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Fast quantification of the exhaled breath condensate of oxidative stress 8-iso-prostaglandin F2α using on-line solid-phase extraction coupled with liquid chromatography/electrospray ionization mass spectrometry

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ABSTRACT

A method using automated on-line solid-phase extraction (SPE) liquid chromatography–electrospray tandem mass spectrometry (LC–ESI-MS/MS) for the determination of 8-iso-PGF2α in human exhaled breath condensate (EBC) was developed and validated. A C18 SPE column with an affinity sorbent was used for on-line extraction. A C18 column was employed for LC separation and ESI-MS/MS was utilized for detection. 8-iso-PGF2α-d_4 was used as an internal standard for quantitative determination. The extraction, cleanup and analysis procedures were controlled by a fully automated six-port switch valve. Identification and quantification were based on the following transitions: m/z 353 → 193 for 8-iso-PGF2α and m/z 357 → 197 for 8-iso-PGF2α-d_4, respectively. Good recoveries from 98.94 to 99.86% were measured and satisfactory linear ranges for these analytical compounds were determined. Intra-day and inter-day precision showed that coefficients of variance (CV) ranged from 6.5 to 8.0% and 5.2 to 6.3%, respectively. The applicability of this newly developed method was demonstrated by analyzing human EBC samples for an evaluation of the future risk of human exposure to nanoparticles.

1. Introduction

Quantification of 8-iso-prostaglandin F2α (8-iso-PGF2α) has been suggested as a reliable indicator of lipid peroxidation [1,2]. Lipid peroxidation may be related to *in vivo* free radical generation, oxidative damage, and antioxidant deficiency [3]. Oxidative stress has been associated with several diseases such as lung cancer [4], asthma [5] and cardiovascular disease [6]. Particular attention has focused on 8-iso-PGF2α, which is stable, specific for lipid peroxidation, produced *in vivo*, and relatively abundant in biological fluids including plasma [7,8], serum [9], urine [8,10,11], expired breath condensate (EBC) [4], and tissues [12] as well as in lipid-rich foods. Exhaled breath condensate (EBC) is a safe, convenient, non-invasive medium for sampling the airway fluid lining the respiratory tract [4,13–15], airway surface liquid, and mediators of airway inflammation and oxidative stress [16,17].

Various traditional analytical approaches with different sensitivities and specificities can be used to measure 8-iso-PGF2α in biological fluids. These include immunological methods (ELISA and RIA) [5,18,19] that are simple and inexpensive, but requires validation with reference analytical techniques. A RIA for 8-isoprostane in EBC has been qualitatively validated with reverse phase-high performance liquid chromatography (RP-HPLC) [20]. Gas chromatography–mass spectrometry (GC/MS) [21,22] has high sensitivity and specificity for individual isoprostanes, but the sample preparation procedures are extensive, complex and time-consuming. Recently developed liquid chromatography tandem mass spectrometric (LC-MS/MS) [23,24] methods have good specificity, good sensitivity, have the capacity to simultaneously quantify multiple analytes, and are compatible with the additional cleanup and sample preparation methodologies required for the testing of urine, plasma and possibly EBC [25]. However, while repeating this procedure, there also was contamination of the curtain plate, which resulted in signal attenuation and reduced sensitivity. In the present study, we developed a new on-line SPE-LC-MS/MS method, that is rapid, automated, and provides accurate and precise measurements of 8-iso-PGF2α in human EBC samples. The aim of the present study was to present and compare different methods currently developed for the extraction, purification and analysis of 8-iso-PGF2α in various biological samples.

2. Experimental

2.1. Chemicals and reagents

8-iso-PGF2α, 8-iso-PGF2α-d_4 and 8-isoprostane affinity sorbent were purchased from Cayman Chemicals Co. (Ann Arbor, MI, USA).

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Table 1
Timetable for the column-switching procedure.

Time (min)	Flow rate (mL/min)	Eluent I (trap)[a]		Flow rate (mL/min)	Eluent II (analysis)[b]		Time (min)	Valve	Remarkers
		Solvent A	Solvent B		Solvent A	Solvent B		Position	
0	1	85	15	0.5	75	25	0	A	Loading and washing samples on trap column
5.5	1	85	15	0.5	75	25	4	B	Inject samples to analytical column
9.5	1	0	100	0.5	0	100	5.5	A	End of injection; cleanup and reconditioning trap column
9.6	1	85	15	0.5	75	25	12	A	
12	1	85	15	0.5	75	25			

[a] Eluent I: solvent A: 1% ACN (v/v) with 0.1% FA; solvent B: 90% ACN (v/v) with 0.1% FA.
[b] Eluent II: solvent A: 100% H_2O (v/v) with 0.1% FA; solvent B: 100% ACN.

All HPLC grade solvents were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Stock solutions and working solutions

A stock standard solution of 8-iso-PGF2α (1000 pg/mL) was prepared in 1% acetonitrile (v/v) with 0.1% formic acid, and it was then stored in polypropylene screw-top tubes at −80 °C. The standard solutions were diluted to 9 and 900 pg/mL with 1% ACN (v/v) with 0.1% formic acid and spiked with a fixed amount of 100 pg/mL 8-iso-PGF2α-d_4 as internal standards (IS).

