Published in final edited form as:

Rapid Commun Mass Spectrom. 2006 ; 20(20): 3023–3029. doi:10.1002/rcm.2697.

Lipidomic Analysis of Twenty Seven Prostanoids and Isoprostanes by Electrospray Liquid Chromatography/Tandem Mass Spectrometry

Mojgan Masoodi and Anna Nicolaou*

School of Pharmacy, University of Bradford, Richmond Road, Bradford BD7 1DP, UK

Abstract

Prostanoids are potent mediators of many physiological and pathophysiological processes. Of the many analytical methodologies used for their qualitative and quantitative analysis, electrospray tandem mass spectrometry coupled to liquid chromatography (ESI-LC-MS/MS) offers a rapid, sensitive and versatile system applicable to lipidomic analyses. We have developed an ESI-LC-MS/MS assay for twenty-seven mediators including prostaglandins, prostacyclins, thromboxanes, dihydroprostaglandins and isoprostanes. The assay was linear over the concentration range 1–100 pg/μL. The limits of detection and quantitation were 0.5–50 pg and 2–100 pg respectively, whilst recoveries were from 83–116 % depending on the metabolite. The assay can be applied to profiling prostanoids produced by a variety of biological fluids and extracts including brain, liver, plasma and urine, facilitating thus our understanding of the role of these lipid mediators in health and disease, as well as assisting in drug development.

INTRODUCTION

Prostanoids, a term that collectively describes prostaglandins, prostacyclins and thromboxanes, are a sub-class of the lipid mediator group known as *eicosanoids* [1]. They derive from C-20 polyunsaturated fatty acids, mainly dihomo- γ -linoleic (20:3 n -6), arachidonic (20:4 n -6), and eicosapentaenoic (20:5 n -3) acids, through the action of cyclooxygenases-1 and -2 (COX-1 and COX-2) [2] (Figure 1 and scheme 1). The reaction product of COX is the unstable endoperoxide prostaglandin H (PGH) that is further transformed to the individual prostanoids by a series of specific prostanoid synthases [3]. Prostanoids are local-acting mediators formed and inactivated within the same or neighbouring cells prior to their release into circulation as inactive metabolites (15-keto- and 13,14-dihydro-keto metabolites) [4]. Non-enzymatic peroxidation of arachidonic acid and other fatty acids *in vivo* can result in prostaglandin-like substances isomeric to the COX-derived prostaglandins that are termed isoprostanes (scheme 1c) [5].

Prostanoids take part in many physiological and pathophysiological processes in practically every organ, tissue and cell, including the vascular, renal, gastrointestinal and reproductive systems [1,6–9]. Their activities are mediated through prostanoid-specific receptors and intracellular signalling pathways, whilst their biosynthesis and action are blocked by nonsteroidal anti-inflammatory drugs (NSAID). Isoprostanes are considered to be reliable markers of oxidant stress status and have been linked to inflammation, ischaemia-reperfusion, diabetes, cardiovascular disease, reproductive disorders, diabetes [10].

*Corresponding author: Dr A. Nicolaou, Email: a.nicolaou@bradford.ac.uk, Tel: +44 1274 234717, Fax: +44 1274 235600.

Methods currently used for the analysis of prostanoids include HPLC with fluorescence or UV detection, GC, LC-MS, GC-MS, enzyme immunoassays and radioimmunoassays [1,11]. Although the immunoassays are very popular, they have low specificity and are not applicable to the simultaneous profiling of more than one metabolites at a time. HPLC and GC-MS methods offer greater sensitivity and flexibility but require derivatisation or the use of radiolabelled fatty acid precursors to facilitate detection and increased sensitivity, since most prostanoids do not absorb UV except at low wavelengths [12]. When analysed by electrospray ionisation (ESI), eicosanoids easily form protonated and deprotonated molecules [13]. Consequently, the use of tandem mass spectrometry (MS/MS) coupled to liquid chromatography (LC-MS/MS) can lead to the development of fast and sensitive analytical protocols with high-throughput potential. Furthermore, LC-MS/MS assays permit the simultaneous profiling and quantitative analysis of prostanoids, dihydroprostaglandins and isoprostanes without the need of derivatisation and lengthy sample preparation, thus overcoming the limitations of GC-MS, HPLC, and other conventional methodologies.

