

Determination of Leukotriene B₄ (LTB₄) in Human Plasma by UFLC-MS/MS – A Novel Approach to Evaluate LTB₄ as a Pharmacodynamic (PD) Biomarker in Pharmaceutical Development

Mike-Qingtao Huang¹, Naidong Weng¹, Weisheng Lin², Futain Han², Hsiaoju Lin², Zhongping (John) Lin², Xiaohua Xue³, Anne Fourie³, Ann Welton³, Kirk Bertelsen³ and Jan de Jong³
¹ Johnson & Johnson Pharmaceutical Research & Development, LLC, 1000 Route 202 South, Raritan, NJ 08869

² Frontage Laboratories, Inc., 105 Great Valley Parkway, Malvern, PA 19355; ³ Johnson & Johnson Pharmaceutical Research & Development, LLC, 3210 Merryfield Row, San Diego, CA 92121

Overview

- A validated method for the determination of Leukotriene B₄ in human plasma using UFLC-MS/MS.

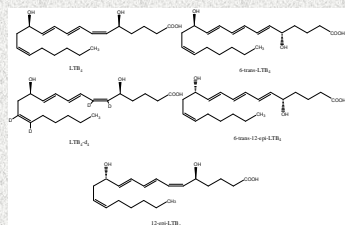
Assay Summary

- Extraction method: Liquid-liquid extraction with methyl tert-butyl ether
- Calibration curve range: 0.2 – 200 ng/mL
- QC concentrations: 0.6, 30, 150 ng/mL
- Sample volume: the assay was initial validated with 200 uL of plasma (BTM-1138-R0); later was partially validated with 100 uL of plasma (BTM-1138-R1) due to the limitation of plasma volume in incurred samples.
- Method: UFLC/MS/MS

Introduction

Production of leukotriene B₄ (LTB₄) is considered important for the inflammatory component in a number of diseases. Thus, agents that reduce LTB₄ levels should have therapeutic value as anti-inflammatory agents. Therefore LTB₄ has been used as a PD biomarker in pharmaceutical development. No LC-MS/MS assays were reported in the literature for the determination of LTB₄ with baseline separation of LTB₄ and its isomers in human plasma. This poster presents a novel UFLC-MS/MS method for the determination of LTB₄ with baseline separation of LTB₄ and its three isomers in ex vivo stimulated human plasma using LTB₄-d₄ as Internal Standard. The structures of LTB₄, its three isomers and LTB₄-d₄ are shown in Figure 1.

Figure 1. Structures of LTB₄, LTB₄-d₄ and LTB₄ Isomers

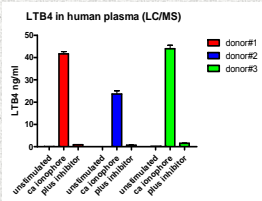


Methods

Ex vivo stimulation and detection for LTB₄

- Dilute human blood 1:1 with RPMI1640
- Incubate in the absence/presence of inhibitor for 15 min
- Stimulate with calcium ionophore for 60 min
- Centrifuge and collect plasma
- Measure LTB₄ in plasma by LC/MS/MS (Figure 2)

Figure 2. Ex vivo stimulation and LC-MS/MS detection of LTB₄



UFLC/MS/MS Condition

- Column: Phenomenex Synergi Hydro-RP, 100 x 3 mm, 2.5 um
- Mobile Phase A: 5 mM Ammonium
- Mobile Phase B: 80:20 Acetonitrile/MeOH
- Flow rate 0.65 mL/min with gradient elution
- Injection Volume: 20 uL
- UFLC/MS/MS system: Sciex API 4000 Q-Trap with Shimadzu pump in negative ion electrospray MRM mode
- MRM transition: m/z 335.0 → 194.9 for LTB₄ and m/z 339.0 → 196.9 for LTB₄-d₄ (IS).

Results and Discussion

Separation of LTB₄ and its isomers

- Baseline separation is needed for LTB₄ and its isomers as well as an unknown interference peak observed in incurred sample.
- Figure 3 shows the separation using regular HPLC system: baseline separation of LTB₄ and the unknown interference peak was not achieved.
- Figure 4 shows the separation using UFLC system: baseline separation was achieved among LTB₄, its isomers and the unknown interference peak. This condition was used for the assay validation.

Linearity and Sensitivity

- Calibration curve range: 0.2 – 200 ng/mL with r² ≥ 0.9975.
- LLOQ was confirmed with 6 replicates (Table 1).

