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A SENSITIVE AND SELECTIVE MICROSCALE SPE METHOD FOR THE DETERMINATION OF F_2a IN RAT PLASMA BY LC/MS/MS

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OVERVIEW -

Previous solid phase extraction (SPE) methods for isoprostanes included using a C18 reversed-phase material with an elution solvent of ethyl acetate:heptane:methanol (5:4:1) that required evaporation and reconstitution before LC/MS/MS analysis. Additionally, using this method often resulted in recoveries of only 60%. We were asked to help a customer develop a method to run 10,000 to 30,000 samples per year with a better SPE method with higher recoveries. Because of the need for higher throughput, we developed a simplified method on an ultra-low elution volume 96-well SPE plate utilizing a mixed-mode material. Sample recoveries were greater than 90%. Using the low elution volume plates, there is a concentration factor of at least 2. The calibration range was 0.1 to 100 pg/uL and was linear for both water and plasma samples. The detection limit was 0.1 pg/uL.

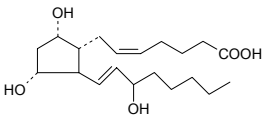
INTRODUCTION –

F_2-iosprostanes are biomarkers for oxidative stress in vivo that are stable and distributed throughout the body. They are formed from nonenzymatic autoxidation of arachidonic acid in cell membranes (1). The extent of systematic damage due to oxidative stress within the body can be determined by measuring levels of these isoprostanes in body fluids such as plasma and urine (1). Our challenge was to develop a simple, robust and high-throughput SPE method followed by LC/MS/MS analysis for prostaglandin F_{2a}, or isoprostane.

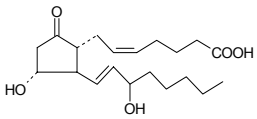
The challenge in this analysis was achieving both high throughput and low detection and quantitation limits (LOD/LOQ's). We know that solid phase extraction (SPE) in a 96-well plate format (2, 3) can meet the demand for low LOD/LOQ's using less sample volume. In this experiment, we chose to develop the method on a novel ultra-low elution volume 96-well SPE plate containing a mixed-mode material. Additionally, our method of chromatographic analysis was an LC/MS/MS system to increase our level of sensitivity.

1. Chu, K. O., Wang, C. C., Rogers, M. S., Pang, C.P. Anal. Biochem. 2003, 316, 111.
2. Zweigenbaum, T., Heing, K., Steinborner, S., Wachst, T., Henion, J., Anal. Chem.,1999, 71, 2294.
3. Show, W.Z., Jiang, X., Beato, B.D., Naidong, W., Rapid Commun. Mass Spectrom., 2001, 15, 466.

STRUCTURES-



Analyte: Prostaglandin F_{2a}
FW: 354.5
MRM 353.0 → 193.0



ISTD: 8-Iso Prostaglandin E_2
FW: 352.5
MRM: 351.0 → 315.0

SPE METHOD-

Device:	Oasis® MAX µElution Plate
Part Number:	186001829
Condition:	200 µL MeOH
Equilibrate:	200 µL H_2O
Mix/Load:	350 µL Spiked H_2O or rat plasma (0.1to 100 pg/µL)and 350 µL ISTD (10 pg/µL) in H_2O
Wash 1:	200 µL H2O with 2% NH_4OH (Lock on acidic analytes and remove salts/polars)
Wash 2:	200 µL MeOH (remove neutral and basic interferences)
Elute:	50 µL ACN:IPA (40:60) with 5% FA
Dilute:	125 µL H_2O with 2% NH_4OH (total volume of 175 µL results in a concentration factor of 2)

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HPLC CONDITIONS-

Column: XTerra® MS C_{18}, 2.1 x 30 mm, 3.5 µm
Part Number: 186000398
Mobile Phase A: Water with 0.5% NH_4OH
Mobile Phase B: ACN with 0.5% NH_4OH
Flow Rate: 0.2 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	95%	5%
1.0	5%	95%