Lipidomics is an emerging area of lipid research aiming to study the profiles and complete composition of lipids in parallel with their functional role in any given tissue or system [14,15]. The study of prostanoid profiles in conjunction with other metabolic profiles, enzyme activities, protein and gene expression can offer valuable insights to tissue and organ function in health and disease. In this study we present a rapid method for the simultaneous qualitative and quantitative assay of twenty-seven prostanoids, dihydroprostanoids and isoprostanes. This method can be applied to elucidate the profiles of prostanoids and their inactivated metabolites in different cellular systems and body fluids, and consequently assist any lipidomic or systems biology application aiming to explore physiological states, disease phenotypes, novel biomarkers or the development of therapeutic approaches.

EXPERIMENTAL

Materials

Prostaglandin D₁ (PGD₁), prostaglandin E₁ (PGE₁), prostaglandin F_{1α} (PGF_{1α}), 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}), prostaglandin B₂ (PGB₂), prostaglandin B₂-d₄ (PGB₂-d₄), prostaglandin D₂ (PGD₂), prostaglandin E₂ (PGE₂), prostaglandin F_{2α} (PGF_{2α}), prostaglandin J₂ (PGJ₂), Δ¹² 2-Prostaglandin J (Δ¹² 2-PGJ), 15-deoxy-Δ^{12,14}-prostaglandin J (15-deoxy-Δ^{12,14} 2-PGJ₂), prostaglandin D₃ (PGD₃), prostaglandin E₃ (PGE₃), prostaglandin F_{3α} (PGF_{3α}), thromboxane B₂ (TXB₂), thromboxane B₃ (TXB₃), 8-*iso*-PGE₂, 8-*iso*-PGF_{2α}, 8-*iso*-15-keto PGE_{2α}, 8-*iso*-15-keto PGF_{2α}, 13,14-dihydro PGE₁, 13,14-dihydro PGF_{1α}, 13,14-dihydro PGF_{2α}, 13,14-dihydro-15-keto PGE₁, 13,14-dihydro-15-keto PGF_{1α}, 13,14-dihydro-15-keto PGE₂ and 13,14-dihydro-15-keto PGF_{2α} were purchased from Cayman Chemicals (Ann Arbor, MI, USA). HPLC grade solvents, glacial acetic acid and all other chemicals were from Sigma-Aldrich (Dorset, UK). Solid phase extraction (SPE) cartridges (C18-E 500 mg, 6 mL) were purchased from Phenomenex (Macclesfield, UK).

Preparation of standards and calibration lines, linearity, detection and quantitation limits

Stock standard solutions of all prostanoids and isoprostanes were prepared in ethanol (400 pg/μL) and stored at -20°C under N₂. Composite standard solutions were prepared by mixing and diluting the appropriate stock solutions to the final concentrations of 100 pg/μL, 50 pg/μL, 20 pg/μL, 10 pg/μL and 1 pg/μL. The internal standard (PGB₂-d₄) was prepared in ethanol (1 ng/μL) and added to all composite standards at a final concentration of 400 pg/μL. The peak-area ratios of every compound to PGB₂-d₄ were calculated and plotted against the concentration of the calibration standards. Calibration lines were calculated by the least

squares linear regression method. To calculate the concentration of any given analyte the peak-area ratio to PGB₂-*d4* was calculated and read off the corresponding calibration line. The limit of detection was calculated by using a signal to noise ratio of 3. The limit of quantitation was determined by using a signal to noise ratio of 10. Peak integrations and signal to noise calculations were performed using the MassLynx™ V4.0 software (Waters) using the manufacturers' instructions.

