDM. ETI levels in NAS were significantly higher than in plasma or sweat, indicating localized accumulation of the drug on the airway surface, a key site of CFTR activity. Sweat samples, while showing lower analyte amounts, contained detectable levels in all analytes within their biologically active concentrations.

Conclusions: This integrated analytical approach provides a holistic view of systemic and local ETI distribution, increasing the potential for personalized TDM, pharmacokinetics/pharmacodynamics study, and optimization of CFTR modulator therapy.

Abbreviations: ACN, Acetonitrile; ADME, Absorption, distribution, metabolism, and excretion; CF, Cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; CI, Confidence interval; CV, Coefficient of variation; DBS, Dried blood spots; EMA, European medicines agency; EPC, Estimated plasma concentration; ETI, Elexacaftor, tezacaftor, and ivacaftor; HCQ, High concentration quality control; Hct, Hematocrit; ICH, International council for harmonisation; IS, Internal standard; LC-MS/MS, Liquid chromatography–tandem mass spectrometry; LLOQ, Lower limit of quantification; MeOH, Methanol; MRM, Multiple reaction monitoring; NAS, Nasal airway swab; PBS, Phosphate-buffered saline; PD, Pharmacodynamic; PK, Pharmacokinetic; pwCF, People with cystic fibrosis; QC, Quality control; QC High, Quality control, high concentration; QC Low, quality control, low concentration; QC Medium, Quality control, medium concentration; RSD, Relative standard deviation; rt, Room temperature; TDM, Therapeutic drug monitoring; ULOQ, Upper limit of quantification.

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1. Introduction

Cystic fibrosis (CF) is a life-threatening autosomal recessive disease, caused by mutations in the CFTR gene that impair chloride cellular transport, and underpinned by non-resolving inflammation in multiple organs [1–3]. The development of CFTR modulators, especially the highly effective triple combination therapy of elexacaftor, tezacaftor, and ivacaftor (ETI), has radically transformed paradigms of CF treatment, shifting the focus from symptom management to targeted, disease-modifying interventions [4–6]. However, despite the long-term improvements in CFTR function and clinical benefits of ETI, there remains considerable inter-individual variability in therapeutic response, especially regarding persistent inflammation [7,8]. These discrepancies are inadequately explained by clinical laboratory endpoints [9] can be partly attributable to differences in pharmacokinetics (PK) such as absorption, distribution, metabolism, and excretion (ADME) processes that are uniquely affected in people with CF (pwCF) [10,11] and often require a therapeutic drug monitoring (TDM). Furthermore, the growing number of women with CF who are completing pregnancies and breastfeeding while under ETI therapy [12], along with the recent expansion of ETI eligibility to include children and individuals with rare CFTR mutations [12,13] is expected to further elevate the demand for reliable, scalable, minimally invasive, and ideally self-administered TDM tools for pwCF [2,14,15].

Traditionally, plasma has served as the gold standard matrix for PK studies and TDM due to its direct reflection of systemic exposure [16]. However, the invasiveness of venipuncture and the difficulties associated with repeated blood draws, especially in outpatient settings and pediatric populations, which are increasing significantly with a growing number of women with CF achieving pregnancy [17,18] have prompted the search for alternative sampling matrices. Two recent studies underscore the viability of alternative matrices for monitoring ETI. Vonk et al. validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for TDM of ETI and their major metabolites in blood spotted post draw onto manually-cut filter paper spots [19]. Similarly, Pigliasco et al. [20] presented a LC-MS/MS method for quantifying ETI in plasma, plasma dried onto filter paper, and whole blood collected via volumetric absorptive microsampling devices [21]. While both studies demonstrated interchangeability between plasma and dried matrices in providing accurate measurements of systemic ETI exposure, despite challenges related to high plasma protein binding, these methods still require venipuncture followed by manual sample processing, making them unideal for large-scale TDM applications. In this context, DBS – largely used in newborn screening programs - is a particularly attractive alternative, offering a minimally invasive, cost-effective, and logistically more convenient platform for TDM [21].

While plasma and DBS capture systemic PK effectively, they do not fully characterize the body distribution of CFTR modulators, particularly within the respiratory tract where CF pathology is most pronounced. Orally administered drugs may exhibit distinct tissue distribution patterns [22], thus, concentrations in the airway mucosa could offer critical insights into the therapeutic efficacy and mechanism of action of ETI. Nasal airway swab (NAS) sampling represents an innovative and non-invasive approach to assess drug deposition at the epithelial surface of the upper airways, serving as a surrogate for drug distribution in the lower respiratory tract. This approach is particularly novel because it moves beyond conventional systemic measures to investigate the “area pathway” distribution, a parameter that may correlate more directly with clinical outcomes, such as improvements in mucociliary clearance and lung function, as well as side effects.

The sweat chloride test is a key diagnostic tool for CF, since it gives a reliable readout of CFTR activity. However, the simultaneous quantification of CFTR modulators in sweat and chloride levels, has not been extensively explored. Measuring drug concentrations in sweat offers a unique opportunity to correlate local drug exposure with changes in sweat chloride, potentially providing a dual PK and pharmacodynamic (PD) biomarker. An integrated assessment of ETI concentrations systemically and tissue-specific could lead to a more nuanced understanding of drug efficacy, particularly in cases where CFTR modulation results not only in systemic improvements but also in the restoration of local ion transport in sweat glands.

Building on our previous research, which established a LC-MS/MS methodology for measuring ETI in maternal plasma, breast milk, and newborn plasma [23], here we introduce novel protocols for ETI quantification integrating localized (in NAS and sweat specimens) and systemic matrices (comparing plasma and truly venipuncture-free, self-administered, and quantitative DBS), to provide a comprehensive PK and PD profile of ETI.

2. Methods

2.1. Study design and population

This study was designed as a prospective, observational pilot study to develop and validate a comprehensive LC–MS/MS method for the simultaneous quantification of CFTR modulators, elexacaftor, tezacaftor, and ivacaftor, in plasma, DBS, NAS, and sweat. A total of 10 adult pwCF (5 males and 5 females, all >18 years of age, each with at least one ΔF508 mutation) were enrolled at the Regional Cystic Fibrosis Center in Atri. Study participants signed an informed consent form, and the Ethics committee gave ethical approval for this work (Protocol 1984/2019, Study Name RECCHI19). Samples from all 10 patients were used for the validation of systemic monitoring methods (plasma and DBS), including Bland–Altman and Passing–Bablok regression analyses. For the comparative analysis across all four matrices, plasma, DBS, NAS, and sweat, a subset of 5 patients was selected based on the availability of complete sample sets. Anthropometric and clinical characteristics of the study participants are reported in Table 1. For all participants, the dosing regimen was as follows: elexacaftor 200 mg / tezacaftor 100 mg / ivacaftor 150 mg administered orally in the morning, followed by an additional ivacaftor 150 mg dose in the evening. Blood samples were collected 12 h after the evening dose.

2.2. Sample collection procedures

Venous blood was drawn into citrate-containing tubes, centrifuged at 3000 rpm for 10 min at 4 °C, and the resulting plasma aliquoted and stored at –80 °C until analysis. Capillary blood was collected via fingerstick and applied onto volumetric DBS collection cards (50 μL). DBS were dried at room temperature (rt) for 3 h and stored at –20 °C. Nasal swabs were gently rotated against the inferior turbinate of one nostril for approximately 10 s and transferred at rt into their transport medium for storage at –80 °C. Nasal epithelias cells in NAS samples were counted and analyzed with flow cytometry. ETI concentrations in NAS were normalized for cell numbers to correct the variability of sample collection. Sweat samples were collected via pilocarpine iontophoresis following the department’s standard clinical protocol and split into aliquots for chloride and drug analysis.

2.3. Reagents, standards, and equipment

Analytical reagents used in this study were of LC-MS/MS grade. Elexacaftor (CAS 2216712–66–0), tezacaftor (CAS 1152311–62–0), ivacaftor (CAS 873054–44–5), and ivacaftor-d_{19} (CAS 1413431–22–7) used as internal standard (IS) were obtained from Cayman Chemical Company (Ann Arbor, MI). Methanol (MeOH), acetonitrile (ACN), and water (H_2O) used for preparation of solutions were purchased from Carlo Erba (Cornaredo, Milan, Italy). Vacutainer® citrate blood collection tubes (BD, Franklin Lakes, NJ, USA) were used for plasma sampling. For DBS sampling, a Capitainer®B device (50 μL) was employed, while for NAS samples, the ESwab system from Copan (Murrieta, CA, USA) was used. Sweat collection was performed according to the standard

Table 1
Characteristics of study participants treated with ETI enrolled for method validation (A) and for paired drug measurement in plasma, DBS, NAS, and sweat (B).