Injection volume: 20 µL
Temperature: Ambient
Instrument: Alliance® 2795 Separsations Module

MS/MS CONDITIONS-

Instrument: Waters Micromass® Quattro Ultima
Source: Electrospray negative
Source temperature: 150 °C
Desolvation temperature: 400 °C
Desolvation gas flow: 520 L/hr
Collision gas cell: 2.0e-3 mbar
Cone voltage: 60 V for both
Collision energy: 25 eV for F_{2a} and 12 eV for E_2

RESULTS-

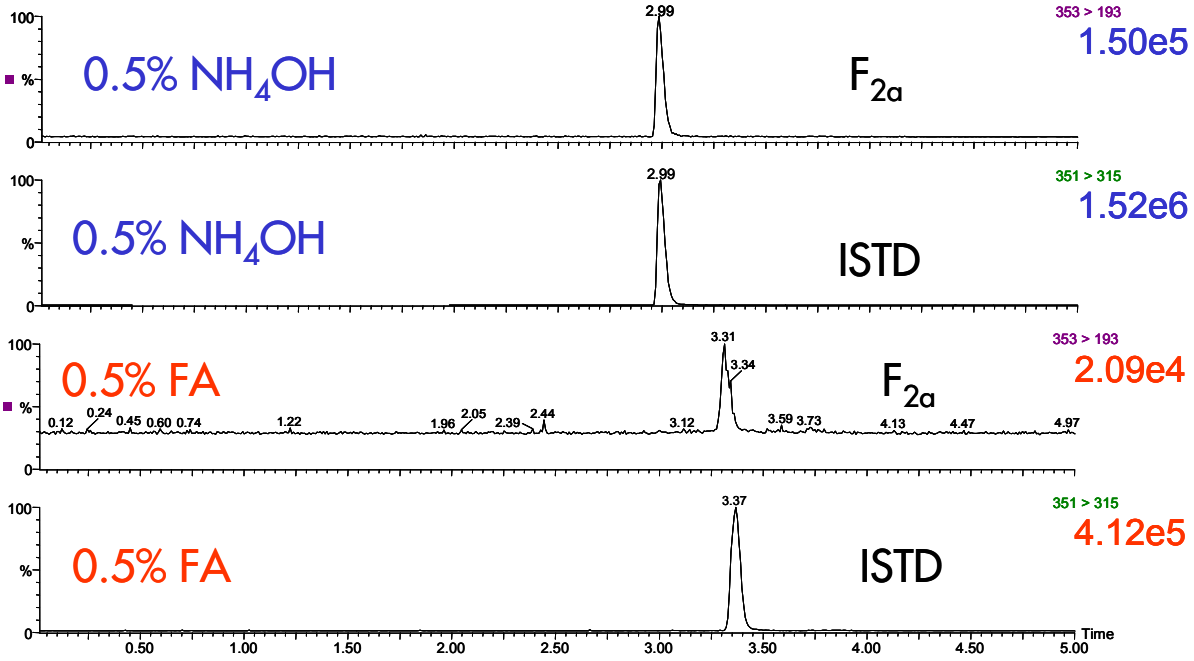

Figure 1. Before developing the SPE method, we needed to develop an LC/MS/MS method for these analytes. We made an infusion of the samples into the MS to determine the MRM transitions and the optimum settings for the MS. We then ran the samples under low and high pH conditions using an XTerra® MS C_{18} column. Under high pH conditions with NH_4OH, we found the best signal-to-noise and sensitivity for the analytes and chose that as our final method.

