Journal of Chromatography A, 1440 (2016) 260–265

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Enhanced performance for the analysis of prostaglandins and thromboxanes by liquid chromatography-tandem mass spectrometry using a new atmospheric pressure ionization source

Arnaud Lubin[a], Suzy Geerinckx[a], Steve Bajic[b], Deirdre Cabooter[c], Patrick Augustijns[c], Filip Cuyckens[a], Rob J. Vreeken[a,*]

[a] *Discovery Sciences, Janssen R&D, Beerse, Belgium*
[b] *Waters Corporation, Wilmslow, United Kingdom*
[c] *Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium*

ARTICLE INFO

Article history:
Received 2 February 2016
Accepted 18 February 2016
Available online 22 February 2016

Keywords:
Prostaglandin
Eicosanoid
LC–MS/MS
Unispray
Electrospray

ABSTRACT

Eicosanoids, including prostaglandins and thromboxanes are lipid mediators synthetized from polyunsaturated fatty acids. They play an important role in cell signaling and are often reported as inflammatory markers. LC–MS/MS is the technique of choice for the analysis of these compounds, often in combination with advanced sample preparation techniques.

Here we report a head to head comparison between an electrospray ionization source (ESI) and a new atmospheric pressure ionization source (UniSpray). The performance of both interfaces was evaluated in various matrices such as human plasma, pig colon and mouse colon. The UniSpray source shows an increase in method sensitivity up to a factor 5. Equivalent to better linearity and repeatability on various matrices as well as an increase in signal intensity were observed in comparison to ESI.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Eicosanoids are autocrine and paracrine lipid mediators involved in a variety of biological functions such as regulation of inflammation, immunity, fertility and cellular growth [1–4]. They have, amongst others, an important role in cell signaling and thus a reliable read-out is essential in understanding biological dynamics in a variety of pheno- and metabotypes. Eicosanoids are divided in subfamilies which include e.g., prostaglandins, thromboxanes, lipoxins and leukotrienes. Prostaglandins (PGs) and thromboxanes (TXs) are generated via enzymatic oxidation by cyclooxygenase (COX) and non-enzymatic oxidation of arachidonic acid (AA), eicosapentaenoic acid (EPA) and dihomo-γ-linolenic acid (DGLA) [5,6]. PGs and TXs derived from AA containing 2 double bonds are known as 2-series PGs and TXs. PGs and TXs containing 1 or 3 double bonds are called 1- and 3-series and are derived from DGLA and EPA, respectively [7]. 2-series PGs and TXs are the most abundant in the human body [8,9].

Techniques used for the analysis of eicosanoids include immunoassays (radioimmunoassay and enzyme immunoassay) [10–12], gas chromatography mass spectrometry (GC–MS) [8,13,14] and liquid chromatography-tandem mass spectrometry (LC–MS/MS) using electrospray ionization (ESI) [8,15–17]. Immunoassays are sensitive and specific, though limited to one or a few compounds per analysis making them less attractive for group specific analysis. Despite great sensitivity and selectivity, GC–MS requires derivatization to increase the volatility of eicosanoids, limiting its application. LC–MS/MS has been proven to be a practical strategy for the analysis of a wide range of eicosanoids from various origins (tissues, plasma, cell cultures, etc.) and species in a single analytical run [17–21].

Typical concentrations of these compounds in tissues and plasma are in the nM to pM-range. Therefore, most methods rely on either solid-phase extraction (SPE) or liquid-liquid extraction (LLE) as sample clean-up and pre-concentration step prior to LC–MS/MS analysis [22]. Especially, in low volume samples the analysis of these compounds becomes rather challenging.

In this paper we report early results on the increased performance of a new atmospheric pressure ionization source, viz. UniSpray (Waters Corp.)[1] in comparison to classical ESI for the analysis of PGs and TXs in biofluids and different tissues. In the UniSpray

* Corresponding author.
E-mail address: rvreeken@its.jnj.com (R.J. Vreeken).