A	
Variable (Unit)	**Median (IQR)**
Sex	
Male (n)	5
Female (n)	5
Age (Year)	29.5 (10.3)
Weight (kg)	64.2 (14.2)
BMI (kg/m^2)	22.8 (3.0)
Genotype	
F508del/F508del (n)	3
F508del/Stop	3
F508del/Other	4
Sweat Chloride (mmol/L)	41.0 (28.5)
$\geq$ 40 mmol/L (n)	5
FEV1pp (%)	71.0 (9.7)
Hct (%)	42.0 (3.1)
Pancreatic insufficiency (n)	8
GOT/AST (IU/L)	23.0 (6.5)
GPT/ALT (IU/L)	27.0 (7.0)
γGT (IU/L)	10.5 (16.8)
B	
Variable (Unit)	**Median (IQR)**
Sex	
Male (n)	2
Female (n)	3
Age (Year)	35.0 (9.0)
Weight (kg)	63.4 (14.2)
BMI (kg/m^2)	24.0 (3.0)
Genotype	
F508del/F508del (n)	2
F508del/Stop	1
F508del/Other	2
Sweat Chloride (mmol/L)	52.0 (29.0)
$\geq$ 40 mmol/L (n)	3
FEV1pp (%)	72.0 (23.0)
Hct (%)	40.7 (1.80)
Pancreatic insufficiency (n)	4
GOT/AST (IU/L)	24.0 (5.0)
GPT/ALT (IU/L)	26.0 (7.0)
γGT (IU/L)	9.0 (17.0)

procedure established by the cystic fibrosis department, utilizing the SS-032 Macroduct® supply kits from Elitech Group; the collected sweat was divided into two aliquots, one for chloride measurement and one for LC–MS/MS analysis of the modulators. The LC/MS-MS system consisted of an Agilent 1260 Infinity II LC system coupled to a Ultivo Triple Quadrupole mass spectrometer, which was equipped with a Jet Stream electrospray (ESI) ionization source (Agilent Technologies, USA). The chromatographic separation was achieved using a Acquity UPLC® BEH C18 column (50 ×2.1 mm, 1.7 μm, Waters) and a gradient elution with a mobile phase consisting of 0.1 % aqueous formic acid and ACN.

2.4. UV spectral analysis and determination of extinction coefficients

UV absorption spectra were acquired using a Cary UV-3500 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) to verify analyte purity and to determine extinction coefficients (ε). Stock solutions of each CFTR modulator (1 μg/mL) were prepared in MeOH and subsequently diluted to obtain absorbance values within the linear response range. Spectra were recorded between 200 nm and 400 nm using 1 cm quartz cuvettes with pure MeOH as baseline reference. Distinct absorption maxima (λ_max) were identified at approximately 279 nm for elexacaftor, 299 nm for tezacaftor, and 310 nm for ivacaftor. Extinction coefficients (ε) were calculated using the Beer–Lambert Law ($A = \varepsilon \times c \times l$), yielding ε values reported here for the first time, critical for quantitative analytical applications of these modulators (Fig. 1a).

2.5. Preparation of standard mix and calibration curves

Stock solutions of ETI were prepared individually and mixed to yield a standard mixture (MIX) at a final concentration of 20 μg/mL in a solvent mixture of MeOH and H_2O (50:50, v/v). The solution was homogenized by vortexing followed by brief sonication (~30 s, 30 °C, power level 9), aliquoted, and stored at –80°C. Calibration standards for plasma and DBS were prepared by serial dilution from the 20 μg/mL standard mix:

Drug-free plasma samples from healthy volunteers were spiked to yield calibration points from 5 μg/mL to 0.019 μg/mL. Aliquots (20 μL) from each dilution point were mixed with 500 μL of MeOH containing 0.01 μg/mL IS, vortexed at 2000 rpm for 1 min, cooled at –20°C for 10 min, vortexed again, and centrifuged at 4000 rpm for 5 min at 4°C. Supernatants (200 μL) were transferred into LC-MS/MS vials for analysis (figure S1).

For DBS calibration curves, drug-free capillary blood samples from one healthy male volunteer (age: 30 years; Hct: 42.0 %) were similarly spiked to create concentrations ranging from 5 μg/mL to 0.019 μg/mL. Capillary blood was incubated at 25 °C for 30 min with spiked calibration standards to facilitate partition between blood cells and plasma. Exactly 50 μL were collected into Capitainer®B devices, dried, and stored at –20°C.

After adding MeOH with IS, spots were extracted using a mixture of MeOH:H_2O (95:5 v/v), vortexed for 2 min, and sonicated at 30 °C for 30 min. After extraction, the samples were evaporated (~30 min, 37 °C) under a gentle stream of nitrogen (2 bar). Residues were reconstituted in 200 μL of MeOH, centrifuged at 14,000 rcf for 2 min at 4°C, and injected into the LC-MS/MS (figure S2).

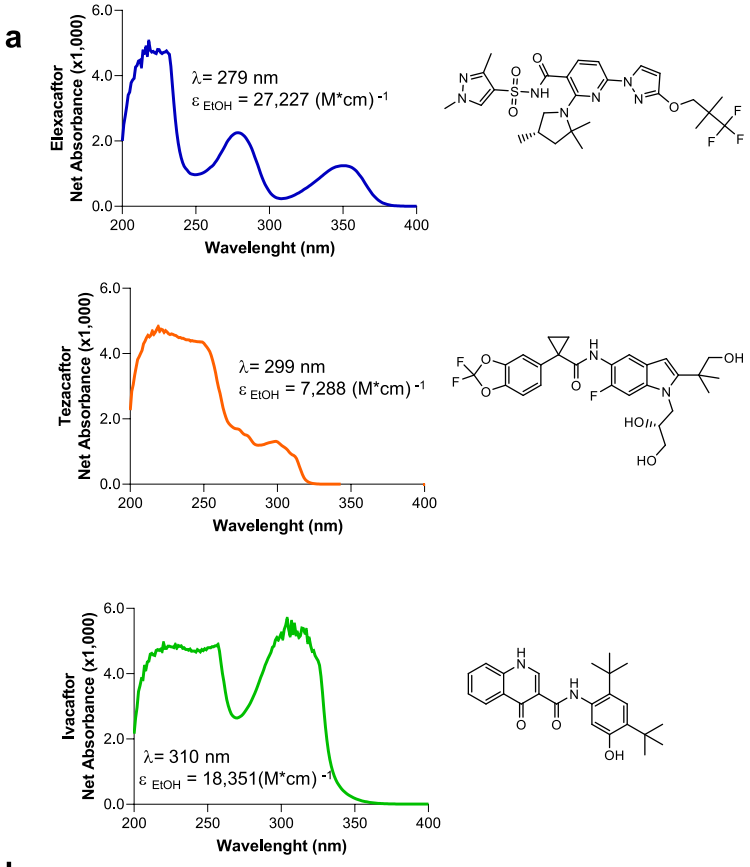
NAS calibration curves were prepared in MeOH by serial dilutions from 0.5 μg/mL down to 0.001 μg/mL. From each dilution point, 100 μL was mixed 100 μL phosphate-buffered saline (PBS) to simulate the surrogate matrix, followed by 500 μL MeOH containing 0.01 μg/mL IS. After vortexing (2 min, 2,000 rpm) and centrifugation (10 min, 14,000 rcf, 4 °C), 200 μL of supernatant were transferred to vials for LCMS/MS analysis (figure S3).

Sweat standards were prepared in MeOH followed by serial dilution from 5 μg/mL to 0.001 μg/mL. Aliquots (10 μL) were combined with H_2O (10 μL) to simulate the sweat matrix, extracted with MeOH (40 μL) containing 0.01 μg/mL IS and chloroform (20 μL). After vortexing and centrifugation (14,000 rcf, 4°C, 5 min), the chloroform phase was evaporated, reconstituted in MeOH (200 μL), and analyzed by LC-MS/MS (figure S4).

