Therapeutic Drug Monitoring System for CFTR Modulators and their Respective Potent Metabolites High-Performance Liquid Chromatography (HPLC)

Ryan D. Willey | Silvia Caceres, MSc | Jerry A. Nick, MD | Jennifer L. Taylor-Cousar, MD, MSCS

NACFC 2023 | Phoenix, AZ

National Jewish Health®

Breathing Science is Life.®

Abstract

CFTR modulators (elexacaftor-tezacaftor-ivacaftor, tezacaftor-ivacaftor, lumacaftor-ivacaftor, ivacaftor) are combination drug therapies directly improves the activity and trafficking of the defective CFTR protein.

The role of therapeutic drug monitoring (TDM) of CFTR modulators in clinical settings has recently been established. TDM can be useful for individualizing drug dosing, particularly when factors such as age, weight, liver and kidney function, and other medications being taken can impact drug metabolism and clearance. Because of these modulators' complex composition, developing a reliable, accurate method is essential for drugs with narrow therapeutic indexes.

This study aimed to develop a high-performance liquid chromatograph (HPLC) method to monitor elexacaftor-tezacaftor-ivacaftor or ETI and two metabolites (M1-IVA, M6-IVA. We aim to better assist clinicians in improving treatment outcomes while minimizing the risk of adverse effects.

Methodology

Patient samples were collected at 3 different time points, post ETI (3 hr, 4.5 hr, 6hr). 200-µL aliquot of plasma (calibrators, quality controls, and patient samples) was protein precipitated with 400 µL of 0.1% FA acetonitrile.

After the addition of internal standard, samples were vortexed then centrifuged (14000xg) for 10 minutes. supernatants were loaded into 1mL HPLC vials for analysis.

Samples were observed using variable wavelength UV Detector set to at 295nm (Figure 1).

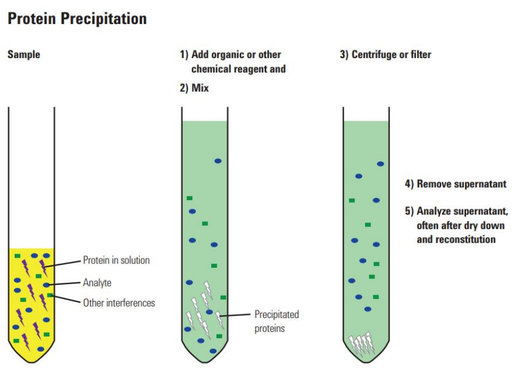


Figure 1. Extraction Technique

Instrument Parameters

An overview of the HPLC system is shown in Figure 2.

ThermoFisher UltiMate 3000 HPLC System

- **Mobile Phase:** 50:50 (v/v) DI Water:ACN
- **Flow Rate**: 0.7 mL/min
- **Column:** C18; Gemini 5µm, (150× 4.6mm).
- **Temperature:** 30° C
- **Detector:** VWR set to 295nm

Reportable Range: 0.2 µg/mL to 15 µg/mL (R^2=0.9883 to 0.9998).

Linear fit with a $1/C^2$ weighting factor.

