SHORT REPORT

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A simple UFLC method for determination of montelukast in human plasma

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Abstract: In this investigation, an UFLC technique utilizing fluorimetric detection was reported for the analysis of Montelukast (MON) in plasma. The method involves pre-column derivatization using 4-bromomethyl-7-methoxycoumarin (BrMmC). The calibration curve linear concentrations from 10 to 1000 ng/mL. The method exhibited an average recovery rate of 95.49%. The newly developed method was effectively utilized to assess the pharmacokinetics of Montelukast (MON) following the administration of a single 10 mg tablet to a healthy male volunteer.

Keywords: Montelukast; ultrafast liquid chromatography; fluorescence detection; 4-bromomethyl-7-methoxy coumarin; human plasma. © 2024 ACG Publications. All rights reserved.

1. Sample Source

A volunteer's peripheral veins provided 5-10 mL of venous blood samples, collected following informed consent under ethical committee approval (Bezmialem Vakif University Hospital of the Istanbul Faculty of Medicine (Türkiye, Ethical Committee approval, No. 15/10, 2021). 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). A sample of Montelukast (MON) (Purity: ≥98%%) and 4-bromomethyl-7-methoxycoumarin (BrMmC) (Purity: 97.00%) was provided by Sigma-Aldrich. Organic solvents (HPLC Grade) were procured from Merck chemicals (Purity: 99.9%).

2. Previous Studies

Montelukast's (MON) acts as a selective leukotriene receptor antagonist [1]. Numerous HPLC methodologies have been developed and documented in the literature for quantifying MON concentrations in plasma [2–8]. This study seeks to establish and validate a straightforward and highly sensitive UFLC-FL method for quantifying Montelukast (MON) in human plasma. A novel, sensitive, and specific UFLC method utilizing fluorimetric detection is introduced, involving the derivatization of Montelukast (MON) with a 4-bromomethyl-7-methoxycoumarin (BrMmC) reagent. This method provides the capability to analyze a diverse range of analytes with high sensitivity and selectivity. BrMmC is commonly employed as a fluorescent reagent for derivatizing analytes containing a carboxylic acid functional group [9-11].

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3. Present study

The study explored various types of analytical columns, including C18, CN, and C8. In this study, C18 column, a mobile phase composed of methanol: acetonitrile: water (50:30:20, v/v/v) at a flow rate of 0.5 mL/min were selected for the measurement of MON. (an excitation wavelength of λext. = 320 nm and an emission wavelength of λem. = 380 nm). The MON derivative exhibited a retention time of approximately 2.0 minutes under the specified chromatographic conditions (Figure 1).

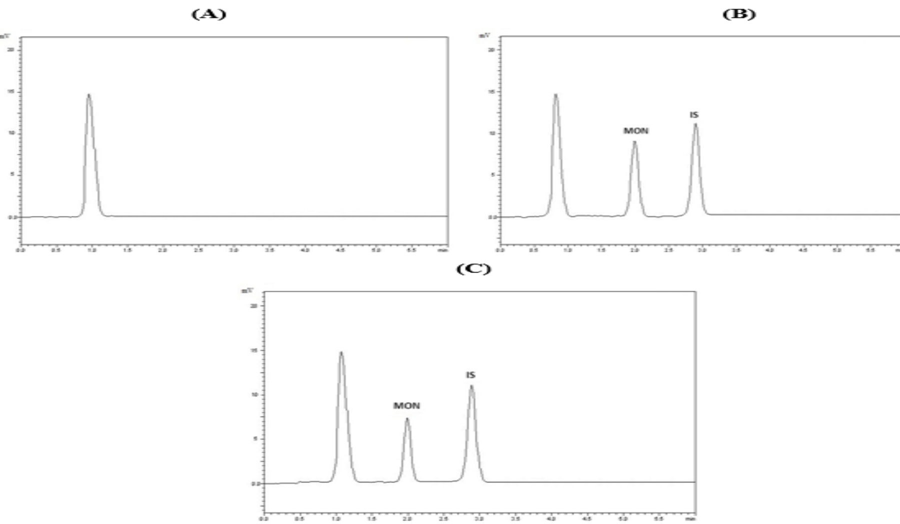

Figure 1. Chromatograms of (A) Blank plasma (B) plasma sample spiked with MON (750 ng/mL) (C) Plasma sample obtained from a healty volunteer 3.5 h after oral administration of 10 mg MON

The optimization process for derivatizing Montelukast (MON) involved investigating several parameters. Initial trials determined that 25 µL of a 50 µg/mL 4-bromomethyl-7-methoxycoumarin (BrMmC) solution in acetonitrile adequately facilitated plasma derivatization. Exploration of reaction temperatures (40, 50, 70, and 80°C) pinpointed 70°C as yielding the highest efficiency. Subsequent assessment of reaction duration at this temperature identified 50 minutes as the optimal period for achieving MON derivatization equilibrium. Minimal derivative formation was observed in its absence, contrasting with significantly enhanced production upon introducing 20 µL of an 18-crown-6 solution (1 µg/mL) and 2 mg of K_2CO_3 suspension during MON derivatization. Stability was observed for up to 24 hours at room temperature and in auto-sampler conditions, and for over 1 month at 4°C.