2.6. LC-MS/MS conditions and data analysis

Gradient elution was performed using mobile phases of 0.1 % formic acid in H_2O (eluent A) and ACN (eluent B), at a flow rate of 0.5 mL/min. The injection volume was 10 μL. Analytes were detected via scheduled MRM transitions as we published [23]: elexacaftor (598.2→96.2, 598.2→55.1), tezacaftor (521.2→131.1, 521.2→103.1), ivacaftor (393.2→337.3, 393.2→172.1), and ivacaftor-d_{19} (412.3→348.4, 412.3→172.1), with compound-specific fragmentation parameters summarized in Table S1. Chromatographic runs lasted 7.10 min plus a 3.50 min post-run equilibration phase. Representative chromatograms obtained from lower limit of quantification (LLOQ), low concentration quality control (QC LOW) (high concentration quality control (QC High), and Upper Limit of Quantification (ULOQ) samples across plasma (a), DBS (b), NAS (c), and Sweat (d) are illustrated in Supplementary Fig. 5–6. These chromatograms demonstrate sharp, well-defined peaks with clear baseline separation, highlighting the robustness of the developed LC-MS/MS method.

MassHunter Quantitative Analysis Software version 12.1 (Agilent) was used for data processing, and analyte quantification was performed using IS peak area ratios versus nominal concentration by weighted linear regression ($1/x^2$).



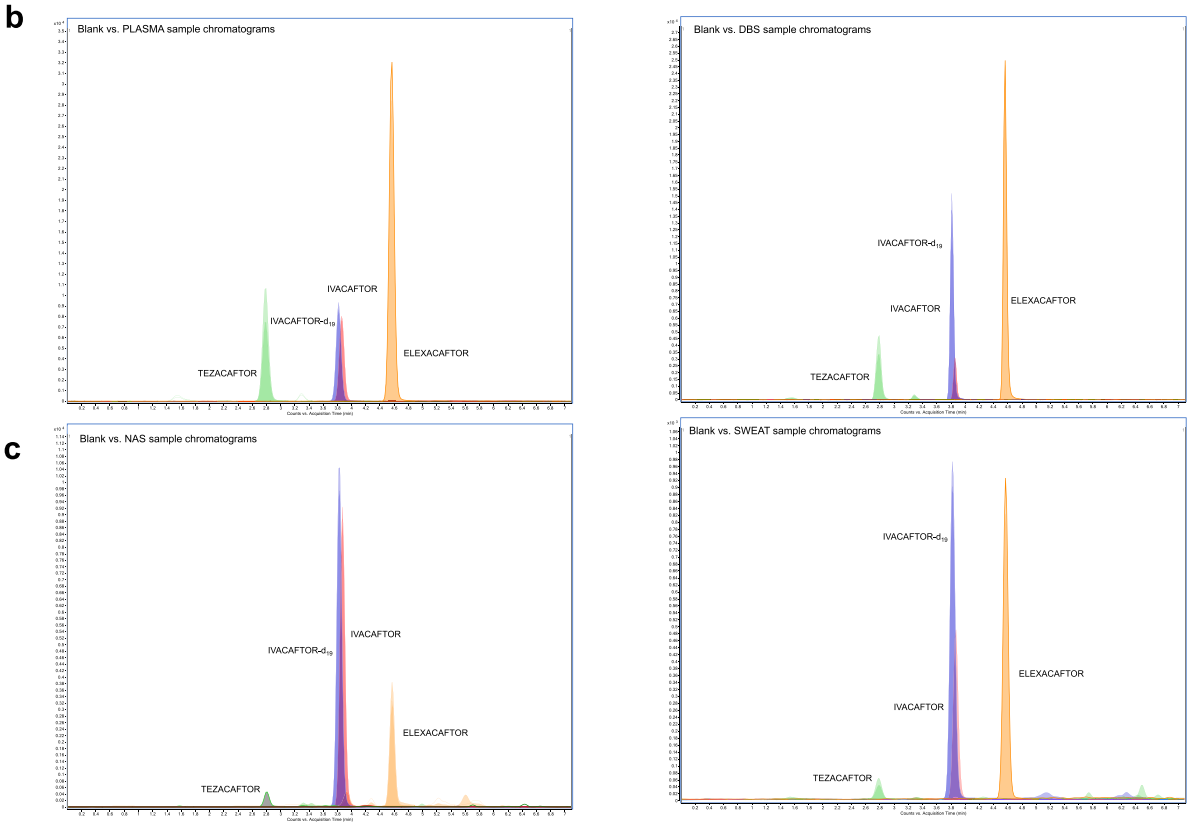


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Fig. 1. a) UV absorption spectra of CFTR modulators. Representative UV absorption spectra of elexacaftor (blue), tezacaftor (orange), and ivacaftor (green), recorded using a Cary UV-3500 spectrophotometer. Distinct absorption maxima (λ_max) were observed at approximately 245 nm for elexacaftor, 260 nm for tezacaftor, and 273 nm for ivacaftor. b) Representative LC-MS/MS chromatograms of CFTR modulators in plasma and DBS. Overlaid chromatograms of blank samples (grey) and high concentration quality control (HCQ) samples in plasma (left) and DBS (right). Peaks corresponding to each analyte are clearly resolved with excellent selectivity: elexacaftor (orange), ivacaftor (red), tezacaftor (green), and the internal standard ivacaftor-d19 (blue). No matrix interferences were observed at the retention times of the analytes or IS. c) Representative LC–MS/MS chromatograms of CFTR modulators in NAS and SWEAT. Overlaid chromatograms of blank samples (grey) and patient samples in NAS (left) and sweat (right). Peaks corresponding to each analyte are clearly resolved with excellent selectivity: elexacaftor (orange), ivacaftor (red), tezacaftor (green), and the internal standard ivacaftor-d19 (blue). All analytes and the internal standard are well separated, confirming assay selectivity and suitability for quantification in minimally invasive sample types.

2.7. Statistics

Qualitative variables were reported as distribution of frequency, absolute and percentage. Continuous variables were reported as mean and SD or median and interquartile range (IQR) according to their distribution, assessed by the Shapiro-Wilks test. Relationships between variables were calculated using the non-parametric Spearmen's rank correlation. Statistical comparisons of ETI in paired specimens of plasma, DBS, NAS, and sweat were performed using the Friedman non-parametric test followed by Dunn's post-hoc analysis. All tests were 2-tailed, with a significance level set at $p < 0.05$. Graphical representations was performed using GraphPad Prism (version 9.0.0; GraphPad Software, San Diego, CA, USA)

3. Results

3.1. Method validation

3.1.1. Validation of systemic monitoring methods: plasma and DBS

Method validation was performed rigorously according to the ICH M10 guidelines for bioanalytical method validation [24] (https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation-scientific-guideline). For DBS, validation followed ICH M10 where applicable, with additional procedures aligned with the IATDMCT consensus recommendations for DBS bioanalysis [25]. Parameters evaluated included selectivity, specificity, linearity, accuracy, precision, matrix effect, and clinical comparability, specifically through a non-parametric Bland–Altman regression analysis for the DBS method.

3.2. Selectivity and specificity

Selectivity and specificity were assessed using blank plasma and DBS samples obtained from six independent donors. No interfering peaks were observed at retention times of elexacaftor, tezacaftor, ivacaftor, or ivacaftor-d_{19} in drug-free blank plasma and DBS. Fig. 1B shows representative blank chromatograms overlaid with each matrix sample (plasma, and DBS) from patients, while representative blank chromatograms overlaid with LLOQ, QC Low, QC High, and ULOQ are shown in Supplementary Fig. 5(a-b). These results confirm selectivity and specificity of the assay, which we demonstrated previously demonstrated for plasma and breast milk [23]

3.3. Linearity

Linearity was demonstrated across the calibration range of 0.019–5 μg/mL for all analytes in both plasma and DBS. Weighted linear regression ($1/x^2$) yielded R^2 values > 0.998, confirming the robustness of the analytical response. (See supplementary Fig S1 and S2).

3.4. Accuracy and precision

Accuracy and precision were assessed using five replicates at five QC levels (LLOQ, QC Low, QC Medium, QC High, and ULOQ) across three consecutive days. All intra- and inter-day accuracy values were within ±15 %, with precision (CV%) below 15 % in line with ICH acceptance criteria (Table 1).