Sample preparation

Brain, liver, plasma and urine samples were collected from male Wistar rats. Tissue samples (approximately 500 mg) were homogenised in water (35 up and down strokes) using a Dounce glass mini homogeniser (2 mL) with tight fitting pestle. During this process the homogeniser was kept on ice. The resulting solution was adjusted to 15% methanol (v/v) (final volume 3 mL). Plasma and urine samples (500 µL) were diluted with water and adjusted to 15 % methanol (v/v), to a final volume of 3 mL. Internal standard PGB₂-*d4* (40 ng) was added to each sample. The samples were incubated on ice for 30 min and then centrifuged at 3000 rpm for 5 min to remove any precipitated proteins. The resulting clear supernatants were acidified with 0.1 M hydrochloric acid to pH 3.0 and immediately applied to SPE cartridges that had been preconditioned with 20 mL methanol followed with 20 mL water. The cartridges were then washed with 20 mL 15% (v/v) methanol, 20 mL water, and 10 mL hexane in succession. Finally, the prostanoids were eluted with 15 mL methyl formate. The extraction procedure was performed using a vacuum manifold (Phenomenex); the vacuum was adjusted so that individual drops could be seen from each cartridge. The organic solvent was evaporated under a fine stream of nitrogen, the residue was dissolved in 100 µL ethanol and stored at -20°C awaiting LC-MS/MS analysis.

ESI-LC-MS/MS analysis

The LC-MS/MS analysis was performed on a Waters Alliance 2695 HPLC pump coupled to an electrospray (ESI) triple quadrupole Quattro Ultima mass spectrometer (Waters, Elstree, Hertsfordshire, UK). Instrument control and data acquisition were performed using the MassLynx™ V4.0 software. The instrument was operated in the negative ionisation mode. For optimisation of MS and MS/MS conditions, standards at a concentration of 10 ng/µL were individually introduced to the spectrometer by direct infusion through a syringe pump (flow rate of 10 µL/min) into the HPLC solvent flow (flow rate 0.2 mL/min). The capillary voltage was set at 3500 V, source temperature 120 °C, desolvation temperature 360 °C, and cone voltage 35 V. The collision energy was optimised for each compound to get optimum sensitivity using argon as collision gas. Dwell times were 0.2 s. The collision energy settings used for each one of the 21 MRM transitions are summarised in Table 1.

Chromatographic analysis was performed on a C18 column (Gemini, 5µ, 150 x 2 mm) (Phenomenex, Macclesfield, UK). Sample injections were performed with a Waters 2690 autosampler and the sample chamber temperature was set at 8 °C. The injection volume was 5 µL and the flow rate 0.2 mL/min. The column was maintained at ambient temperature. The analysis was performed using an acetonitrile-based gradient system mixing two solvents: Solvent A was acetonitrile : water : glacial acetic acid, 45:55:0.02 (v/v/v); Solvent B was acetonitrile : water : glacial acetic acid, 90:10:0.02 (v/v/v). The analytes were separated using the following gradient: 0.0-8.0 min, 0% solvent B; 8.0-8.1 min, 0 to 50 % solvent B; 8.0-12.0 min 50% solvent B; 12.0-12.1 min, 50 to 70 % solvent B; 12.1-20.0 min 70 % solvent B; 20.0-20.1 min, 70 to 0 % solvent B; 20.1-30.0 min 0% solvent B.

Recovery

Rat brain, liver, plasma and urine samples were spiked with 200 pg of a mixed prostanoid and isoprostane standard. The metabolites were extracted as described above, and analysed

in parallel with the extracts of un-spiked tissue samples to estimate the recovery. The recovery of 8-*iso*-PGE₂ was calculated at the absence of PGE₂. In detail: the extracts of un-spiked tissue samples allowed us to estimate the peak-area value corresponding to the amount of naturally occurring metabolites in those tissues. This figure was then subtracted from the corresponding peak-area value obtained from the spiked samples. The difference is equivalent to the amount of metabolite added to each tissue sample. This figure was then compared to the peak-area value that was obtained from the analysis of metabolites that did not undergo extraction thus representing 100% of the initial concentration. The resulting value shows the recovery of each metabolite expressed as percent of the initial concentration. Three separate sets of recovery experiments were performed per type of tissue or body fluid.

