

An Efficient Sample Preparation Method for High-Throughput Analysis of 15(S)-8-iso-PGF₂α in Plasma and Urine by Enzyme Immunoassay

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Although several methods have been reported on the analysis of the oxidative stress marker 15(S)-8-iso-prostaglandin-F₂α (8-iso-PGF₂α) in biological fluids, they either involve extensive sample preparation and costly technology or require high sample volume. This study presents a sample preparation method that utilizes low sample volume for 8-iso-PGF₂α analysis in plasma and urine by an enzyme immunoassay (EIA). In brief, 8-iso-PGF₂α in deproteinized plasma or native urine sample is complexed with an antibody and then captured by molecular weight cut-off filtration. This method was compared with two other sample preparation methods that are typically used in the analysis of 8-iso-PGF₂α by EIA: Cayman's affinity column purification method and solid-phase extraction on C-18. The immunoaffinity purification method described here was superior to the other two sample preparation methods and yielded recovery values of 99.8 and 54.1% for 8-iso-PGF₂α in plasma and urine, respectively. Analytical precision (relative standard deviation) was ±5% for plasma and ±15% for urine. The analysis of healthy human plasma and urine resulted in basal 8-iso-PGF₂α levels of 31.8 ± 5.5 pg/mL and 2.9 ± 2.0 ng/mg creatinine, respectively. The robustness and analytical performance of this method makes it a promising tool for high-throughput screening of biological samples for 8-iso-PGF₂α.

Introduction

Oxidative stress is implicated in the development and progression of many human diseases, including atherosclerosis and diabetes (1–3). One of the biochemical changes associated with oxidative damage is lipid peroxidation. The F₂-isoprostanes are a series of prostaglandin-like molecules formed by non-enzymatic free radical-catalyzed oxidation of arachidonic acid (4). These compounds are produced in esterified form in tissue and enter the circulatory system in the free acid form (5). F₂-isoprostanes can be detected in biological fluids, including plasma, urine and bronchoalveolar lavage fluid. One of the most abundant isoprostanes *in vivo*, 15(S)-8-iso-prostaglandin-F₂α (abbreviated here as 8-iso-PGF₂α; also known as 9α,11α-15S-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid) (3, 22, 23), has been shown to be biologically active and a potent vasoconstrictor in the renal, pulmonary and coronary arteries (6, 7). 8-iso-PGF₂α is recognized as a reliable marker of oxidative injury and lipid peroxidation *in vivo* (2, 4, 5, 8, 9). High levels of 8-iso-PGF₂α in biological fluids have been reported in subjects that smoke and consume alcohol (9, 10), and in patients with diabetes (2), cardiovascular (8, 11) and liver diseases (12).

Many methods have been developed to isolate and quantify F₂-isoprostanes and their metabolites in urine and plasma. They include gas chromatography–mass spectrometry (GC–MS)

(13), liquid chromatography–mass spectrometry (LC–MS) (14), GC–tandem MS (16), LC–tandem MS (17) and enzyme immunoassays (9, 15). The GC–MS and LC–MS methods generally require multiple sample preparation and steps to clean up the biological matrices, which can lead to contamination, measurement of more than one isomer of 8-iso-PGF₂α, and poor recoveries of 8-iso-PGF₂α (13–15). For instance, sample preparation by solid-phase extraction (SPE) on C-18 followed by GC–MS analysis for 8-iso-PGF₂α is hampered by co-eluting peaks (18). Therefore, further sample purification by thin-layer chromatography or high-performance liquid chromatography prior to GC–MS analysis of 8-iso-PGF₂α is recommended (19, 20). To improve the specificity of the sample preparation techniques, methods have been pursued that use immunoaffinity purification of biological samples using 8-iso-PGF₂α specific antibodies bound to an inert stationary phase, followed by GC–tandem MS (16, 21). These methods involve lengthy sample preparation steps, including derivatization processes that can impact the analytical recovery values. Alternatively, LC–MS and LC–tandem MS analyses of 8-iso-PGF₂α in biological matrices generally suffer from insufficient resolution between isomers of isoprostanes, and vigorous fragmentation during collision-induced dissociation. Moreover, these procedures require high sample volume, costly technologies and well trained analysts. Conversely, immunoassays can be relatively easy to use and comparably less time consuming, and are therefore suitable for high-throughput screening of 8-iso-PGF₂α; however, they can be limited in their reliability and specificity due to cross-reactivity depending upon the sample pretreatment techniques used (14, 15). Currently used sample preparation and purification methods for immunoassays include SPE and immunoaffinity column chromatography (14–17).

The aim of this study was to develop an optimal sample preparation method for the reliable assessment of free 8-iso-PGF₂α in plasma and urine, suitable for high-throughput screening of this oxidative stress marker. The procedure consists of immunoaffinity purification (IAP) followed by clarification using molecular weight cut-off filtration (MWCO). The performance of this modified IAP method was compared with two commonly used procedures for immunoassays: Cayman's original affinity column purification, and SPE by C-18. Purified samples were then analyzed by competitive enzyme immunoassay (EIA).

Materials and Methods

Preparation of 8-iso-PGF₂α spiking solution

A 1 μg/mL solution of 8-iso-PGF₂α was prepared in 95% ethanol from an 8-iso-PGF₂α crystalline solid (Cayman

Chemical Company, Ann Arbor, MI). A stock solution at a concentration of 50 ng/mL was made by diluting the 1 µg/mL solution with 0.15 M NaCl solution.