Recovery and Dilution Integrity

- Recovery was determined 99% -106% for LTB₄ at three conc. Levels and 101% for IS indicating good recovery.
- A 20 X dilution was established with a high QC at 2000 ng/mL diluted with control plasma.

Precision and Accuracy

- Excellent assay accuracy and precision results were obtained using QC concentrations at 0.6, 30 and 150 ng/mL for both Method BTM-1138-R0 and Method BTM-1138-R1.
- The assay accuracy and precision results for Method BTM-1138-R0 are shown in Table 2.
- The assay accuracy and precision results for Method BTM-1138-R1 are shown in Table 3.

Table 1. Precision and accuracy of LLOQ

Sample No.	Method BTM-1138-R0	Method BTM-1138-R1
	LTB ₄ 0.2 ng/mL	LTB ₄ 0.2 ng/mL
1	0.217	0.191
2	0.206	0.167
3	0.230	0.193
4	0.230	0.212
5	0.187	0.199
6	0.226	0.175
Mean	0.216	0.190
SD	0.017	0.016
%CV	7.9	8.4
%Nominal	108.0	95.0

Stability

- Minimum sample stability was established and summarized in Table 4.

Table 2. Precision and Accuracy of QC samples

Day ID		LTB ₄ Concentration, ng/mL		
		0.6	30	150
Intraday 1	Mean	0.608	30.7	158
	SD	0.027	0.431	1.910
	%CV	4.4	1.4	1.2
	%Nominal	101.3	102.2	105.1
Intraday 2	Mean	0.585	30.0	156
	SD	0.022	0.332	2.606
	%CV	3.8	1.1	1.7
	%Nominal	97.5	100.2	103.9
Intraday 3	Mean	0.572	29.7	154
	SD	0.018	0.279	2.468
	%CV	3.1	0.9	1.6
	%Nominal	95.3	99.1	102.3
Interday Results	Mean	0.588	30.1	156
	SD	0.026	0.516	2.808
	%CV	4.4	1.7	1.8
	%Nominal	98.0	100.5	103.8

Table 3. Precision and Accuracy of QC samples

Day ID	Sample No.	LTB ₄ Concentration, ng/mL		
		0.6	30	150
Intraday 1	1	0.626	29.8	148
	2	0.588	29.9	151
	3	0.553	30.0	149
	4	0.594	29.5	148
	5	0.564	29.2	150
	6	0.599	29.3	149
	Mean	0.587	29.6	149
	SD	0.026	0.344	1.258
	%CV	4.4	1.2	0.8
	%Nominal	97.8	98.7	99.5

Figure 3. Separation of LTB₄, its isomers and an interference peak in incurred plasma sample by regular HPLC

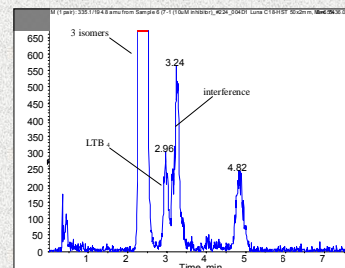


Figure 4. Separation of LTB₄, its isomers and an interference peak by UFLC

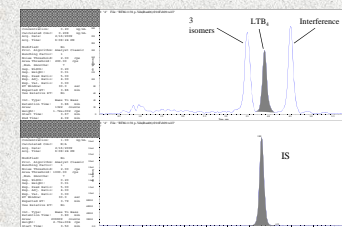


Figure 5. Plasma blank (A) and Lower Limit of Quantitation (B) (0.2 ng/mL)

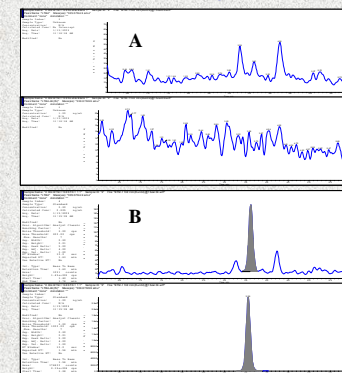


Table 5. Stability Summary

Bench-top stability	At least 6 hours at room temperature
Stock solution stability	At least 53 days at 4 °C and 20 hours at room temperature for both LTB ₄ and LTB ₄ -d ₄ (IS) prepared in acetonitrile/water, 50:50
Processed sample stability	At least 72 hours at room temperature
Freeze/thaw stability	3 freeze (-20 °C)/thaw cycles
Long-term storage stability	At least 53 days at -20 °C

Conclusions

A novel UFLC-MS/MS method was developed and validated for the determination of LTB₄ in ex vivo stimulated human plasma. The method was sensitive, robust and reliable. The validated method has been successfully used to support a clinical study.