RESULTS AND DISCUSSION

Prostanoid profiles are tissue and stimuli-dependent and cannot be predicted. Furthermore, quantitative methodologies require versatility, high sensitivity and selectivity because of the low concentrations, short half-lives and structural similarities of these metabolites [1]. Electrospray ionisation (ESI) has been readily applied in the analysis of all classes of eicosanoids. Since prostanoids and isoprostanes have free carboxylic acid moieties, ESI results in an abundant [M-H]⁻ carboxylate ion that allows for relatively low concentrations to be detected [16]. Multiple reaction monitoring assays (MRM) allow for the further improvement of the detection and quantitation limits of LC-MS assays. So far, only a small number of prostanoids, dihydroprostaglandins and isoprostanes have been studied together for the development of LC-MS/MS assays that detect more than one class of eicosanoids [13,17-20]. We have developed a sensitive ESI-LC-MS/MS assay applicable to the simultaneous detection and quantitation of twenty-seven eicosanoids including sixteen prostanoids (scheme 1a and b), seven dihydroprostanoids and four isoprostanes (scheme 1c).

In order to develop the LC-MS/MS assay, production scanning experiments were conducted using argon as collision gas and the collision energy was optimised for each individual compound to generate the most abundant product ions. These product ion spectra were then used to select the precursor-product ion pairs for the development of MRM assays (spectra presented as Supplementary Data, see attached file). Table 1 summarises the optimal collision energies and ion pairs selected for the ESI-LC-MS/MS assay. The choice of product ions for MRM was in agreement with the available literature values, i.e.: PGE₂ 251→271, PGF_{2α} 353→193; 6-keto-PGF_{1α} 369→163, PGE₃ 349→269, TXB₂ 369→169; 8-*iso*-PGF_{2α} 353→193; 8-*iso*-15-keto PGF_{2α} 351→315 [17-20]. Although PGD₃ has been detected using the transition 349→233 [18], we opted for the transition 349→269 that is the same one used to detect PGE₃. The isobaric compounds PGD₃ and PGE₃ are chromatographically separated therefore this choice of transition allows the use of only one MRM to detect both compounds (Figure 2A). Furthermore, the product ion m/z 269 is of higher abundance in the relevant spectra under the conditions used in our study. Overall, the analysis of all the twenty-seven metabolites included in the present study was performed using twenty-one MRM transitions (Table 1).

The compounds were chromatographically resolved on a C18 column using a gradient of two acetonitrile-based solvents. Figure 2 shows representative chromatograms of the analysis of these lipid mediators, i.e. 16 prostanoids including prostaglandins, cyclopentanone prostaglandins and thromboxanes, 7 dihydroprostanoids and 4 isoprostanes. The run time of the assay was 30 min including a 10 min wash cycle programmed to run before the next injection. Since the retention time (r.t) of the last eluting compound was 18 min (Figure 2A), the wash cycle and therefore the run time of the assay can be further reduced depending on the application (e.g. quality of biological sample; need for high-

throughput). Overall, this run time is comparable to other HPLC-based reported methods of eicosanoid analysis and provides the basis for a rapid assay [16,21].

