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Low Level Quantitative Analysis of Prostaglandins in Human Serum by LC/MS/MS Utilizing Dual Ion Funnel Technology

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Introduction

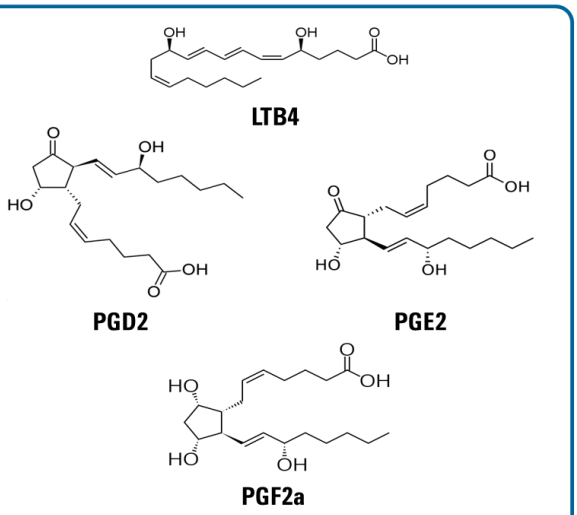
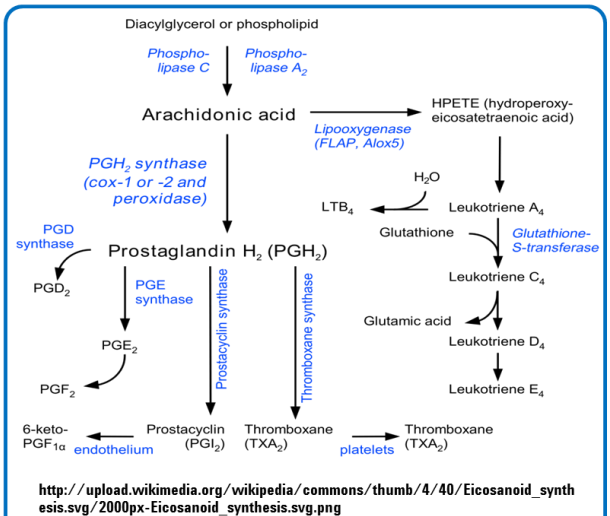


Figure 1. Structures

Mass spectrometry has become an essential tool for small molecule quantitation due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis of multiple analytes. Prostaglandins can be challenging compounds to analyze due to the low levels in biological matrices relevant to clinical research. In order to address this challenge, a sensitive liquid chromatography tandem mass spectrometry (LC/MS/MS) method for the simultaneous analysis of four prostaglandins in serum samples has been developed without derivatization.



http://upload.wikimedia.org/wikipedia/commons/thumb/4/40/Eicosanoid_synthesis.svg/2000px-Eicosanoid_synthesis.svg.png

Figure 2. Biosynthesis of Eicosanoids

Experimental

Sample Preparation:

Sample information: Four prostaglandin standards were purchased from Cayman Chemical Co.

Calibration curve: Calibration range is from 0.005 to 100 ng/mL. The dilution solvent is acetonitrile.

Serum sample preparation: 250 µL human serum (obtained from UTAK Laboratories, Inc.) was precipitated with 500 µL acetonitrile, votexed for 1 minute and centrifuged for 4 min at 10,000 rpm. 500 µL. Supernatant was transferred and diluted with 500 µL of water. 2 µL was injected for LC-MS/MS analysis.

LC Method:

Agilent 1290 Infinity UHPLC series binary pump, well plate sampler, thermostatted column compartment
Column: Eclipse Plus C18,RRHD, 2.1x100mm 1.8 um
Column temperature: 50 °C
Injection volume: 2 µL
Autosampler temp: 4 °C
Needle wash: flushport (MeOH:water 75:25), 10 sec
Mobile phase: A = 0.01 % formic acid in water:acetonitrile 70:30
B = acteonitrile:isopropanol 50:50
Flow rate: 0.4 mL/min
Gradient for neat solution: 0% B to 35% B in 5 minutes and up to 95% B in 0.1 min, hold at 95% B for 2.0 min, post run is 2.0 min
Gradient for serum samples: 0% B to 40% B in 8 minutes and up to 95% B in 0.1 min, hold at 95% B for 2.0 min, post run is 4.0 min

MS Method:

Agilent 6490 triple quadrupole mass spectrometer
Ion mode: Jet Stream negative mode

Gas temperature:	250 °C
Drying gas (nitrogen):	11 L/min
Nebulizer gas (nitrogen):	35 psi
Sheath gas (nitrogen):	350 °C
Sheath flow:	12 L/min
Capillary voltage:	-3500V
Nozzle voltage:	-1250V
Q1/Q2 Resolution:	0.7/0.4 amu
Delta EMV:	200V
Cell acceleration voltage	2V
Cycle time	300 ms
Min/max dwell time	15.6/48.9 ms

Results and Discussion

This method utilizes triggered MRM (tMRM). In addition to the two primary MRM transitions for each analyte, 4 to 7 more product ions were selected and triggered when the primary transitions reached a target threshold (Table 1). Three replicate measurements were made, which only takes about 1 second with 18 concurrent MRMs. Note that PGD2 and PGE2 are isobaric isomers and baseline LC separation is necessary for accurate quantitation.

