

# Supporting Information

*J. Chem. Metrol.* X:X (202X) XX-XX

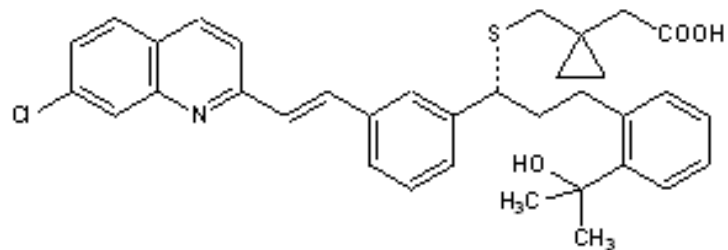
## A Simple UFLC Method for the Determination of Montelukast in Human Plasma

Cem Onal <sup>1</sup>, S. Evrim Kepekci Tekkeli <sup>1</sup> and Armagan Onal <sup>2</sup>

*Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul Health and Technology  
University, Istanbul, Türkiye*

*Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul University, Istanbul, Türkiye*

<b>Table of Contents</b>	<b>Page</b>
<b>Figure S1:</b> Chemical structure of Montelukast	2
<b>S1 : Experimental</b>	3
<i>S.1.1. Materials and Methods</i>	3
<i>S.1.2. Sample Preparation and Derivatization</i>	3
<i>S.1.3. Method Validation</i>	3
<i>S.1.4. Applicability in Clinical Trial</i>	4



**Figure S1** : Chemical structure of Montelukast

## **S1 : Experimental**

### *S.1.1. Materials and Methods*

Montelukast (MON) was procured from Sigma Aldrich (St. Louis, USA), and Singulair® tablets containing 10 mg of MON were obtained from a local pharmacy. All chemicals and reagents used were of analytical-reagent grade. The derivatizing agent, 4-bromomethyl-7-methoxycoumarin, and the catalyst dibenzo-18-crown-6-ether were sourced from Sigma-Aldrich Chemie (Steinheim, Germany). Instrumentation and chromatographic conditions for the UFLC analyses included a Shimadzu (Japan) LC 20 liquid chromatograph featuring a binary LC-20AT pump, SIL AT-HT autosampler, SPD-20A HT fluorimetric detector set at an excitation wavelength of 320 nm and an emission wavelength of 380 nm, along with a CTO 10 AC column oven. To prepare standard solutions, a stock solution of Montelukast (MON) was initially prepared in methanol at a concentration of 1 mg/mL. From this stock solution, standard solutions of MON were then created by diluting with methanol. For the derivatizing reagent, 4-bromomethyl-7-methoxycoumarin (BrMmC), daily stock solutions were prepared at a concentration of 50 µg/mL. This concentration was achieved by dissolving the appropriate amount of BrMmC in acetonitrile to mitigate decomposition due to light exposure. Additionally, a solution of dibenzo-18-crown-6-ether in acetonitrile was prepared at a concentration of 1 µg/mL to serve as a catalyst in the derivatization process.

### *S.1.2. Sample Preparation and Derivatization*

A volunteer's peripheral veins provided 5-10 mL of venous blood samples, collected following informed consent under ethical committee approval. The blood was preserved in tubes containing disodium EDTA and then subjected to centrifugation at  $4500 \times g$  for 10 minutes. The resulting plasma samples were stored at  $-20\text{ }^{\circ}\text{C}$  for subsequent analysis. To extract Montelukast (MON) from the plasma samples, 0.5 mL aliquots of plasma were mixed with working solutions of MON and captopril as an internal standard (IS). Following this, 1 mL of acetonitrile was added, and the mixture was vortexed at moderate speed for 3 minutes. The organic layer was evaporated to dryness under a stream of nitrogen at  $40^{\circ}\text{C}$ . The dried residue underwent derivatization by adding 25 µL of a 4-bromomethyl-7-methoxycoumarin (BrMmC) acetonitrile solution (50 µg/mL), along with 20 µL of an 18-crown-6 solution (1 µg/mL), and 2 mg of finely suspended  $\text{K}_2\text{CO}_3$ . The reaction mixture was shaken for 50 minutes at  $70^{\circ}\text{C}$  in a thermostated water bath. Subsequently, the resulting reaction mixture was diluted to 100 µL with the mobile phase. This protocol delineates the steps involved in processing and derivatizing plasma samples for subsequent analysis of Montelukast using the developed UFLC method.

### *S.1.3. Method Validation*

The method validation conducted for the analysis of Montelukast (MON) in plasma samples followed stringent ICH Guidelines, encompassing various critical parameters to ensure the reliability and robustness of the developed UFLC method. A series of MON concentrations ranging from 100 to 1000 ng/mL were prepared by spiking seven standard solutions into 200.0 µL of blank plasma extract after deproteinization. Each sample underwent derivatization, and the resulting calibration curve was constructed by plotting MON concentrations against their respective peak area ratio. This curve enabled the determination of unknown MON concentrations using the established regression equation. The Limit of Quantitation (LOQ) was defined as the lowest MON concentration that produced a chromatographic peak with an acceptable coefficient of variation. Intra-day and inter-day precision

were evaluated by analyzing MON samples (100, 500, and 1000 ng/mL) with six replicates within a single day and over five consecutive days, respectively. The relative standard deviations (RSD) calculated from these analyses demonstrated the method's precision and reproducibility. The stability of MON in plasma was assessed at concentrations of 100, 500, and 1000 ng/mL with three replicates after storage for 24 hours at ambient temperature and on the 3rd day under similar conditions. Additionally, stability after three freeze–thaw cycles and long-term stability at -20°C were also investigated. These studies ensured that MON remained stable under various storage conditions relevant to clinical sample handling. Recovery of MON at three concentration levels (100, 500, and 1000 ng/mL) was determined by comparing the peak areas obtained from individual plasma samples to those obtained from standard solutions spiked with blank plasma residues. The recovery rates indicated the method's ability to accurately quantify MON in plasma samples.

#### *S.1.4. Applicability in Clinical Trial*

The validated UFLC method was successfully applied in a clinical trial involving a healthy male participant who received a single 10 mg MON tablet under fasting conditions. Venous blood samples collected at specified intervals post-dose were processed to obtain plasma samples, which were then analyzed using the validated method. This approach allowed for a comprehensive assessment of MON pharmacokinetics over a 24-hour period, crucial for understanding its absorption, distribution, metabolism, and elimination profiles in the body. In conclusion, the rigorous validation of the UFLC method ensures its suitability and reliability for pharmacokinetic studies of MON in human plasma samples, adhering to international guidelines and facilitating accurate assessment of the drug's behavior in clinical settings.