3.5. Stability

The stability of ETI in plasma was investigated in accordance with ICH M10 guidelines. QC samples at low (0.039 μg/mL) and high concentrations (2.5 μg/mL) were analyzed immediately after preparation and following different storage conditions to reflect the handling of study samples. Freeze–thaw stability was assessed over three cycles, in which samples were frozen for at least 12 h at - 80 °C, thawed for at least 12 h at room temperature, and subsequently refrozen. Short-term stability was determined by keeping samples for 72 h at room temperature (25 °C) and at refrigerated conditions (2–8 °C), while long-term stability was assessed after storage at −80 °C for 9 days. In all cases, the mean concentrations at both QC levels deviated by less than ± 15 % from nominal values, demonstrating compliance with the ICH M10 acceptance criteria. For DBS, stability was studied following the IATDMCT consensus recommendations [22] QC Low and QC High were prepared and analyzed at baseline (freshly collected blood, T0), after storage for 2 days at 60 °C (T1), after 1 week at room temperature (T2), and after 2 weeks at room temperature (T3). Across all conditions, the mean concentrations of all three analytes differed by less than ± 15 % from baseline values, confirming that ETI compounds remained stable in DBS under relevant environmental conditions, in agreement with IATDMCT acceptance criteria. [25]

3.6. Matrix effect

Matrix effects (ME) were evaluated to assess potential ion suppression or enhancement caused by co-eluting matrix components. The effect was quantified by comparing the peak area of each analyte spiked into the extracted blank matrix (plasma or DBS) with that of the same analyte spiked into a neat solution (MeOH). The IS-normalized ME was calculated by dividing the analyte ME by the internal standard ME. According to EMA guidelines, the coefficient of variation (RSD%) of the IS-normalized ME across six different matrix sources should not exceed 15 %. Matrix effect assessments were performed at both low and high QC levels using six independent sources of plasma and DBS. As shown in Table S2, all analytes demonstrated acceptable variability. In plasma, RSD% values ranged from 2.76% to 12.86 %, while in DBS, values ranged from 6.58 % to 14.83 %. These results confirm that the method is robust and not significantly affected by matrix variability, meeting the acceptance criteria for both matrices.

3.7. Comparison of DBS and gold standard plasma method

DBS and plasma concentrations, which are the references for PK/PD and TDM because clinical trials typically measure drug concentrations only in plasma, can differ. These differences arise from the distinct composition of capillary blood (e.g., varying protein concentrations, presence of interstitial fluid), drug partitioning between plasma and erythrocytes, and the influence of hematocrit (Hct). To address this, we calculated plasma concentrations (CPL) of ETI from DBS measurements using Eq. 1, where CPL is the plasma concentration, CB is the DBS concentration, and Hct is the hematocrit expressed as a fraction of total blood volume. This calculation allowed us to account for the confounding effect of Hct on DBS concentrations [26]:

$$CPL = \frac{CB}{1 - Hct} \quad (1)$$

To assess the agreement between CPL and DBS values, we carried out a non-parametric Passing-Bablok regression analysis. This approach also provides insights into the partitioning behavior of ETI between plasma and blood cells, which may be influenced by the drugs' physicochemical properties (e.g., lipophilicity, protein binding) and pharmacokinetics.

As shown in Fig. 2A, the regression between CPL and DBS concentrations for each of the three drugs yielded a positive slope of approximately 0.5. This slope corresponds to the blood/plasma concentration ratio (R), as described in Eq. 2:

$$R = \frac{CBC}{CPL} \quad (2)$$

Where CBC is the drug concentration within erythrocytes. A positive slope indicates a direct relationship between the two compartments. The observed R values, ranging from 0.4 to 0.6, are consistent with the known physicochemical characteristics of the ETI compounds, which are reported to be poorly distributed in red blood cells and preferentially partitioned into plasma. The tight 95 % CI and a significant Spearman correlation ($P < 0.001$) demonstrate the goodness of the fitting model.

A Bland-Altman statistical analysis was carried out to determine the mean difference between CPL derived from DBS and measured drug concentration, which should fall within the ± 1.96 SD limits of agreement [15]. As shown in Fig. 2B, Bland–Altman analysis revealed a tighter agreement between the two measurements, being the systematic bias small and not statistically significant and most of the data points residing within the limits of agreement. These results confirm that ETI concentrations can be reliably calculated from DBS samples with satisfactory accuracy when hematocrit and the blood/plasma ratio are taken into account.

A major drawback of conventional DBS paper cards lays in the lack of control of blood volume and Hct, which can hamper quantitative analysis. On the contrary, the volumetric Capitainer B devices provide an exact pre-defined blood volume, thus overcoming the Hct influence on downstream analyses, including volcano effect and blood volume. Nonetheless, other Hct-related issues can persist. Regarding the possible effect of Hct, this parameter is generally not modified in pwCF and all the participants to this study had Hct within the normal range (males: 40–50 %; female: 36–47 %) as shown in Table 1.

3.8. Validation of local monitoring methods: NAS and sweat

To extend PK monitoring to sites more reflective of CF pathophysiology, we adapted and validated the LC-MS/MS method to NAS and sweat. These matrices, unlike plasma or DBS, provide insights into local drug penetration and epithelial exposure, critical for evaluating the efficacy of CFTR modulators at their primary sites of action. Validation was performed in accordance with ICH M10 bioanalytical method validation guidelines [24], with parameters evaluated including selectivity, specificity, linearity, accuracy, precision, matrix effect, and stability.

3.9. Selectivity and specificity

Selectivity and specificity were assessed using blank NAS and SWEAT samples obtained from six independent donors. No interfering peaks were observed at retention times of elexacaftor, tezacaftor, ivacaftor, or ivacaftor-d_{19} in NAS and sweat, confirming selectivity and specificity of the assay. Fig. 1C shows representative blank chromatograms overlaid with each matrix sample (NAS, and SWEAT) from patients, while representative blank chromatograms overlaid with LLOQ, QC Low, QC High, and ULOQ are shown in Supplementary Fig. 6c-d.

3.10. Linearity

Linearity was achieved over the concentration range of 0.001–5 μg/mL, with excellent correlation coefficients ($R^2 > 0.998$) across all three analytes in both matrices (See supplementary Fig 3 and 4).

3.11. Accuracy and precision

Accuracy and precision were assessed as above. All intra- and inter-day values were within the ±15 % range, with CV% consistently below the threshold of 15 % at all QC levels. Results are summarized in Table 3.

3.12. Stability

For NAS and sweat, stability was assessed in accordance with ICH M10 using the same experimental conditions applied to plasma, using QC low and QC high concentrations. Specifically, freeze–thaw stability was tested through three cycles (≥12 h frozen at −80 °C and ≥12 h thawed at room temperature), autosampler stability at 10 °C for 72 h, short-term stability at room temperature (15–25 °C) and 2–8 °C for 72 h, and long-term stability after storage at −80 °C for 9 days. In all cases, deviations from nominal concentrations were within ±15 %, confirming the stability of analytes in these matrices.

3.13. Matrix effect

ME for NAS and sweat were evaluated as described above to assess potential ion suppression or enhancement due to endogenous components. As shown in Table S3, ME assessments were performed at both low and high QC levels using six independent sources of NAS and sweat. For NAS, RSD% values ranged from 2.03 % to 14.07 %, and for sweat, from 6.46 % to 14.69 %. Hence, all values were within the ±15 % acceptance threshold, confirming that the method is robust and not significantly affected by matrix variability in these local matrices.

These results affirm the method's validity for local matrices. To our knowledge, this is the first study to achieve full quantitative validation of ETI in both NAS and sweat matrices using a single harmonized LC-MS/MS protocol. The inclusion of these matrices not only broadens the landscape for non-invasive therapeutic monitoring but also opens a novel avenue for assessing localized drug delivery and response to treatment in pwCF.

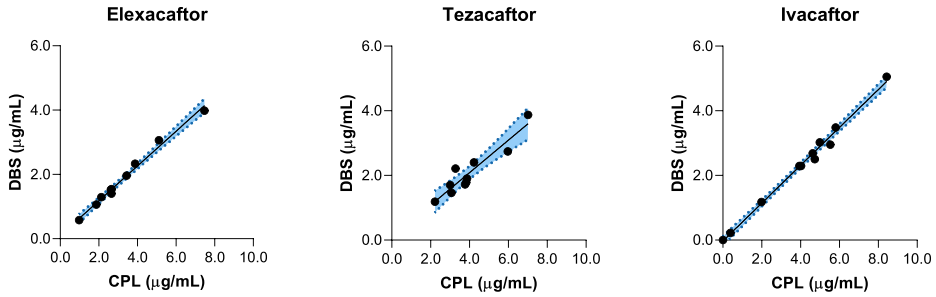
3.14. Multimatrix measurement of ETI

Lastly, in a cohort of five pwCF receiving ETI, we compared drug concentrations across the four matrices (Fig. 3a). The Friedman's and Dunn's tests confirmed significant differences in matrix-specific concentrations for each CFTR modulator. Elexacaftor, tezacaftor, and ivacaftor levels were significantly higher in NAS than in sweat. Elexacaftor levels were also higher in plasma than in sweat. These results suggest accumulation in the airway epithelium, the primary target site for CFTR modulation, compared to systemic circulation. ETI concentrations in sweat were in the μM range, ~100–1000 times lower than those measured in DBS and NAS, yet detectable (Fig. 3b), supporting the suitability of sweat as a TDM matrix.