Table 1. Triggered MRM acquisition table (Primary and Triggered transitions)

Compound Name	Precursor	Product	Primary	Trigger	Threshold	Collision Energy
LTB4	335.2	317.2	True	True	500	10
LTB4	335.2	195.1	True	True	500	13
LTB4	335.2	129.1, 123.1, 71.0, 59.0			0	20, 16, 12, 20
PGD2/PGE2	351.2	315.2	True	True	500	8
PGD2/PGE2	351.2	271.2	True		0	16
PGD2/PGE2	351.2	333.1, 235.2, 233.2, 217.2, 189.1, 175.1, 135.1			0	12, 12, 8, 20 , 18,12,8
PGF2a	353.2	309.2	True	True	500	16
PGF2a	353.2	291.2	True		0	20
PGF2a	353.2	335.2, 317.2, 247.2, 209.2, 193.1, 171.2, 165.1			0	12,8, 24, 20, 26,16, 20

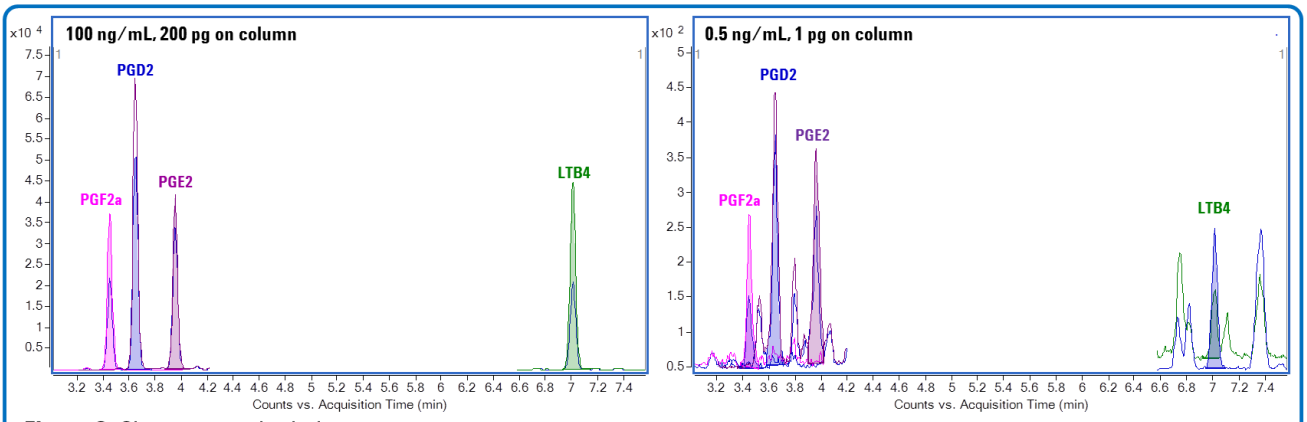


Figure 3. Chromatography in human serum

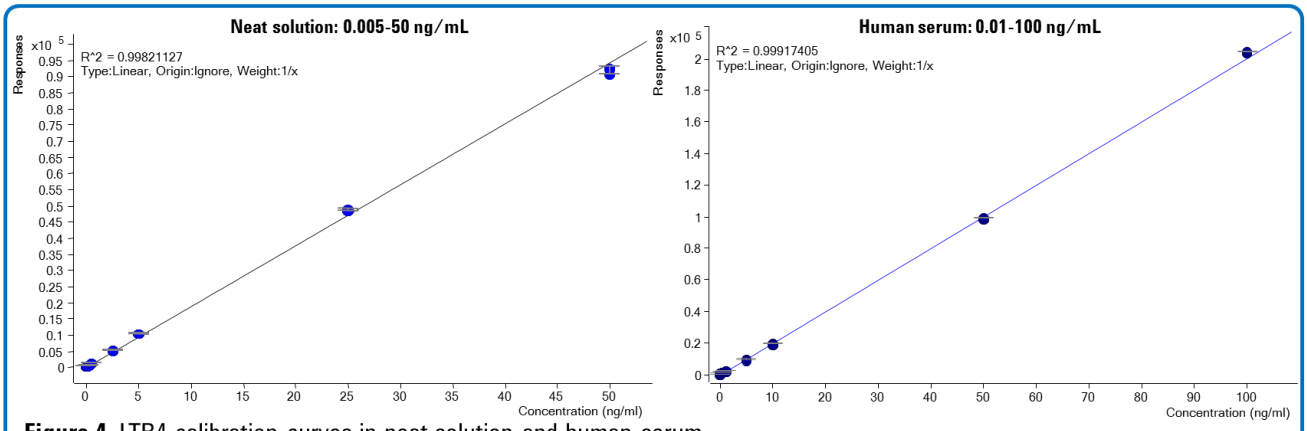
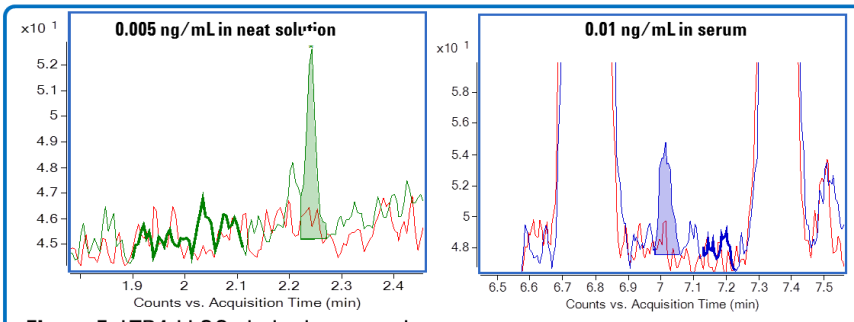