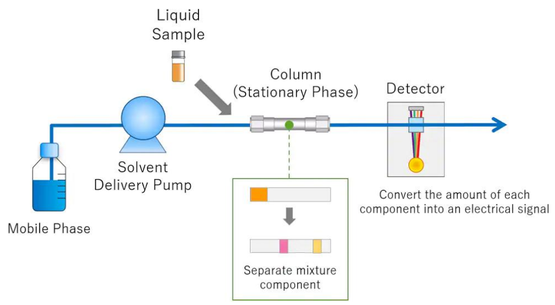
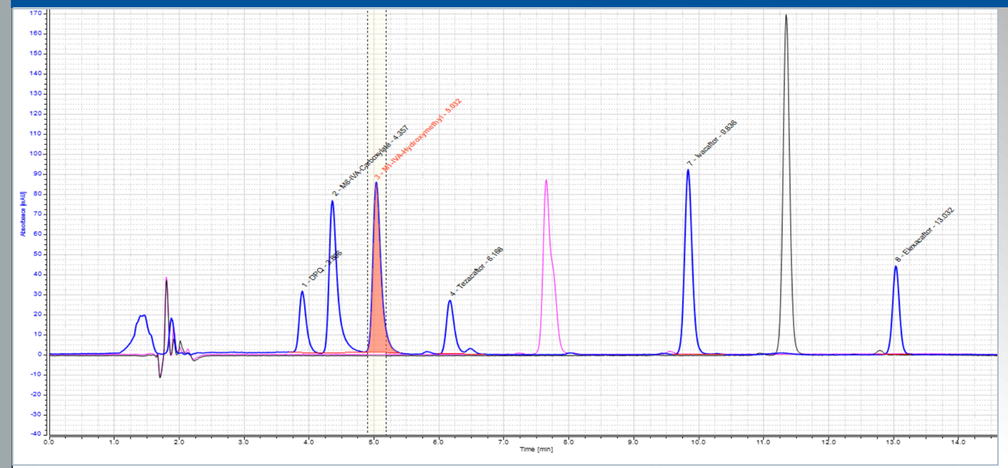
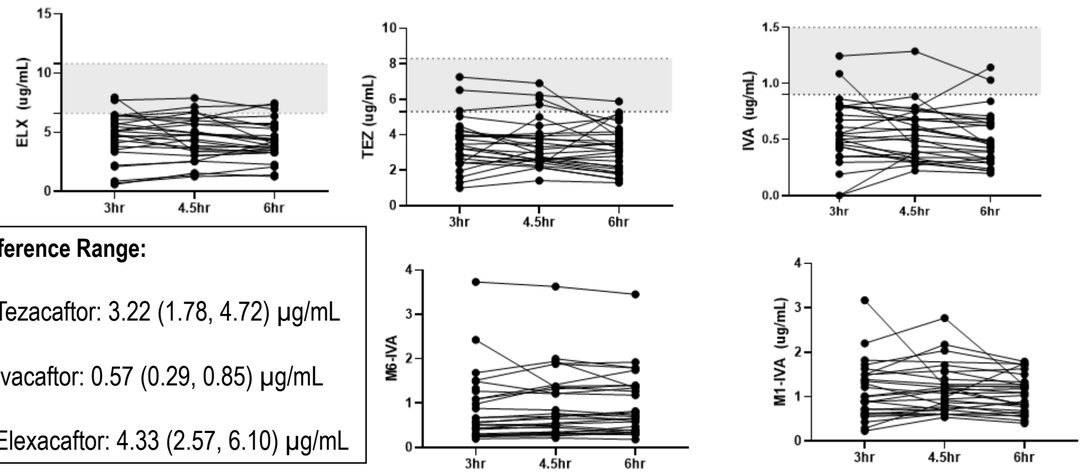


Figure 2. HPLC System Overview.

Results



Shown above is an individual patient chromatogram. Retention times were the following:
Primary | TEZA (6.16 min), IVA (9.83 min) ELEXA (13.03 min).
Secondary | M6-IVA (4.36 min), M1-IVA (5.03 min), M23 ELEXA (7.75 min), M1 TEZA (11.3 min).



Study Reference Range:

- Tezacaftor: 3.22 (1.78, 4.72) µg/mL
- Ivacaftor: 0.57 (0.29, 0.85) µg/mL
- Elexacaftor: 4.33 (2.57, 6.10) µg/mL

Figure 4. Patient Results

Conclusion

- We were able to detect ETI and the 2 metabolites from plasma samples of CF patients.
- Therapeutic Drug Monitoring (TDM) is crucial for patients as it aids at tailoring medication doses to individual needs, prevents adverse effects, and ensures drug effectiveness.
- It is especially important for conditions with narrow therapeutic ranges, chronic illnesses, and precision medicine.
- TDM not only benefits patients but also contributes to overall healthcare system improvement by identifying and addressing medication-related issues
- It leads to safer and more effective treatments.
- Its ongoing development and integration into clinical practice are vital for patient well-being and healthcare advancement.