4. Discussion

This study presents the comprehensive development and validation of an LC-MS/MS method for the quantification of the CFTR modulators ETI across four biological matrices: plasma, DBS, NAS, and sweat. While plasma remains the clinical gold standard for pharmacokinetic monitoring, our findings demonstrate the robustness and applicability of alternative and minimally invasive matrices that may offer enhanced utility in specific clinical or research contexts. Our validation results,

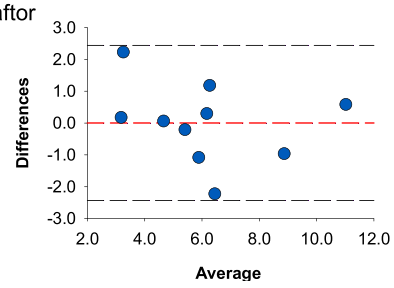
a



Drug	b (slope) (95 % CI)	a (y-intercept) (95 % CI)	R^2	P
Elexacaftor	0.552 (0.517; 0.586)	0.052 (-0.064; 0.168)	0.992	< 0.001
Tezacaftor	0.513 (0.430; 0.596)	0.026 (-0.296; 0.347)	0.951	< 0.001
Ivacaftor	0.585 (0.551; 0.620)	0.030 (-0.184; 0.124)	0.993	< 0.001

b

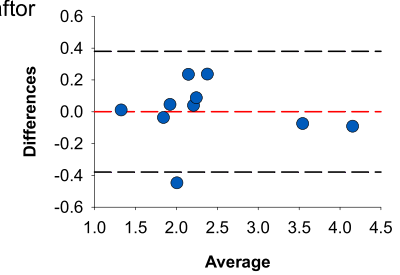
Elexacaftor



Bland-Altman

	Estimate	95% Confidence Interval Lower	Upper
Bias (n = 10)	-0.004	-0.894	0.885
Lower limit of agreement	-2.441	-4.016	-0.867
Upper limit of agreement	2.432	0.858	4.007

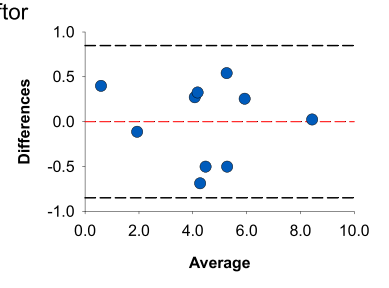
Tezacaftor



Bland-Altman

	Estimate	95% Confidence Interval Lower	Upper
Bias (n = 10)	0.005	-0.141	0.142
Lower limit of agreement	-0.380	-0.625	-0.134
Upper limit of agreement	0.379	0.135	0.624

Ivacacaftor



Bland-Altman

	Estimate	95% Confidence Interval Lower	Upper
Bias (n = 10)	0.019	-0.307	0.345
Lower limit of agreement	-0.874	-1.451	-0.297
Upper limit of agreement	0.912	0.335	1.489

Fig. 2. a) Passing–Bablok regression of DBS concentrations versus CPL for elexacaftor, tezacaftor, and ivacaftor. Analysis was performed on paired samples from 10 pwCF. The black line represents the regression line; blue dashed lines and shades are the 95 % CI of the regression. b) Bland–Altman plots comparing DBS-derived CPL and measured CPL for elexacaftor, tezacaftor, and ivacaftor. The red line in ach graph displays the mean difference (bias) and 95 % limits of agreement (dashed black lines) between paired samples from the same 10 pwCF.

Table 2
Intra-day and inter-day accuracy and precision of elexacaftor, tezacaftor, and ivacaftor in plasma, and DBS matrices.

		INTER-DAY			INTRA-DAY			
QC Level	Conc. mean (μg/mL)	SD	CV (%)	Accuracy (%)	Conc. mean (μg/mL)	SD	CV (%)	Accuracy (%)
PLASMA - ELEXACAFTOR								
LLOQ	0.017	0.002	12.60	89.87	0.016	0.002	10.96	85.50
QC High	2.618	0.338	12.90	104.70	2.512	0.251	9.97	100.49
QC Low	0.031	0.003	9.68	78.77	0.034	0.003	10.34	86.46
QC Medium	0.327	0.014	4.40	104.63	0.333	0.020	6.03	106.63
ULOQ	4.860	0.303	6.230	97.206	4.666	0.388	8.316	93.330
DBS - ELEXACAFTOR								
LLOQ	0.024	0.003	11.56	124.84	0.018	0.005	25.47	94.60
QC High	2.487	0.046	1.86	99.47	2.486	0.027	1.08	99.44
QC Low	0.046	0.002	4.04	118.97	0.041	0.004	10.65	104.89
QC Medium	0.376	0.004	1.00	120.63	0.369	0.006	1.75	118.16
ULOQ	4.726	0.320	6.770	94.526	4.906	0.220	4.474	98.124
PLASMA - TEZACAFTOR								
LLOQ	0.016	0.002	11.69	85.16	0.016	0.002	13.21	86.46
QC High	2.592	0.221	8.526	103.698	2.560	0.133	5.204	102.400
QC Low	0.041	0.001	1.97	104.82	0.040	0.002	5.92	103.04
QC Medium	0.337	0.013	3.79	107.78	0.350	0.025	7.26	111.87
ULOQ	4.686	0.412	8.79	93.72	4.613	0.315	6.84	92.25
DBS - TEZACAFTOR								
LLOQ	0.017	0.001	5.36	90.95	0.017	0.001	8.40	90.25
QC High	2.506	0.008	0.32	100.25	2.472	0.025	1.03	98.89
QC Low	0.036	0.001	3.22	91.85	0.036	0.001	3.36	93.06
QC Medium	0.356	0.006	1.70	114.13	0.358	0.004	1.05	114.63
ULOQ	5.015	0.022	0.429	100.297	4.953	0.048	0.972	99.063
PLASMA - IVACAFTOR								
LLOQ	0.017	0.004	24.62	86.95	0.018	0.002	13.95	94.21
QC High	2.503	0.083	3.317	100.138	2.467	0.069	2.781	98.663
QC Low	0.034	0.004	10.85	86.15	0.035	0.003	8.13	89.52
QC Medium	0.342	0.002	0.72	109.50	0.359	0.020	5.69	114.85
ULOQ	4.933	0.089	1.802	98.669	4.955	0.065	1.319	99.102
DBS - IVACAFTOR								
LLOQ	0.022	0.001	4.87	114.53	0.017	0.004	21.12	91.44
QC High	2.514	0.034	1.346	100.570	2.473	0.035	1.434	98.909
QC Low	0.049	0.001	1.03	125.28	0.040	0.006	15.38	103.56
QC Medium	0.346	0.007	2.12	110.92	0.354	0.007	2.08	113.61
ULOQ	4.998	0.027	0.540	99.951	4.962	0.044	0.885	99.235

Accuracy is expressed as percent deviation from the nominal value; precision is presented as coefficient of variation (CV%) calculated from five replicates at each QC level (LLOQ, QC Low, QC Medium, QC High, and ULOQ) across three separate days.

conducted under ICH M10 and IATDMCT guidelines, confirmed excellent linearity ($R^2 > 0.99$), accuracy, precision, and reproducibility across all matrices. Importantly, the method performed equally well in low- and high-concentration ranges, with intra- and inter-day coefficients of variation generally below 10 % and accuracy within the ±15 % acceptance criteria. The utility of DBS as a practical alternative to plasma for systemic drug monitoring was reinforced by our Bland–Altman analysis, which revealed strong concordance between paired DBS-derived CPL and plasma samples. This confirms previous literature [16,17] on the suitability of DBS as an instrument for TDM in pwCF and supports its adoption in decentralized or home-based TDM settings. Crucially, our study extends the application of LC-MS/MS to matrices not yet standard in TDM that can be relevant for assessing PK of ETI in pwCF and/or compliance, namely nasal epithelial lining fluid via NAS and eccrine sweat. This dual utility—biochemical and pharmacological—makes sweat a particularly interesting platform for future studies aimed at integrating PK with PDs in CF.

