UHPLC (X-LC®) APPLICATION NOTE 01-09

Rapid Profiling of Prostanoids

Rapid Profiling of Prostanoids in Biological Samples using the JASCO X-LC® system

Deepti Varma, Dr. Susan Jansen, Temple University, Philadelphia, PA
Atsushi Tsukamoto, JASCO

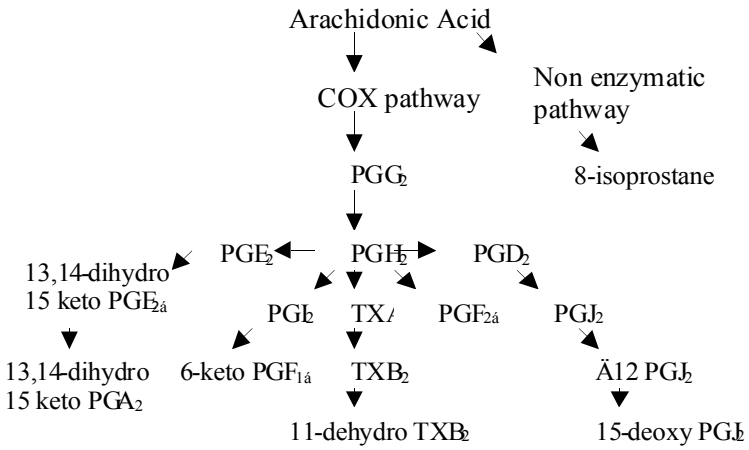
Introduction

Inflammation is implicated in number of diseases including, hypertension[1], ischemic heart injury[2] rheumatoid arthritis[3] and atherosclerosis[4]. Therefore, a mechanistic understanding of the inflammation process at the molecular level would contribute to the mechanism of the disease state, injury and recovery. Eicosanoids are specific biomarkers for inflammation[5] Arachidonic acid (AA) which is an omega-6 polyunsaturated fatty acid can undergo oxidative metabolism by cyclooxygenase (COX), lipoxygenase (LOX) or cytochrome (P450) to produce a number of these inflammatory lipid biomolecules. The oxidation of AA by COX-2 results in a number of prostanoids which include PGE_2, PGD_2 and PGF_2, PGJ_2, prostacyclin (PGI_2) and thromboxane A_2 (TXA_2)[6]. Prostacyclin is considered to be a potent vasodilator whereas thromboxane A_2 is considered to be a potent vasoconstrictor. PGI_2 and TXA_2 are unstable at physiological pH and are readily converted to 6-keto $PGF_{1\alpha}$ and 11-dehydro TXB_2 respectively. Hence these are considered to be reliable for the in vivo estimation of PGI_2 and TXA_2 respectively. PGE_2, PGD_2 and PGF_2 are considered to be pro inflammatory biomarkers.13, 14 –dihydro-15-keto PGE_2 and 13, 14 –dihydro-15-keto PGA_2 are major metabolites of PGE_2 in plasma.15-deoxy PGJ_2 which is the major metabolite of PGJ_2 is thought to have anti inflammatory properties especially in rheumatoid arthritis. Free radical peroxidation of AA produces a series of prostaglandin like compounds known as the isoprostanes[7] of which F_2 isoprostane which are PGF_2 like compounds is considered to be the most important.8-iso PGF_2 also known as 8-isoprostane the most abundant form of the F_2 isoprostane is considered to be an indicator of *in vivo* oxidative stress[8]. Figure 1 shows the understood pathways for the metabolism of arachidonic acid.

This application note describes a 6.8 min method for identifying a mixture of 9 prostanoids using Jasco X-LC. Separation of these compounds using conventional HPLC typically takes around 21 minutes.

Figure 1: Arachidonic acid metabolism by COX-2 and non-enzymatic pathway.