Figure 4. LTB4 calibration curves in neat solution and human serum

Results and Discussion



In the neat solution, the mass resolutions of both Q1 and Q2 MRM acquisition were 0.7 amu. In the biological matrix, the Q2 resolution was increased to 0.4 amu due to the complexity and interference from the matrix, which is simply cleaned up by protein precipitation.

Figure 5. LTB4 LLOQs in both neat and serum.

Table 2. Summary

Compound	in neat solution				in serum			
	LOQ (ng/mL)	fg on column	Range (ng/mL)	R^2	LOQ (ng/mL)	fg on column	Range (ng/mL)	R^2
PGF2a	0.005	10	0.005-50	0.9971	0.01	20	0.01-100	0.9993
PGD2	0.005	10	0.005-50	0.9960	0.05*	100	0.05-100	0.9995
PGE2	0.005	10	0.005-50	0.9982	0.05*	100	0.05-100	0.9993
LTB4	0.005	10	0.005-50	0.9978	0.01	20	0.01-100	0.9991

* The endogenous PGD2 and PGE2 are detected in human serum blank. The LOQ could be lower if spiked into cleaned biological matrix.

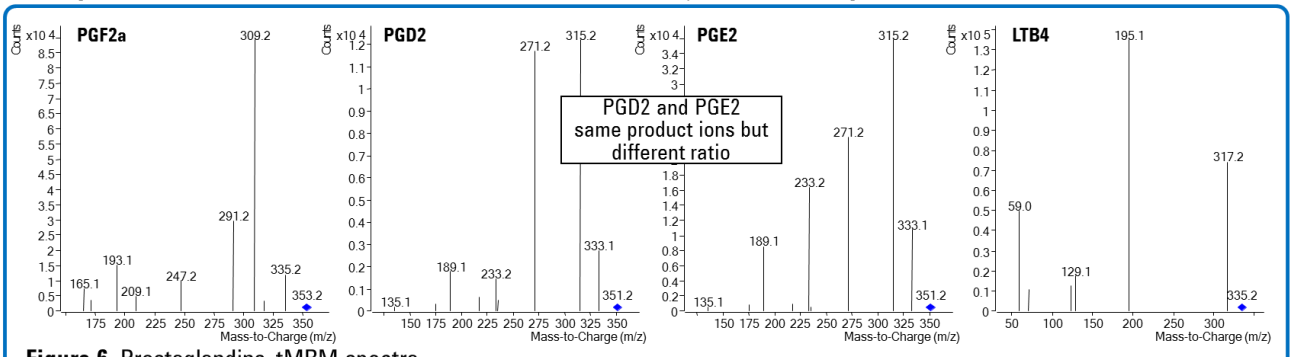


Figure 6. Prostaglandins tMRM spectra.

tMRM acquisition combines MRM with the generation of a product ion spectrum which can then be used for library identification and confirmation. As a result, tMRM analysis decreases analysis time, increases throughput, and allows for fast, sensitive, quantitative and qualitative analysis on a single instrument, in a single analytical run. In a future study, a heart-cutting LC method with highest separation power and larger injection volume will be applied to achieve a high sensitive quantitative analysis on challenging biological matrices.

Conclusions

- Baseline separation of four target and other endogenous prostaglandins is achieved under 9 minutes.
- Isobaric isomers, PGD2 and PGE2, are distinguished by LC separation and by a tMRM method.
- The calibration curves show excellent linearity (>0.996) with four orders of dynamic range in both neat solution and human serum.
- Excellent accuracy, precision, reproducibility, and signal stability of LC-MS/MS (QQQ) analyses were observed for all the target compounds.
- This fast and simple LC-MS/MS method is suitable for analyzing multiple prostaglandins in biological matrices in a single run.