Beyond demonstrating accurate quantification of ETI in multiple matrices, our findings directly address the crucial need to better understand the clinical applications and therapeutic relevance of these alternative sample types, providing a framework for a holistic view of ETI pharmacotherapy, which is crucial for personalized therapy. The response of pwCF to ETI is highly heterogeneous [7,27,28]. In the attempt to identify predictors of response to ETI, Alicandro et al. found that clinical outcomes predict only partially the degree of the response [9], highlighting the need of other instruments for improving therapies of pwCF, including TDM. The results presented here indicate that volumetric DBS can facilitate acceptability and adherence to TDM, especially in adults requiring frequent measurements or children [9,14].

The integrated analytical approach validated here provides a platform for a holistic assessment of ETI concentrations in pwCF, which can be particularly relevant in patients receiving ETI off-label for rare CFTR mutations, for which data on drug regimen and PK/PD may not exist. In this regards, the multimatrix quantification of ETI can adjuvate clinicians to establish and compare drug concentrations reached in vivo with those tested in vitro (e.g., 3D organoids), which are the only available option for therapying [14]. Additionally, measuring ETI in several compartments can help to investigate cause of adverse effects or monitor changes in emerging risk factors, such as cardiometabolic complications, following CFTR modulator therapy [29]. Finally, the multimatrix monitoring validated here for ETI can be extended to other CFTR modulators in clinical trials or recently approved.

Although the small sample size precludes robust correlation analysis across all matrices for each individual pwCF, a consistent trend in relative drug concentrations—where individuals tend to exhibit uniformly higher or lower levels across matrices—can still be observed. These findings underscore the potential of NAS as a non-invasive, locally relevant matrix for assessing drug distribution in respiratory tissues.

Measurement of ETI in NAS provides an additional and clinically relevant dimension, since the airway surface is the primary site of CFTR dysfunction. Drug concentrations in NAS may better reflect the local exposure–response relationship than plasma levels and could therefore help bridge PK and PD to clinical outcomes, such as improvements in lung function or airway inflammation, which remains higher in pwCF

Table 3
Intra-day and inter-day accuracy and precision of elexacaftor, tezacaftor, and ivacaftor in NAS, and sweat matrices.

		INTER-DAY			INTRA-DAY			
QC Level	Conc. mean (μg/mL)	SD	CV (%)	Accuracy (%)	Conc. mean (μg/mL)	SD	CV (%)	Accuracy (%)
NAS - ELEXACAFTOR								
LLOQ	0.001	0.000	3.67	104.55	0.001	0.00	2.89	103.29
QC High	0.251	0.001	0.25	100.28	0.251	0.001	0.43	100.27
QC Low	0.004	0.000	0.80	100.36	0.004	0.000	0.87	100.17
QC Medium	0.032	0.000	0.57	99.70	0.032	0.000	0.67	99.71
ULOQ	0.500	0.001	0.22	100.02	0.500	0.003	0.54	100.02
SWEAT - ELEXACAFTOR								
LLOQ	0.018	0.003	14.636	93.895	0.018	0.002	13.766	93.158
QC High	2.500	0.016	0.649	100.007	2.503	0.023	0.919	100.137
QC Low	0.039	0.001	1.870	99.538	0.039	0.001	1.587	99.436
QC Medium	0.313	0.003	0.903	100.006	0.313	0.003	1.046	100.094
ULOQ	5.030	0.023	0.455	100.604	5.038	0.040	0.803	100.767
NAS - TEZACAFTOR								
LLOQ	0.001	0.000	2.677	103.214	0.001	0.000	2.566	103.525
QC High	0.250	0.001	0.319	99.940	0.250	0.001	0.301	100.058
QC Low	0.004	0.000	0.764	101.014	0.004	0.000	1.028	100.983
QC Medium	0.031	0.001	2.923	98.249	0.032	0.001	1.817	99.371
ULOQ	0.499	0.002	0.482	99.816	0.500	0.002	0.377	99.923
SWEAT - TEZACAFTOR								
LLOQ	0.019	0.000	2.088	100.211	0.019	0.000	2.472	98.386
QC High	2.493	0.009	0.347	99.720	2.519	0.021	0.825	100.742
QC Low	0.039	0.000	0.405	100.000	0.039	0.000	0.455	99.709
QC Medium	0.325	0.022	6.754	104.038	0.328	0.021	6.295	104.960
ULOQ	5.022	0.029	0.577	100.442	5.074	0.047	0.935	101.482
NAS - IVACAFTOR								
LLOQ	0.001	0.000	3.394	104.866	0.001	0.000	3.220	106.093
QC High	0.251	0.001	0.297	100.282	0.251	0.001	0.578	100.493
QC Low	0.004	0.000	1.428	101.200	0.004	0.000	1.315	100.480
QC Medium	0.032	0.000	0.601	100.253	0.032	0.000	0.919	99.676
ULOQ	0.501	0.001	0.265	100.222	0.000	0.002	0.395	100.038
SWEAT - IVACAFTOR								
LLOQ	0.019	0.000	2.231	100.632	0.019	0.000	2.242	99.544
QC High	3.004	1.124	37.422	120.173	3.025	1.048	34.656	121.014
QC Low	0.039	0.001	3.369	99.231	0.039	0.001	3.140	99.026
QC Medium	0.312	0.001	0.177	99.904	0.314	0.002	0.502	100.503
ULOQ	5.014	0.016	0.317	100.275	5.049	0.032	0.630	100.982

Accuracy is expressed as percent deviation from the nominal value; precision is presented as coefficient of variation (CV%) calculated from five replicates at each QC level (LLOQ, QC Low, QC Medium, QC High, and ULOQ) across three separate days.

[7,8]. Incorporating airway matrices into TDM could support more individualized dosing by linking systemic exposure to therapeutic concentrations at the target organ.

Our results demonstrate, for the first time, the presence of ETI in eccrine sweat at concentrations in the micromolar range, which are comparable to their in vitro EC50 values [30]. From a clinical standpoint, this finding opens the possibility of monitoring ETI concentrations alongside sweat chloride levels in routine laboratory specimens, offering insights into drug exposure at a primary cellular target, i.e., the sweat glands. In support of this, a pooled analysis of phase 3 CFTR modulator trials investigating the relationship between sweat chloride and improvements in lung function showed that sweat chloride is a reliable biomarker of CFTR activity and correlates with clinical outcomes following ETI therapy [31].

To the best of our knowledge, this is the first report demonstrating that ETI concentrations can be measured in sweat, a finding that may hold significant clinical relevance for therapeutic monitoring and understanding drug distribution at the site of action.

Sweat represents a unique case, as it simultaneously offers PK and PD information. The detection of ETI in sweat not only confirms systemic distribution into eccrine glands but, when combined with sweat chloride, provides a dual PK/PD biomarker. Sweat chloride is a well-established surrogate of CFTR activity, and its marked reduction upon ETI initiation is consistently correlated with clinical benefit [31]. Thus, sweat sampling provides a minimally invasive means to integrate drug exposure with CFTR functional readouts in a single specimen, which may be particularly useful for pediatric patients, for patients in whom airway sampling is difficult, or for long-term therapy monitoring.

In summary, the demonstration of a multimatrix approach for TDM of ETI addresses a critical need for more comprehensive and patient-friendly monitoring tools. By establishing the simultaneous feasibility of ETI measurement across both systemic (plasma, DBS) and compartmental (NAS, sweat) matrices, this work expands the methodological foundation for individualized therapy. This integrated approach, provides a robust basis for a holistic view of drug distribution and effects, paving the way for a new form of personalized CF care. In an era where CF treatments are increasingly prolonged and patient populations are diversifying (including young children, pregnant and breastfeeding women, and individuals with rare CFTR mutations), the need less tedious more comprehensive TDM tools is more pressing than ever.

CRediT authorship contribution statement

Matteo Mucci: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Data curation, Conceptualization. **Antonio Recchiuti**: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Data curation, Conceptualization. **Pietro Ripani**: Conceptualization, Supervision. **Marta Di Nicola**: Data curation. **Martina Colarelli**: Formal analysis. **Maria Di Sabatino**: Formal analysis. **Marianna Del Ciotto**: Formal analysis. **Mario Romano**: Supervision. **Francesca Collini**: Formal analysis.