Experimental



Sample Preparation:
8-iso PGF_2 was supplied in methanol with a stock mass concentration of 1 mg/ml and 13, 14 –dihydro-15-keto PGE_2 and 13, 14 –dihydro-15-keto PGA_2 were supplied in methyl acetate with a stock mass concentration of 1 mg/ml. All the other prostanoids were dissolved in 1 ml methanol such that the stock mass concentration for all was 1mg/ml.Working standards for the analytes were prepared by serial dilution using ACN.

X-LC system and operating conditions:

System: Jasco X-LC
Mobile Phase A: 0.1 % Formic Acid
Mobile Phase B: ACN
Flow Rate: 0.2 ml/min
Gradient:

Time (min)	%B
0.0	35
4.0	35
6.0	90
6.5	90
6.6	35
6.8	35

Injection Volume: 2µl

Stationary Phase:

Column: Restek Pinnacle DB C_{18} 1.9µm (50*2.1 mm)
Column Temperature: 25°C

UV Detection:

Time (min)	*Wavelength (nm)*
0.0	220
1.0	196

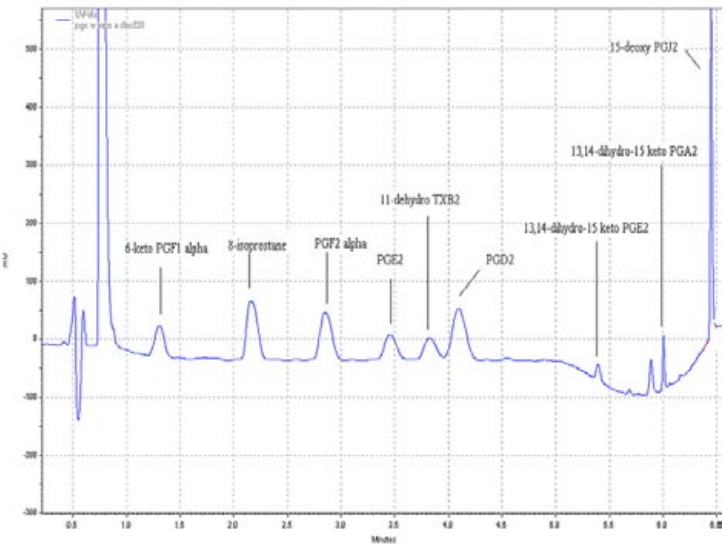
Results and Discussion:

Table 1 provides a list of the compounds from the method and figure 1 shows an X-LC chromatogram of a standard mixture of prostanoids (150 ng each). The compounds are separated by a gradient elution of 0.1 % formic acid and acetonitrile on a Restek Pinnacle DB C_{18} 1.9µm (50*2.1 mm) at a flow rate of 0.2 ml/min.

Table 1: Prostanoids from Arachidonic Acid metabolites from the COX-2 pathway and a non-enzymatic pathway.

Compound Name	Symbol	RT (min)
6-keto-Prostaglandin-$F_{1\alpha}$	6-keto $PGF_{1\alpha}$	1.25
8-isoprostane	8-iso $PGF_{2\alpha}$	2.25
Prostaglandin $F_{2\alpha}$	$PGF_{2\alpha}$	2.88
Prostaglandin E_2	PGE_2	3.49
11-dehydro Thromboxane B_2	11-dehydro TXB_2	3.86
Prostaglandin D_2	PGD_2	4.18
13,14-dihydro-15 keto Prostaglandin E_2	13,14-dihydro-15 keto PGE_2	5.43
13,14-dihydro-15 keto Prostaglandin A_2	13,14-dihydro-15 keto PGA_2	6.01
15-deoxy Prostaglandin J_2	15-deoxy PGJ_2	6.48

Figure 1: X-LC Chromatogram of Prostanioids fromTable 1.



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Contact:
JASCO
28600 Mary's Court Easton, MD 21601
Phone: 800-333-5272 Fax: 410-822-7526
www.jascoinc.com