2.3. Sample preparation

EBC was collected using a commercially available condenser (ECoScreen turbo, Viasys GmbH, Höchberg, Germany) that allows non-invasive sampling of condensable parts of expired air when cooled to about −10 °C, which was performed according to the manufacturer's specified method, and as described in previous publications [9,13,26,27]. All subjects breathed in a relaxed manner (tidal breathing) for 15 min without wearing a noseclip [28]. The EBC produced during a 15-min period (2–3 mL) was collected in polypropylene tubes and stored at −80 °C until analysis. For EBC analysis, each 0.6 mL EBC sample was spiked with 60 μL of the 8-iso-PGF2α-d_4 internal standard and 60 μL of affinity sorbent, vortexed, kept in an ultrasonic bath for 60 min at 4 °C, then centrifuged (12,000 × *g* for 10 min at 4 °C) and the supernatant separated. The affinity sorbent was washed with 0.3 mL water, centrifuged (12,000 × *g* for 10 min at 4 °C), and then the analytes were eluted with 0.6 mL ACN. The supernatants were collected and filtered using a 0.22-μm filter membrane, followed by drying under nitrogen. Finally, 30 μL of solvent (1% ACN, v/v with 0.1% formic acid) were added and the solution was mixed by vortexing for subsequent LC-MS/MS analysis.

2.4. Inter-day and intra-day analysis

Experiments on the intra-day and inter-day variation in 8-iso-PGF2α in EBC were assessed by identical analyses of pooled EBC from 3 volunteers. The intra-day accuracy and recovey of the assay were determined by analyzing replicates ($n = 5$) at spiked 20 and 100 pg/mL in pooled EBC from 3 volunteers and calculating the CV (coefficient of variation). The inter-day was assessed by analyzing replicates of pooled EBC from 3 volunteers ($n = 5$) obatined on different days from the same subject, as described below. Aliquots (1 mL) of mixed EBC sample were transferred to eppendorf tubes. There were a total of 15 samples, which were separated into 3 groups: group I was spiked with 100 μL 8-iso-PGF2α-d_4 internal standard (500 pg/mL), 50 μL solvent (1% ACN, v/v with 0.1% FA) and 110 μL adsorbent; group II was spiked with 100 μL 8-iso-PGF2α-d_4 internal standard (500 pg/mL), 50 μL 8-iso-PGF2α (20 pg/mL) and 110 μL of adsorbent; and group III was spiked with 100 μL 8-iso-PGF2α-d_4 internal standard (500 pg/mL), 50 μL 8-iso-PGF2α (100 pg/mL) and 110 μL of adsorbent. The solutions were mixed in an ultrasonic bath for 60 min at 4 °C, followed by centrifugation at 12,000 × *g* for 10 min at 4 °C. The affinity sorbent was washed with 0.5 mL water, centrifuged (12,000 × *g* for 10 min at 4 °C), and then the analytes were eluted with 1.0 mL ACN. The supernatants were collected and filtered using a 0.22-μm filter membrane followed by drying under nitrogen. Finally, 50 μL of solvent (1% ACN, v/v with 0.1% formic acid) was added and the solution mixed by vortexing for LC-MS/MS analysis.

2.5. Automated on-line SPE

The column-switching system used in the present study is described in detail elsewhere. This system consisted of a switching valve (10-port, 2-position microelectric actuator from Valco Instrument Co., Ltd.) and an Inertsil ODS (33.3 mm × 4.6 mm × 5 μm) column. The switching valve function was controlled by Analyst1.4.2™ software (AB SCIEX, Canada). The column-switching operation, including the LC gradients, that was used during the on-line cleanup and the analytical procedures are summarized in Table 1. When the switching valve was at position A, 30 μL of prepared EBC sample was loaded on the cartridge by an autosampler (Agilent 1200SL; Agilent Technology, U.S.A.), and a binary pump (Agilent 1200; Agilent Technology) delivered the 85% solvent A (1% ACN, v/v with 0.1% formic acid) and 15% solvent B (90% ACN, v/v with 0.1% formic acid) at a flow rate of 1 mL/min as the loading and washing buffer (Eluent I). After the column was flushed with the loading buffer for 4 min, the valve was switched to the injection position (position B) to inject the sample into the LC system. At 5.5 min after injection, the valve was switched back to position A, and the column was eluted with solvent A (Eluent I) using a linear gradient from 85% solvent A to 100% solvent B (90% ACN, v/v with 0.1% formic acid) for 4.0 min, followed by 85% solvent A for 2.4 min for equilibration of the trap column and preparation for the next analysis. The total run time was 12 min.

2.6. Liquid chromatography

The HPLC system consisted of a quaternary pump, an autosampler (Agilent 1200SL, Agilent Technology, U.S.A.), and an Inertsil 5 μm, ODS-80A, 150 mm × 2.1 mm. The chromatography elution using eluent II was used to separate the analytes. After automatic sample cleanup for 4 min, the sample was automatically eluted from the trap column into the analytical column. The mobile phase A (solvent A) was 100% H_2O (v/v) with 0.1% FA, solvent B was 100% ACN, and each was delivered at a flow rate of 0.5 mL/min. The column was incubated at 60 °C.