The co-eluting prostanoids PGE₁ and PGE₂ (r.t. 4.47 min, Figure 2A and 2B) were detected separately using two different MRM transitions (i.e. 353→317 and 351→271 respectively). Conversely, the isobaric compounds PGE₁ and PGD₁ are identified through the same MRM transition but were separated chromatographically (353→317; r.t. 4.47 and 4.79 min respectively; Figure 2B). The same principle was applied to PGE₂ and PGD₂ (351→271; r.t.: 4.47 and 5.18 min respectively), PGE₃ and PGD₃ (349→269; r.t. 3.85 and 4.08 min respectively) and PGJ₂ and Δ¹²-PGJ₂ (333→271; r.t. 9.17 and 9.80 min respectively) (Figure 2A), and PGF_{2α} and its isomer 8-*iso*-PGF_{2α} (353→193; r.t. 3.38 and 3.77 respectively, Figure 2B). The tailing peak observed for 6-keto-PGF_{1α} (Figure 2B) is a result of the chromatography and not the MS assay (the single standard gives a chromatographic peak of similar shape and it is not contaminated with impurities). Although this badly shaped peak may interfere with the reproducibility of the integration, it has not affected the linearity of the calibration line (Table 2).

The two isobaric isoprostanes 8-*iso*-15-keto PGF_{2α} and 8-*iso*-PGE₂ presented an extra challenge: Although the transition 351→315 is the most selective for both compounds, the product ion m/z 315 is also generated from PGE₂ and PGD₂. As a result this transition brings up all four compounds when simultaneously present in the sample (Figure 2A). Whereas 8-*iso*-15-keto PGF_{2α}, PGE₂ and PGD₂ are chromatographically well resolved, 8-*iso*-PGE₂ coelutes with PGE₂. Therefore, the identification of the isoprostane 8-*iso*-PGE₂ may not be possible at the presence of PGE₂. Further development of the gradient system could resolve the issue of separating those two isomers and thus overcome this limitation of the assay.

Standard calibration lines were typically constructed for each analyte over the range of 5 to 500 pg per injection. Calibration lines were calculated by the least squares linear regression method and the results confirmed that the assay was linear over this range (Table 2). In order to assess the recovery of the extraction methodology, rat brain, liver, plasma and urine samples were spiked with mixed standards (200 pg/compound) and analysed. The peak area for each extracted analyte was compared with the one for non-extracted standard from which recovery was calculated. The average recoveries ranged from 83 to 116 % depending on the compound and type of biological sample (Results summarised in Table 3). Finally, the limit of detection (LoD) was found to be in the range of 0.5-50 pg whilst the limit of quantitation (LoQ) was in the range of 2-100 pg, depending on the compound (results are summarised in Table 2). Overall, the assay presented in this paper has been found to perform well, showing good linearity, recovery and sensitivity.

CONCLUSION

The reported ESI-LC-MS/MS assay can be used to identify and quantify a large number of prostanoids in different types of biological samples. The method is simple, sensitive, high-throughput and lends itself to lipidomic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work has been partially supported by The Wellcome Trust, Grant WT077714. The authors thank Andrew Healey (Analytical Centre, University of Bradford) for excellent technical assistance and Dr N Blagden for useful advice.