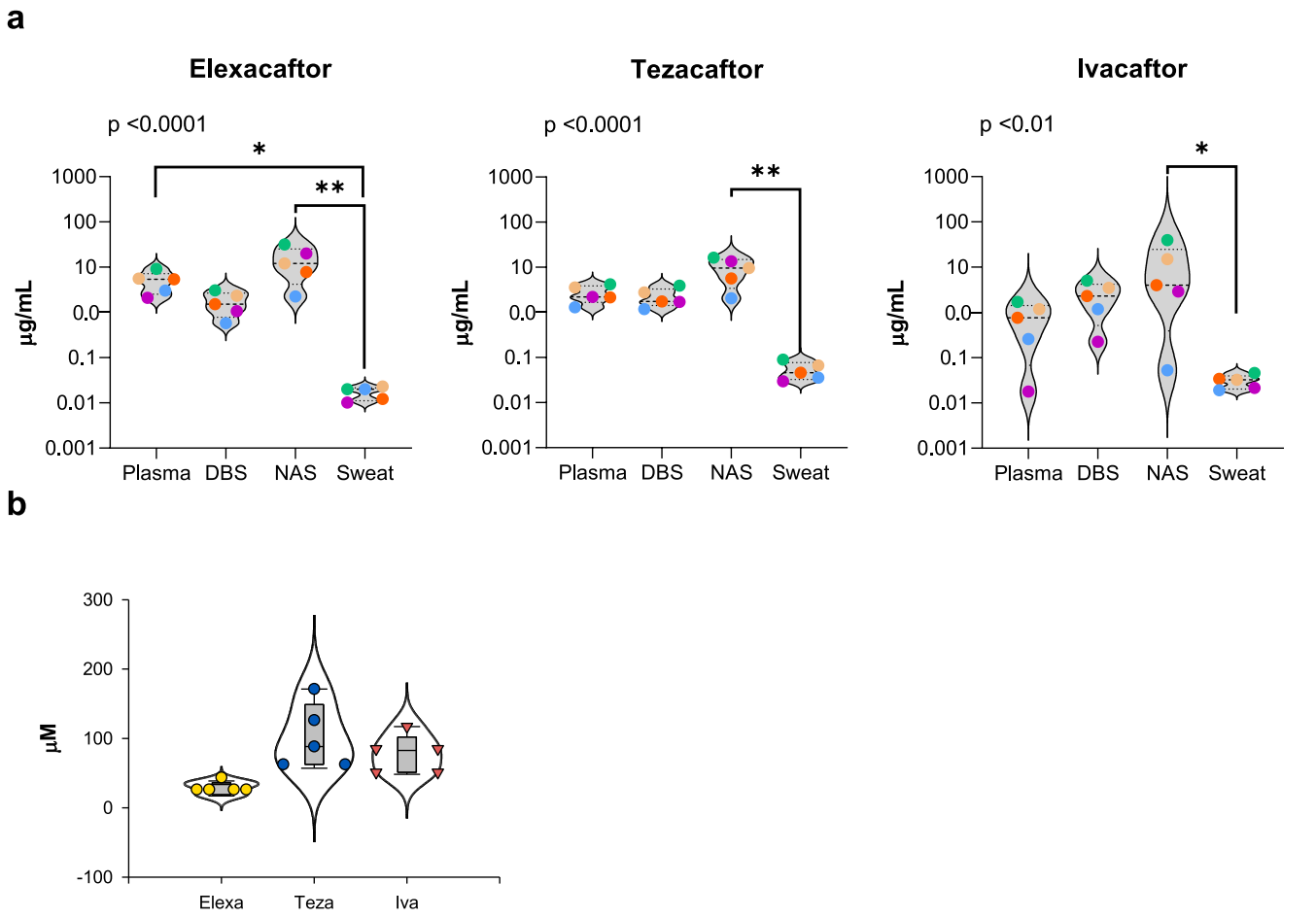


Fig. 3. Concentrations of CFTR modulators in plasma, DBS, NAS, and sweat in five pwCF. a) Violin plots represent individual patient (n = 5) data across plasma, DBS, NAS, and sweat, with dashed lines indicating the matrix-specific median concentration. Statistical analysis was performed using the Friedman test followed by Dunn's test. *, $p < 0.05$, **, $p < 0.01$, *** $p < 0.001$. b) Violin and box plots represent ETI concentrations in sweat (median and 25/75 percentiles). Data are from n = 5 patients.

Declaration of Competing Interest

The authors declare no conflicts of interest related to the content of this manuscript. All authors have reviewed and approved the final version of the manuscript and confirm that they have no financial or personal relationships that could have inappropriately influenced or biased the work presented.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118558.

Data availability

Data will be made available on request.

References

[1] J.F. Chmiel, Anti-inflammatory strategies: what remains to be done if infection and mucus stasis are resolved? Pediatr. Pulmonol. 54 (2019). S18-79.
[2] S.C. Bell, M.A. Mall, H. Gutierrez, M. Macek, S. Madge, J.C. Davies, P.-R. Burgel, E. Tullis, C. Castaños, C. Castellani, C.A. Byrnes, F. Cathcart, S.H. Chotirmall, R. Cosgriff, I. Eichler, I. Fajac, C.H. Goss, P. Drevinek, P.M. Farrell, A.M. Gravelle, T. Havermans, N. Mayer-Hamblett, N. Kashirskaya, E. Kerem, J.L. Mathew, E. F. McKone, L. Naehrlich, S.Z. Nasr, G.R. Oates, C. O'Neill, U. Pypops, K.S. Raraigh, S.M. Rowe, K.W. Southern, S. Sivam, A.L. Stephenson, M. Zampoli, F. Ratjen, The future of cystic fibrosis care: a global perspective, Lancet Respir. Med. 8 (2020) 65–124.
[3] M.A. Mall, P.-R. Burgel, C. Castellani, J.C. Davies, M. Salathe, J.L. Taylor-Cousar, Cystic fibrosis, Nat. Rev. Dis. Prim. 10 (2024) 53.
[4] P.G. Middleton, M.A. Mall, P. Dřevínek, L.C. Lands, E.F. McKone, D. Polineni, B. W. Ramsey, J.L. Taylor-Cousar, E. Tullis, F. Vermeulen, G. Marigowda, C. M. McKee, S.M. Moskowitz, N. Nair, J. Savage, C. Simard, S. Tian, D. Waltz, F. Xuan, S.M. Rowe, R. Jain, Elexacaftor–tezacaftor–ivacaftor for cystic fibrosis with a single Phe508del allele, N. Engl. J. Med. 381 (2019) 1809–1819.
[5] D. Keating, G. Marigowda, L. Burr, C. Daines, M.A. Mall, E.F. McKone, B. W. Ramsey, S.M. Rowe, L.A. Sass, E. Tullis, C.M. McKee, S.M. Moskowitz, S. Robertson, J. Savage, C. Simard, F. Van Goor, D. Waltz, F. Xuan, T. Young, J. L. Taylor-Cousar, VX-445–Tezacaftor–Ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles, N. Engl. J. Med. 379 (2018) 1612–1620.
[6] J.L. Taylor-Cousar, M.A. Mall, B.W. Ramsey, E.F. McKone, E. Tullis, G. Marigowda, C.M. McKee, D. Waltz, S.M. Moskowitz, J. Savage, F. Xuan, S.M. Rowe, Clinical development of triple-combination CFTR modulators for cystic fibrosis patients with one or two F508del alleles, ERJ Open Res. 5 (2019) 00082–02019.