[1] Granted patent: S. Bajic, United States Patent, US 8,809,777.

http://dx.doi.org/10.1016/j.chroma.2016.02.055
0021-9673/© 2016 Elsevier B.V. All rights reserved.

source, a high velocity spray from a, in contrast to ESI, grounded nebulizer is arranged to impact on a Ø1.6 mm stainless steel (SS) rod, at high voltage, that is positioned in close proximity and perpendicular to the spray axis (see scheme in Fig. 1 and picture in Supplemental Fig. 1). The rod target is positioned upstream of the ion inlet aperture of the MS. As the obtained spray is non-charged, ionization takes place at the rod, hence a different ionization mechanism is active in this mode.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.02.055.

2. Material and methods

2.1. Chemicals and reagents

Acetic acid, acetonitrile, butylated hydroxytoluene (BHT), ethyl acetate, ethylenediaminetetraacetic acid (EDTA), isopropanol and methanol, all analytical grade, were purchased from Merck (Darmstadt, Germany). Ultrapure water was produced with a MilliQ system (Millipore, Billerica, MA, USA).

(d_4) 15-deoxy-Δ12,14-PGJ_2, TXB_1, Δ17-6-keto $PGF_{1\alpha}$, (d_4) Δ17-6-keto $PGF_{1\alpha}$, (d_4) TXB_2, (d_4) $PGF_{2\alpha}$, (d_4) PGE_2 and (d_4) PGD_2, 6-keto $PGF_{1\alpha}$, 6-keto PGE_1, TXB_3, $PGF_{3\alpha}$, PGE_3, 8-iso $PGF_{2\alpha}$, TXB_2, 11β-$PFG_{2\alpha}$, PGD_3, 5-$iPF_{2\alpha}$, $PGF_{2\alpha}$, PGE_2, 15-keto $PGF_{2\alpha}$, PGE_1, PGD_2, PGD_1, 13,14-dihydro $PGF_{2\alpha}$, 13,14-dihydro-15-keto PGE_2, 13,14-dihydro-15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto PGD_2, PGA_2, 15-deoxy-Δ12,14-PGJ_2, Δ12-PGJ_2 and PGJ_2 were purchased from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Sample preparation

1.2.1.Standard and internal standard solutions

To prevent oxidation of these compounds, all stock-solutions, subsequent dilutions and all sample extracts, contained BHT and EDTA as anti-oxidants (concentration level 0.2 mg/mL).

Stock solutions for the calibration curves contained (d_4) Δ17-6-keto $PGF_{1\alpha}$, (d_4) TXB_2, (d_4) $PGF_{2\alpha}$, (d_4) PGE_2 and (d_4) PGD_2, each at a concentration of 10 ng/μL, and were prepared in a MeOH/H_2O 50/50 (v/v) solution.

A MeOH/H_2O 50/50 (v/v) solution containing (d_4) 15-deoxy-Δ12,14-PGJ_2, TXB_1 and Δ17-6-keto $PGF_{1\alpha}$ standards, each at a concentration of 20 ng/μL, was used as internal standard solution.

The quality control (QC) stock solution contained (d_4) Δ17-6-keto $PGF_{1\alpha}$, (d_4) TXB_2, (d_4) $PGF_{2\alpha}$, and (d_4) PGD_2 at a concentration of 1 ng/μL and (d_4) PGE_2 at 5 ng/μL in MeOH/H_2O 50/50 (v/v).

Working standard solutions were prepared by dilution of these stock-solutions in MeOH/H_2O 50/50 (v/v), containing the anti-oxidants, to create the necessary concentrations.