REFERENCES

- [1]. Nicolaou, A. 'Bioactive Lipids'. Nicolaou, A.; Kokotos, G., editors. The Oily Press; Bridgwater, England: 2004. p. 197
- [2]. Smith WL, DeWitt DL, Garavito RM. *Annu. Rev. Biochem.* 2000; 69:145. [PubMed: 10966456]
- [3]. Helliwell RJ, Adams LF, Mitchell MD. *Prostaglandins Leukot. Essent. Fatty Acids.* 2004; 70:101. [PubMed: 14683687]
- [4]. Tai HH, Ensor CM, Tong M, Zhou H, Yan F. *Prostaglandins Other Lipid Mediat.* 2002; 69:483. [PubMed: 12432938]
- [5]. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. *Proc Natl Acad Sci USA.* 1990; 87:9383. [PubMed: 2123555]
- [6]. Wise H, Wong YH, Jones RL. *Neurosignals.* 2002; 11:20. [PubMed: 11943879]
- [7]. Samad TA, Sapirstein A, Woolf CJ. *Trends Mol. Med.* 2002; 8:390. [PubMed: 12127725]
- [8]. Bishop-Bailey D, Calatayud S, Warner TD, Hla T, Mitchell JA. *J. Environ. Pathol. Toxicol. Oncol.* 2002; 21:93. [PubMed: 12086407]
- [9]. Simmons DL, Botting RM, Hla T. *Pharmacol. Rev.* 2004; 56:387. [PubMed: 15317910]
- [10]. Cracowski JL, Durand T, Bessard G. *Trends Pharmacol. Sci.* 2002; 23:360. [PubMed: 12377577]
- [11]. Misuraki, M.; Hishinuma, T.; Suzuki, N.; Goto, J. 'The Eicosanoids'. Curtis-Prior, P., editor. John Wiley & Sons, Ltd.; 2004. p. 117
- [12]. Terragno A, Rydzik R, Terragno NA. *Prostaglandins.* 1981; 21:101. [PubMed: 6894197]
- [13]. Murphy RC, Barkley RM, Zemski Berry K, Hankin J, Harrison K, Johnson C, Krank J, McAnoy A, Uhlson C, Zarini S. *Anal. Biochem.* 2005; 346:1. [PubMed: 15961057]
- [14]. van Meer G. *Cellular lipidomics.* *Embo J.* 2005; 24:3159. [PubMed: 16138081]
- [15]. Serhan CN. *Prostaglandins Other Lipid. Mediat.* 2005; 77:4. [PubMed: 16099386]
- [16]. Margalit A, Duffin KL, Isakson PC. *Anal. Biochem.* 1996; 235:73. [PubMed: 8850549]
- [17]. Takabatake M, Hishinuma T, Suzuki N, Chiba S, Tsukamoto H, Nakamura H, Saga T, Tomioka Y, Kurose A, Sawai T, Mizugaki M. *Prostaglandins Leukot. Essent. Fatty Acids.* 2002; 67:51. [PubMed: 12213436]
- [18]. Yang P, Felix E, Madden T, Fischer SM, Newman RA. *Anal. Biochem.* 2002; 308:168. [PubMed: 12234478]
- [19]. Kempen EC, Yang P, Felix E, Madden T, Newman RA. *Anal. Biochem.* 2001; 297:183. [PubMed: 11673886]
- [20]. Taylor AW, Bruno RS, Frei B, Traber MG. *Anal. Biochem.* 2006; 350:41. [PubMed: 16448621]
- [21]. Nithipatikom K, Laabs ND, Isbell MA, Campbell WB. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2003; 785:135.

Figure 1.

Schematic outline of the prostanoids and isoprostanes produced by arachidonic (AA), eicosapentaenoic (EPA) and dihomo- γ -linoleic (DHGLA) acids via the cyclooxygenase (COX) or free radical catalysed pathways.

Figure 2. Representative chromatograms showing the ESI-LC-MS/MS analysis of twenty-seven prostanoids, dihydroprostaglandins and isoprostanes. IS: internal standard (PGB₂-d₄)

Scheme 1a-c.

Structures of prostaglandins, dihydroprostaglandins and isoprostanes.

Table 1

Multiple reaction monitoring (MRM) transitions for the liquid chromatography tandem mass spectrometry (ESI-LC-MS/MS) assay of prostanoids, dihydroprostaglandins and isoprostanes.