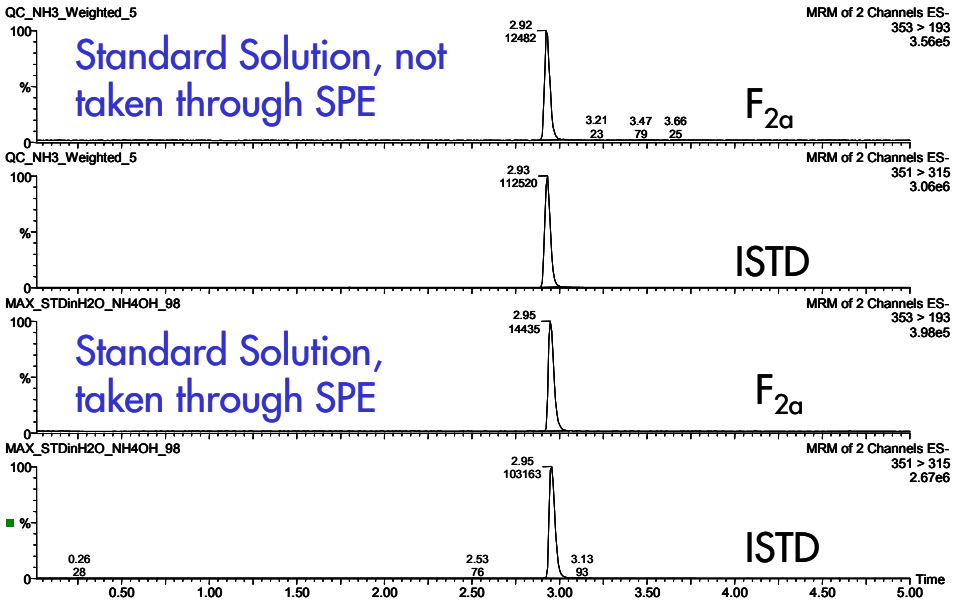

Figure 2. Since these analytes are acids, we chose to use the Oasis® MAX (mixed-mode anion exchange) SPE sorbent for our clean-up step. This sorbent l is highly selective for the analytes and allowed us to obtain an extremely clean extract. Additionally, in order to pre-concentrate the sample and to eliminate the evaporation and reconstitution steps, we utilized the Oasis® µElution Plate which enables low elution volumes. We first tried the generic SPE method that is recommended for the MAX material on a sample dissolved in water. We obtained recoveries of 115% and 92% for the F_{2a} and ISTD, respectively.

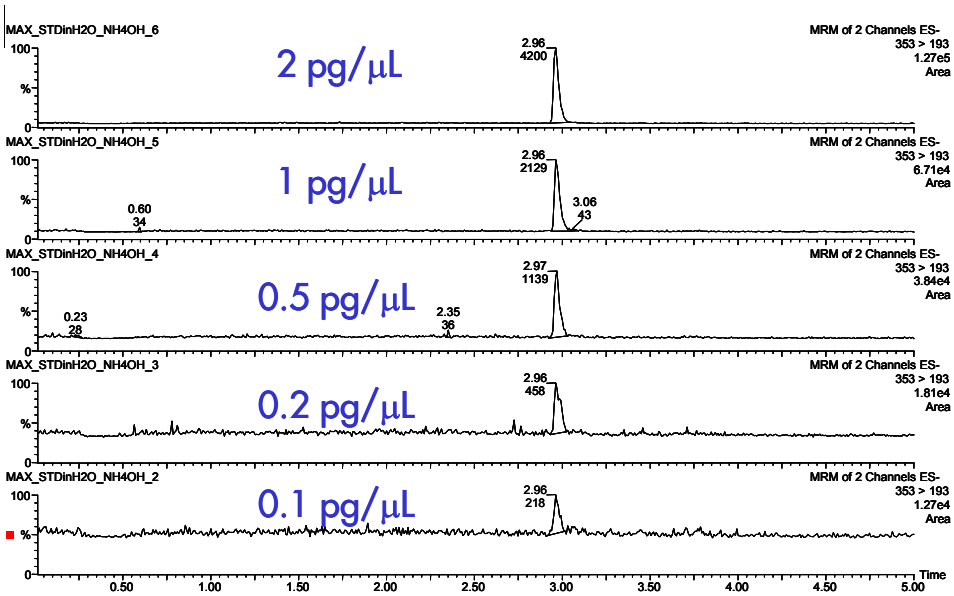

Figure 3. Calibration curve date for the Prostaglandin standards, our calibration range is 0.1 to 100 pg/µL. Our LOD is 0.1 pg/µL.