Calibration curves covered a range of MON plasma concentrations from 10 to 1000 ng/mL. The linear equation correlating MON plasma concentrations (ng/mL) to peak area ratio was established as $y = 1178x + 1967$ ($r^2 = 0.9996$). The limit of quantitation (LOQ) and the limit of detection (LOD) were found using following formula: LOD or LOQ = κSDa/b, where 10 for LOQ and κ= 3 for LOD, SDa represents the standard deviation of the intercept, and b represents the slope. Limits of Detection (LOD) and Quantitation (LOQ) were defined, resulting in an LOQ of 3.3 ng/mL and an LOD of 1 ng/mL. Mean recovery following extraction was evaluated using plasma samples spiked with MON at concentrations of 10, 500, and 1000 ng/mL, yielding an average recovery rate of 95.49% (See supporting information for details)

Table 1. Extraction recovery of MON from plasma samples

Added concentration (ng/mL)	Found concentration (ng/mL)± SD	Recovery%	RSD% *
10	9.538 ± 0.099	95.38	1.04
500	472.74 ± 5.21	94.55	1.10
1000	965.41± 10.45	96.54	1.08

*RSD: Relative Standard Deviation, *n*= 6

Intra-day and inter-day variations of the proposed method showed a small variance between low concentrations and high concentrations. Relative standard deviation values (RSD) according to concentrations are given in Table 2.

Table 2. Intra-day and Inter-day precision results of MON from plasma samples

Added concentration (ng/mL)	Found Concentration (ng/mL)± SD	RSD% *
Intra-day		
10	9.954± 0.075	0.75
500	496.82 ± 6.94	1.40
1000	1000.78± 11.85	1.19
Inter-day		
10	9.971 ± 0.175	1.76
500	498.73 ± 7.07	1.42
1000	997.26± 19.20	1.93

*RSD: Relative Standard Deviation; *n*=12 for each concentration per day.

The measurement uncertainty budget estimation was carried out based on the EURACHEM CITAC Guide and related literature data [12-15]. When the method validation data were taken into consideration, the main uncertainty sources affecting the measurement result were determined as the purity of the standard, calibration curve, recovery and repeatability. Since all of the uncertainties from the balance, volumetric sample preparation and derivatization were included in the repeatability data, they were not taken into account again. For the *Cmax* which is 500 ng/mL, the measurement uncertainty parameters were calculated as $u_{standard}$ 0.06, $u_{calibration\ curve}$ 0.75, $u_{recovery}$ 0.45 and $u_{repeatability}$ 0.29. When these data were processed in Equation 1, the combined measurement uncertainty was calculated as 2.10. The expanded uncertainty value was determined as 4.20 at the 95% confidence interval (k = 2). Uncertainty estimation of method in lower concentration estimated for 10 ng/mL and, the measurement uncertainty was found to be higher as expected (expanded 5.06). These results show that the developed method gives reliable results within a measurement uncertainty budget of around 5% at both high and low concentrations.

$$u = \sqrt{(u_{\mathrm{standard}})^2 + (u_{calibration\ curve})^2 + (\mathrm{u}_{\mathrm{recovery}})^2 + (\mathrm{u}_{\mathrm{repeatability}})^2} \qquad (1)$$

It was found that the calibration curve contributed the largest amount to the uncertainty budget, approximately 66.10%, while the second largest contribution was the recovery data of the proposed method, 23.80%. The contribution from the standard material purity was found to be very low and almost negligible.

The proposed method was effectively employed to examine Montelukast (MON) in plasma for a pharmacokinetic investigation. After administering a single 10 mg oral dose of MON to a healthy male volunteer, approximately 5-10 mL of venous blood samples were collected before dosing and at intervals of 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,14,16, and 24 hours post-dose. Pharmacokinetic parameters a were evaluated in Table 3 and shown as in Figure 2.

Table 3. Pharmacokinetic parameters of Montelukast

Parameter	Found value
T_{max} (h)	3.49
C_{max} (ng/mL)	502.37
$t_{1/2}$(h)	3.67
AUC0−t (ng h/mM) (hr ng/mL) (ng.h./mL)	3687.247

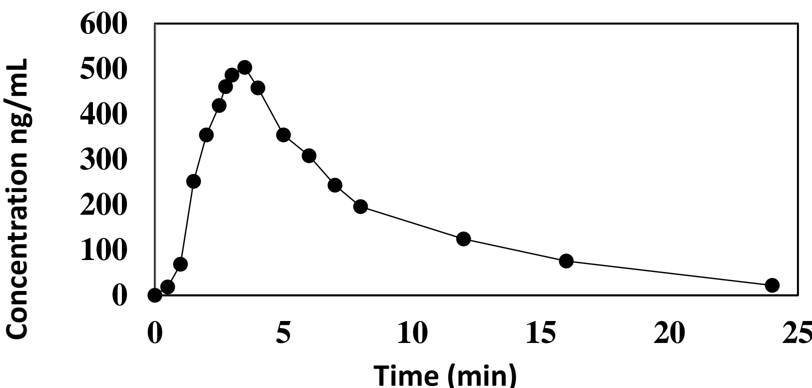

Figure 2. Plasma concentration–time profile of MON in a healthy volunteer after a single oral administration of 10 mg dose MON

In conclusion, the proposed and validated method for the determination of Montelukast in body fluids as a fast and easily applicable method in terms of sample preparation steps and chromatographic stages. With this method, which is extremely robust and resistant to experimental conditions, a total runtime of 6 minutes was obtained in plasma samples, where Montelukast peak was obtained after a period of 3 minutes. The method not only reduces the sample preparation and measurement time, but also demonstrates its sensitivity for the measurements of plasma samples, especially in pharmacokinetic studies, with a limit of detection (LOD) value of 1 ng/mL. This method can be applicable in the clinical trials and new drug form development studies for montelukast.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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