Compound	MRM (<i>m/z</i>)	Collision energy (eV)
PGD ₁	353→317	15
PGE ₁	353→317	15
PGF _{1α}	355→311	25
6-keto PGF _{1α}	369→163	23
PGB ₂	333→175	20
PGB ₂ - <i>d4</i>	337→179	20
PGD ₂	351→271	17
PGE ₂	351→271	17
PGF _{2α}	353→193	25
PGJ ₂	333→271	15
Δ ¹² -PGJ ₂	333→271	15
15-deoxy-Δ ^{12,14} PGJ ₂	315→271	15
PGD ₃	349→269	15
PGE ₃	349→269	15
PGF _{3α}	351→193	25
TXB ₂	369→169	17
TXB ₃	367→169	15
8- <i>iso</i> -PGE ₂	351→315	15
8- <i>iso</i> -15-keto PGE ₂	349→113	23
8- <i>iso</i> -PGF _{2α}	353→193	25
8- <i>iso</i> -15-keto PGF _{2α}	351→315	15
13,14-dihydro PGE ₁	355→337	15
13,14-dihydro-15-keto PGE ₁	353→335	12
13,14-dihydro-PGF _{1α}	357→113	38
13,14-dihydro-15-keto PGF _{1α}	355→193	32
13,14-dihydro-15-keto PGE ₂	351→333	12
13,14-dihydro PGF _{2α}	355→311	30
13,14-dihydro-15-keto PGF _{2α}	353→113	28

Table 2

Linearity, limit of detection (LoD) and limit of quantitation (LoQ) of the ESI-LC-MS/MS assay for prostanoids and isoprostanes. (LoD and LoQ are expressed as pg on the column).

Compound	Equations	Correlation Coefficient	LoD (pg)	LoQ (pg)
PGD ₁	$y = 1.864x - 0.184$	0.970	2	5
PGE ₁	$y = 3.991x - 0.395$	0.951	2	5
PGF _{1a}	$y = 0.758x - 0.034$	0.963	10	50
6-keto PGF _{1a}	$y = 0.530x - 0.032$	0.958	10	50
PGB ₂	$y = 0.935x - 0.080$	0.991	10	50
PGD ₂	$y = 5.689x - 0.098$	0.999	5	10
PGE ₂	$y = 0.186x - 0.230$	0.980	0.5	2
PGF _{2a}	$y = 2.218x - 0.015$	0.999	0.5	5
PGJ ₂	$y = 1.610x - 0.049$	0.999	10	50
Δ^{12} -PGJ ₂	$y = 1.662x - 0.295$	0.999	5	10
15-deoxy- $\Delta^{12,14}$ PGJ ₂	$y = 12.23x - 0.636$	0.989	2	5
PGD ₃	$y = 0.328x - 0.008$	0.993	10	50
PGE ₃	$y = 1.580x - 0.090$	0.993	2	5
PGF _{3a}	$y = 0.353x - 0.008$	0.989	50	100
TXB ₂	$y = 1.738x - 0.078$	0.981	2	5
TXB ₃	$y = 1.695x - 0.064$	0.988	2	5
8- <i>iso</i> -PGE ₂	$y = 0.171x + 0.017$	0.947	5	50
8- <i>iso</i> -15-keto PGE ₂	$y = 0.217x - 0.002$	0.985	5	50
8- <i>iso</i> -PGF _{2a}	$y = 0.334x + 0.004$	0.984	2	5
8- <i>iso</i> -15-keto PGF _{2a}	$y = 0.913x + 0.029$	0.990	5	50
13,14-dihydro PGE ₁	$y = 0.191x - 0.063$	0.983	5	10
13,14-dihydro-15-keto PGE ₁	$y = 0.114x + 0.035$	0.985	5	10
13,14-dihydro-PGF _{1a}	$y = 0.018x + 0.002$	0.999	50	100
13,14-dihydro-15-keto PGF _{1a}	$y = 0.007x - 0.001$	0.985	50	100
13,14-dihydro-15-keto PGE ₂	$y = 1.790x + 0.047$	0.991	5	10
13,14-dihydro PGF _{2a}	$y = 0.004x + 0.006$	0.977	50	100
13,14-dihydro-15-keto PGF _{2a}	$y = 0.015x - 0.008$	0.997	5	50

Table 3

Recovery of 200 pg prostanoids and isoprostanes from spiked rat liver and brain tissues, plasma and urine. Data is shown as mean $\pm$ sd (n=3 experiments).