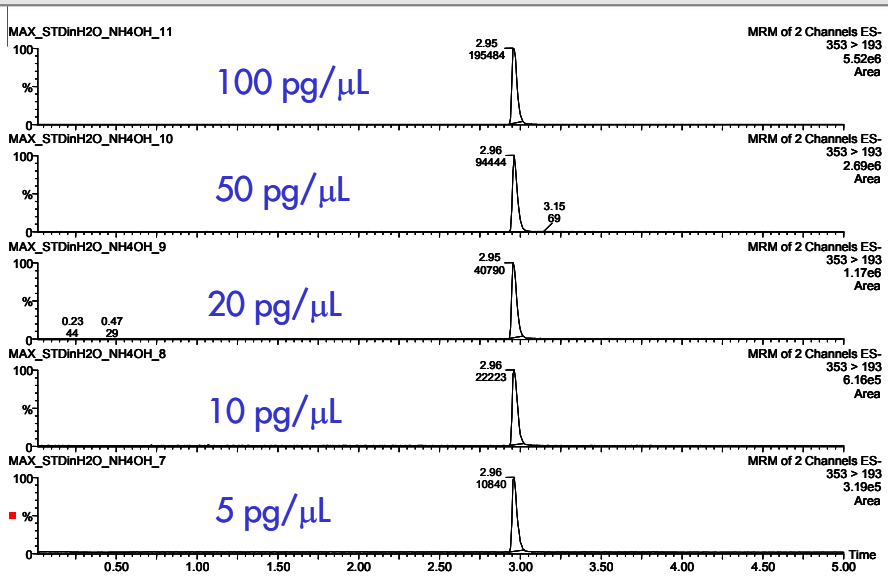

Figure 4. Calibration curve date for the Prostaglandin standards, our calibration range is 0.1 to 100 pg/µL. Our LOD is 0.1 pg/µL.

Coefficient of Determination: 0.997131
Calibration curve: 2004.29 * x + 71.7943
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

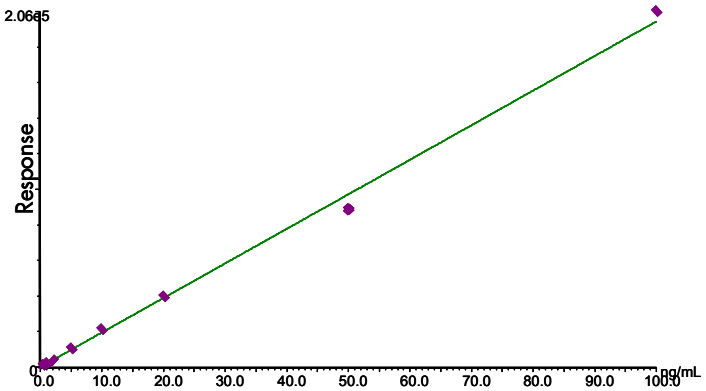

Figure 5. We then generated a calibration curve for the standards to determine our concentration range and our detection limits. Our calibration range is 0.1 to 100 pg/µL. Our LOD is 0.1 pg/µL.