2.2.1. Extraction of PGs and TXs from tissue and plasma

(Proximal and distal) colon samples were obtained from CB56/bl6 mice and colon samples were obtained from mini-pig (Ellegaard Göttingen mini pig, male, age 19 months (August 2013), BW 27.4 kg, fasted state at the time of euthanasia). Human plasma was purchased from Bioreclamation (Hickville, NY, USA). Tissue samples were separately homogenized for 2 min using an Ultra-Turrax laboratory blender (T25, IKA, Staufen, Germany). Sample preparation was performed as follows: 50 μL of a homogenized sample or plasma sample, was mixed with 5 μL of 0.2 mg/mL BHT/EDTA in MeOH/H_2O 50/50 (v/v), 50 μL of internal standard mix, 800 μL of H_2O and 50 μL of MeOH/H_2O 50/50 (v/v) or 50 μL of standard solutions for calibration points and quality controls.

Hereafter, PGs and TXs were extracted using an Oasis HLB LP 96-well (60 μm, 60 mg) SPE plate (Waters, Milford, MA, USA), washed with 1 mL of MeOH and conditioned with 1 mL of ethyl acetate, 1 mL of MeOH and 2 mL of H_2O. After samples were loaded, the cartridges were rinsed with 200 μL of H_2O/MeOH 90/10 (v/v) containing 0.1% of formic acid. Impurity removal was accomplished by flushing the cartridge with 1 mL of H_2O and 1 mL of H_2O/MeOH 90/10 (v/v) containing 0.1% of formic acid. After drying under vacuum for 20 min, PGs and TXs were eluted using 0.3 mL of acetonitrile and 1.2 mL of ethyl acetate, dried under N_2 (25 °C) and re-dissolved in 100 μL of ACN/MeOH 50/50 (v/v).

2.3. Liquid chromatography–mass spectrometry:

All experiments were carried out on an Acquity UPLC system equipped with a sample organizer (maintained at 15 °C) and a 2.1 × 150 mm Acquity UPLC BEH C18 (1.7 μm) column held at 40 °C (Waters, Milford, MA, USA). 0.1% acetic acid in water (solvent A)

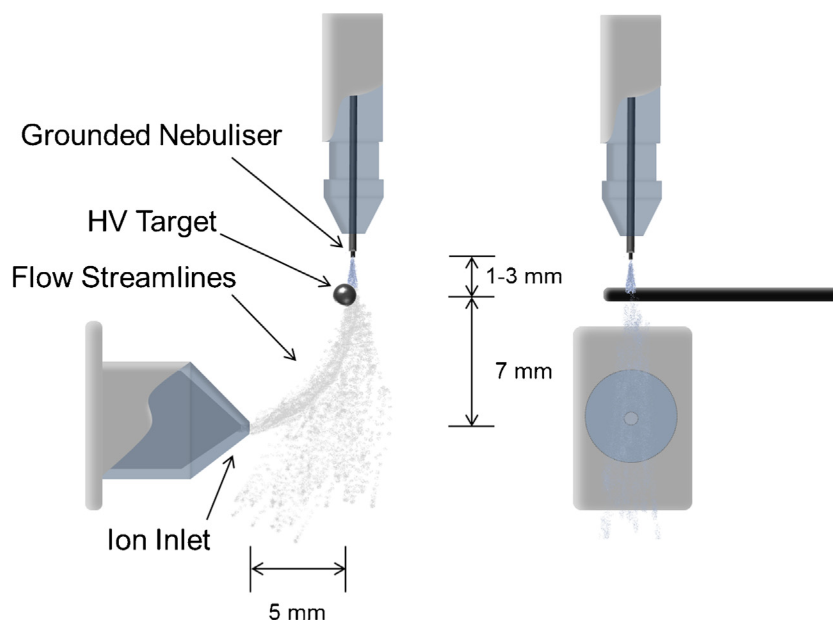

Fig 1. Scheme of the UniSpray source.

Download English Version:

https://daneshyari.com/en/article/7610141

Download Persian Version:

https://daneshyari.com/article/7610141

Daneshyari.com