Compound	% Recovery			
	Liver	Brain	Plasma	Urine
PGD ₁	116 $\pm$ 18	96 $\pm$ 1	104 $\pm$ 7	100 $\pm$ 3
PGE ₁	110 $\pm$ 2	104 $\pm$ 4	100 $\pm$ 1	87 $\pm$ 6
PGF _{1α}	108 $\pm$ 5	103 $\pm$ 4	105 $\pm$ 1	98 $\pm$ 4
6-keto PGF _{1α}	100 $\pm$ 4	98 $\pm$ 4	106 $\pm$ 3	99 $\pm$ 2
PGB ₂	101 $\pm$ 5	102 $\pm$ 3	99 $\pm$ 2	100 $\pm$ 4
PGD ₂	93 $\pm$ 10	100 $\pm$ 1	97 $\pm$ 5	100 $\pm$ 1
PGE ₂	97 $\pm$ 6	101 $\pm$ 8	98 $\pm$ 17	97 $\pm$ 10
PGF _{2α}	102 $\pm$ 12	92 $\pm$ 7	96 $\pm$ 1	100 $\pm$ 1
PGJ ₂	100 $\pm$ 4	110 $\pm$ 6	92 $\pm$ 3	101 $\pm$ 1
Δ ¹² -PGJ ₂	94 $\pm$ 10	102 $\pm$ 4	98 $\pm$ 4	105 $\pm$ 5
15-deoxy- Δ ^{12,14} PGJ ₂	94 $\pm$ 13	100 $\pm$ 1	112 $\pm$ 1	102 $\pm$ 4
PGD ₃	102 $\pm$ 4	100 $\pm$ 1	100 $\pm$ 3	108 $\pm$ 5
PGE ₃	101 $\pm$ 2	98 $\pm$ 2	103 $\pm$ 3	115 $\pm$ 10
PGF _{3α}	91 $\pm$ 10	100 $\pm$ 3	88 $\pm$ 8	100 $\pm$ 1
TXB ₂	93 $\pm$ 14	100 $\pm$ 19	98 $\pm$ 7	83 $\pm$ 3
TXB ₃	98 $\pm$ 2	97 $\pm$ 1	83 $\pm$ 1	75 $\pm$ 2
8- <i>iso</i> -PGE ₂	97 $\pm$ 2	105 $\pm$ 1	106 $\pm$ 2	99 $\pm$ 2
8- <i>iso</i> -15-keto PGE ₂	91 $\pm$ 10	96 $\pm$ 6	103 $\pm$ 1	96 $\pm$ 2
8- <i>iso</i> -PGF _{2α}	102 $\pm$ 12	92 $\pm$ 7	96 $\pm$ 1	100 $\pm$ 1
8- <i>iso</i> -15-keto PGF _{2α}	95 $\pm$ 5	103 $\pm$ 1	105 $\pm$ 3	101 $\pm$ 1
13,14-dihydro PGE ₁	97 $\pm$ 5	104 $\pm$ 2	96 $\pm$ 2	87 $\pm$ 5
13,14-dihydro-15-keto PGE ₁	100 $\pm$ 6	104 $\pm$ 2	97 $\pm$ 2	85 $\pm$ 3
13,14-dihydro-PGF _{1α}	102 $\pm$ 1	84 $\pm$ 2	94 $\pm$ 5	96 $\pm$ 6
13,14-dihydro-15-keto PGF _{1α}	93 $\pm$ 4	100 $\pm$ 1	103 $\pm$ 5	107 $\pm$ 5
13,14-dihydro-15-keto PGE ₂	95 $\pm$ 2	99 $\pm$ 2	103 $\pm$ 2	104 $\pm$ 5
13,14-dihydro PGF _{2α}	95 $\pm$ 2	110 $\pm$ 1	112 $\pm$ 9	102 $\pm$ 2
13,14-dihydro-15-ketoPGF _{2α}	100 $\pm$ 4	100 $\pm$ 1	92 $\pm$ 1	96 $\pm$ 2