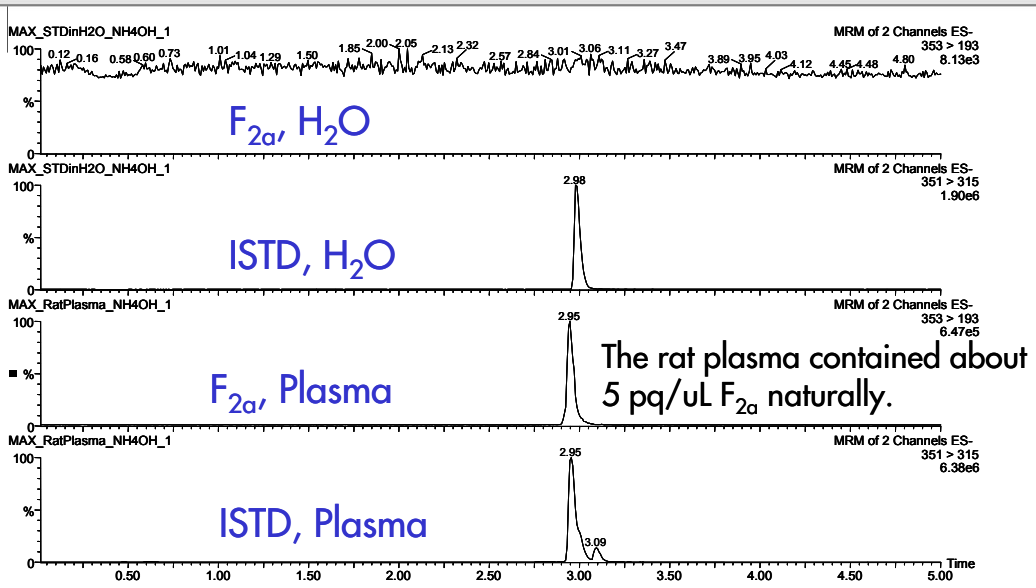

Figure 6. We then ran blank plasma through the SPE procedure and found that the blank plasma contains about 5 pg/µL of F_{2a}.

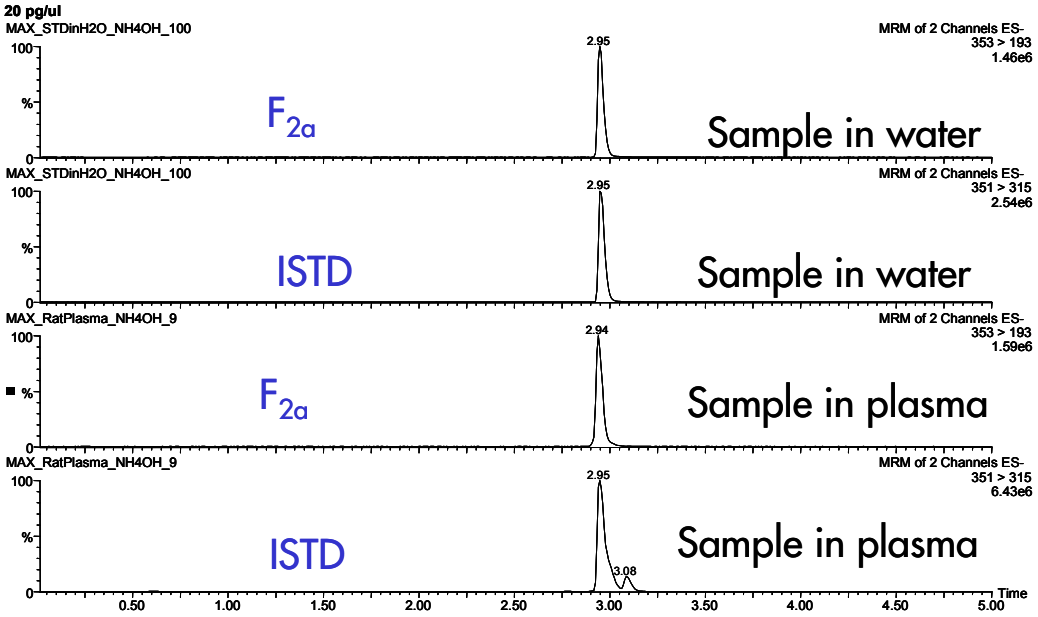

Figure 7. We spiked rat plasma with 20 pg/µL of F_{2a} and ran both the standard and the plasma through the clean up procedure and obtained good recoveries, after accounting for the naturally occurring F_{2a}.

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CONCLUSIONS-

Using the generic SPE protocol on the Oasis® MAX µElution plate, we developed a high-throughput method for prostaglandin F_{2a} with:

-Good sample recoveries
-Concentration factor at least 2x
-A calibration range of 0.1 – 100 pg/µL
-LOD of 0.1 pg/µL

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