[7] S. Sheikh, R.D. Britt, N.A. Ryan-Wenger, A.Q. Khan, B.W. Lewis, C. Gushue, H. Ozuna, D. Jaganathan, K. McCoy, B.T. Kopp, Impact of elexacaftor–tezacaftor–ivacaftor on bacterial colonization and inflammatory responses in cystic fibrosis, Pediatr. Pulmonol. 58 (2023) 825–833.
[8] J. Loske, M. Völler, S. Lukassen, M. Stahl, L. Thürmann, A. Seegebarth, J. Röhmel, S. Wisniewski, M. Messingschlager, S. Lorenz, S. Klages, R. Eils, I. Lehmann, M. A. Mall, S.Y. Graeber, S. Trump, Pharmacological improvement of cystic fibrosis transmembrane conductance regulator function rescues airway epithelial homeostasis and host defense in children with cystic fibrosis, Am. J. Respir. Crit. Care Med. 209 (2024) 1338–1350.
[9] G. Alicandro, A. Gramegna, F. Bellino, S.C. Sciarrabba, C. Lanfranchi, M. Contarini, M. Retucci, V. Daccò, F. Blasi, Heterogeneity in response to Elexacaftor/Tezacaftor/Ivacaftor in people with cystic fibrosis, J. Cyst. Fibros. (2024). S1569199324000572.
[10] A. Awaness, R. Elkeeb, S. Afshari, E. Atef, The pharmacokinetic changes in cystic fibrosis patients population: narrative review, Medicines 12 (1) (2024).
[11] F.A. Elzinga, P.R.V. Malik, O.W. Akkerman, B.L. Rottier, H. van der Vaart, D. J. Touw, G.H. Koppelman, P. Mian, Pharmacokinetics of ivacaftor, tezacaftor, elexacaftor, and lumacaftor in special cystic fibrosis populations: a systematic review, Clin. Pharm. (2025).
[12] J.L. Taylor-Cousar, R. Jain, Maternal and fetal outcomes following elexacaftor-tezacaftor-ivacaftor use during pregnancy and lactation, J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc. 20 (2021) 402–406.
[13] V. Terlizzi, M. Lopes-Pacheco, Cystic fibrosis: new challenges and perspectives beyond elexacaftor/tezacaftor/ivacaftor, Ther. Adv. Respir. Dis. 19 (2025), 17534666251323194.
[14] E. Dreano, P.R. Burgel, A. Hatton, N. Bouazza, B. Chevalier, J. Macey, S. Leroy, I. Durieu, L. Weiss, D. Grenet, N. Stremler, C. Ohlmann, P. Reix, M. Porzio, P. Roux Claude, N. Rémus, B. Douvry, S. Montcouquiol, L. Cosson, J. Mankikian, J. Languepin, V. Houdouin, L. Le Clainche, A. Guillaumot, D. Pouradier, A. Tissot, P. Priou, L. Mély, F. Chedevergne, M. Lebourgeois, J. Lebihan, C. Martin, F. Zavala, J. Da Silva, L. Lemonnier, M. Kelly-Aubert, A. Golec, P. Foucaud, C. Marguet, A. Edelman, A. Hinzpeter, P. De Carli, E. Girodon, I. Sermet-Gaudelus, I. Pranke, Theratyping cystic fibrosis patients to guide elexacaftor/tezacaftor/ivacaftor out-of-label prescription, Eur. Respir. J. 62 (2023) 2300110.
[15] S. Naehrig, C. Shad, M. Breuling, M. Goetschke, K. Habler, S. Sieber, J. Kastenberger, A.K. Kunzelmann, O. Sommerburg, U. Liebchen, J. Behr, M. Vogeser, M. Paal, Therapeutic drug monitoring of elexacaftor, tezacaftor, and ivacaftor in adult people with cystic fibrosis, J. Pers. Med. 14 (2024) 1065.
[16] R. Longuespée, D. Theile, M. Fresnais, J. Burhenne, J. Weiss, W.E. Haefeli, Approaching sites of action of drugs in clinical pharmacology: new analytical options and their challenges, Br. J. Clin. Pharm. 87 (2021) 858–874.
[17] J.L. Taylor-Cousar, CFTR modulators: impact on fertility, pregnancy, and lactation in women with cystic fibrosis, J. Clin. Med. 9 (2020) 2706.
[18] S. Gautier, B. Coulm, M.-A. Thompson-Bos, L. Chouchana, C. Audousset, E. Elefant, J. Mankikian, P.-R. Burgel, C. Garabedian, B. Marin, A.-P.J.- Bera, Pregnancy and neonatal outcome following in utero exposure to CFTR modulators: a multicentre prospective case series, J. Cyst. Fibros. 0 (2025).
[19] S.E.M. Vonk, M. van der Meer-Vos, R. Kos, A.H. Neerincx, S.W.J. Terheggen-Lagro, J. Altenburg, A.H. Maitland-van der Zee, R.A.A. Mathôt, E.M. Kemper, Group, on behalf of the A. M. C. D. (AMCD) research, Dried blood spot method development and clinical validation for the analysis of elexacaftor, Elexacaftor-M23, tezacaftor, Tezacaftor-M1, ivacaftor, ivacaftor carboxylate, and hydroxymethyl ivacaftor using LC-MS/MS, Ther. Drug Monit. 46 (2024) 804.
[20] F. Pigliasco, A. Cafaro, S. Barco, F. Cresta, R. Casciaro, N. Pedemonte, F. Mattioli, C. Castellani, G. Cangemi, A novel LC-MS/MS method for the measurement of elexacaftor, tezacaftor and ivacaftor in plasma, dried plasma spot (DPS) and whole blood in volumetric absorptive microsampling (VAMS) devices, Pharmaceutics 17 (2025) 200.
[21] N.N.B. Zailani, P.C.-L. Ho, Dried blood Spots—a platform for therapeutic drug monitoring (TDM) and Drug/Disease response monitoring (DRM), Eur. J. Drug Metab. Pharm. 48 (2023) 467–494.
[22] L. Dong, X. Zhuang, Insights into inhalation drug disposition: the roles of pulmonary Drug-Metabolizing enzymes and transporters, Int. J. Mol. Sci. 25 (2024) 4671.
[23] P. Ripani, M. Mucci, S. Pantano, M. Di Sabatino, F. Collini, G. Ferri, M. Romano, A. Recchiuti, Maternal, newborn and breast milk concentrations of elexacaftor/tezacaftor/ivacaftor in a F508del heterozygous woman with cystic fibrosis following successful pregnancy, Front. Med. 10 (2023), 1274303.
[24] EMA. (2023) M10 on bioanalytical method validation—scientific guideline. Eur. Med. Agency Publ. 14.
[25] S. Capiau, H. Veenhof, R.A. Koster, Y. Bergqvist, M. Boettcher, O. Halmingh, B. G. Keevil, B.C.P. Koch, R. Linden, C. Pistos, L.M. Stolk, D.J. Touw, C.P. Stove, J.-W. C. Alffenaar, Official international association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood Spot–Based methods for therapeutic drug monitoring, Ther. Drug Monit. 41 (2019) 409–430.
[26] D. Milosheska, I. Grabnar, T. Vovk, Dried blood spots for monitoring and individualization of antiepileptic drug treatment, Eur. J. Pharm. Sci. 75 (2015) 25–39.
[27] D.P. Nichols, A.C. Paynter, S.L. Heltshe, S.H. Donaldson, C.A. Frederick, S. D. Freedman, D. Gelfond, L.R. Hoffman, A. Kelly, M.R. Narkewicz, J.E. Pittman, F. Ratjen, M. Rosenfeld, S.D. Sagel, S.J. Schwarzenberg, P.K. Singh, G.M. Solomon, M.S. Stalvey, J.P. Clancy, S. Kirby, J.M. Van Dalfsen, M.H. Kloster, S.M. Rowe, Clinical effectiveness of Elexacaftor/Tezacftor/Ivacaftor in people with cystic fibrosis, Am. J. Respir. Crit. Care Med. (2021).
[28] D.P. Nichols, A.C. Paynter, S.L. Heltshe, S.H. Donaldson, C.A. Frederick, S. D. Freedman, D. Gelfond, L.R. Hoffman, A. Kelly, M.R. Narkewicz, J.E. Pittman, F. Ratjen, M. Rosenfeld, S.D. Sagel, S.J. Schwarzenberg, P.K. Singh, G.M. Solomon, M.S. Stalvey, J.P. Clancy, S. Kirby, J.M. Van Dalfsen, M.H. Kloster, S.M. Rowe, Clinical effectiveness of Elexacaftor/Tezacaftor/Ivacaftor in people with cystic fibrosis: a clinical trial, Am. J. Respir. Crit. Care Med. 205 (2022) 529–539.
[29] A. Gramegna, M. Ruscica, G. Leonardi, C. Macchi, I. Fichtner, G. Putti, M. C. Schianca, L. Terranova, G. Alicandro, F. Blasi, Cardiometabolic risk factors in adults with cystic fibrosis undergoing elexacaftor/tezacaftor/ivacaftor therapy, J. Cyst. Fibros. 24 (2025) 53–56.
[30] G. Veit, A. Roldan, M.A. Hancock, D.F.D. Fonte, H. Xu, M. Hussein, S. Frenkiel, E. Matouk, T. Velkov, G.L. Lukacs, Allosteric folding correction of F508del and rare CFTR mutants by elexacaftor-tezacaftor-ivacaftor (Trikafta) combination, JCI Insight 5 (2020).
[31] E.T. Zemanick, B. Ramsey, D. Sands, E.F. McKone, I. Fajac, J.L. Taylor-Cousar, M. A. Mall, M.W. Konstan, N. Nair, J. Zhu, E. Arteaga-Solis, F. Van Goor, L. McGarry, V. Prieto-Centurion, P.R. Sosnay, C. Bozic, D. Waltz, N. Mayer-Hamblett, Sweat chloride reflects CFTR function and correlates with clinical outcomes following CFTR modulator treatment, J. Cyst. Fibros. 24 (2025) 246–254.