Biological samples

Plasma and urine samples from five female subjects were purchased from Innovative Research Inc. (Novi, MI) and stored at -80°C until analysis. Master mixes were prepared by pooling plasma or urine samples, which were used in investigations to validate analytical performance: recovery, analytical precision, linearity, accuracy, inter-analyst variation and EIA assay kit batch-to-batch variation. Urine samples were centrifuged at 9,800 rpm for 5 min to remove any particulate matter (e.g., dead cells). Aliquots (200 µL) of individual or pooled plasma or urine samples were treated separately with 20 µL of 0.3 M butylated hydroxytoluene and 20 µL of 0.1 M diethylene triamine pentaacetic acid (prepared in 100% isopropanol; Sigma-Aldrich, St. Louis, MO) to prevent any postmortem oxidative changes. These plasma or urine samples were used in the following experiments.

Spiked plasma and urine sample preparation

Aliquots (200 µL) of pooled plasma or urine samples were spiked with various concentrations of 8-iso-PGF 2α (0.25, 0.5, 1 and 5 ng/mL) to determine spiked recovery values. All spiked samples were prepared in triplicate. To determine the basal levels of 8-iso-PGF 2α , unspiked plasma and urine samples were prepared by adding a volume equivalent to the spike volume of 0.15 M NaCl to the pooled aliquots (200 µL) of plasma or urine samples. Unspiked plasma or urine samples for analytical performance tests were prepared in triplicate. Sample preparation procedures and analysis of free 15(S)-8-iso-PGF 2α by the EIA assay were conducted within 24 h.

Procedure I (modified IAP method)

A set of aliquots of spiked or unspiked plasma samples (200 µL) were deproteinized with 375 µL of ice cold acidified acetone (acetone–1 N HCl–deionized water = 40:1:5 by volume), vortexed and centrifuged at 9,800 rpm for 10 min. Supernatants were evaporated under a gentle stream of N_2 to a final volume of 200 µL. Deproteinization and evaporation steps were repeated. These samples (200 µL) were treated with 200 µL of ethyl acetate, vortexed, and the upper layer was collected. This step was repeated thrice. Combined ethyl acetate layers were evaporated to dryness under a stream of N_2 . The plasma extracts were reconstituted with 200 µL of 0.15 M NaCl solution and mixed with 3 µg of a polyclonal goat 8-iso-PGF 2α antibody (Oxford Biomedical Research, Oxford, MI). This antibody exhibits cross-reactivity with 9 α , 11 β -PGF 2α (4.1%), 13, 14-dihydro-15-keto-PGF 2α (3%) and $<0.01\%$ for the other isoforms. Urine samples did not go through deproteinization or ethyl acetate extraction and were directly mixed with the polyclonal goat 8-iso-PGF 2α antibody. All samples were incubated with gentle mixing (2 h) to allow 8-iso-PGF 2α -antibody complex formation and were then passed through a 30 kDa MWCO filter (Ultrafree MC 30-kDa NMWL filter unit; Millipore, Billerica, MA) by centrifugation (5,000 g, 4°C) for 10 min. Each

filter sample was washed with 50 µL solution of 0.15 M NaCl by centrifugation (5,000 g, 4°C) for 10 min and the flow-through was discarded. The 8-iso-PGF 2α -antibody complex was treated with 95% ethanol (200 µL) and sonicated (1 min) to dissociate 8-iso-PGF 2α . The solution was transferred to a second 30 kDa MWCO filter and centrifuged (5,000 g, 4°C) for 10 min. Eluted 8-iso-PGF 2α was evaporated, reconstituted in 200 µL of 95% ethanol and stored at -80°C until analysis.

Procedure II (Cayman's affinity purification)

The column preparation and purification protocol was performed as per manufacturer's instructions (Cayman Chemical Company). Briefly, 8-iso-PGF 2α affinity columns were prepared by packing 0.5 mL (per sample) of an 8-isoprostane (8-iso-PGF 2α) affinity sorbent (Cat. No. 10010365; Cayman Chemical Company) containing a mouse monoclonal anti-8-iso-PGF 2α antibody (cross-reactivity with 8-isoprostaglandin F 3α was 7.6%, prostaglandin F 1α was 2.85%, and for other isoforms was $<1\%$), and washed with eicosanoid affinity column buffer (Cayman Chemical Company). Spiked or unspiked biological samples were passed through the affinity columns, which were then washed with column buffer and deionized water. The 8-iso-PGF 2α in each sample was eluted with 2 mL of elution solution (95% ethanol; Cayman Chemical Company), evaporated to dryness under a stream of N_2 , reconstituted in elution solution (200 µL), and stored at -80°C until analysis.

Procedure III (SPE–C18 method)

Aliquots of spiked or unspiked urine or plasma samples (200 µL) were applied to a SPE–C-18 column (Sep-Pak cartridge; Waters Associates, Milford, MA), which had been previously primed with 1 mL methanol and washed with 2 mL deionized water. Air (2 mL) was then displaced through the column, which was followed by a 1 mL deionized water wash. The 8-iso-PGF 2α was slowly eluted with 3 mL of methanol, collected into an amber glass vial and evaporated to dryness under a stream of N_2 . Dried samples were reconstituted in 200 µL of 95% ethanol and stored at -80°C until analysis.

EIA assay

Aliquots from each sample were evaporated, reconstituted in EIA buffer at a dilution of 1:10 (for the experiments with spiking concentrations of 0.25, 0.5 and 1 ng/mL) or 1:25 (for the experiments with a spiking concentration of 5 ng/mL) to fit within the mid-range of the EIA standard curve, and plated in duplicate according to the manufacturer's protocol for the 8-isoprostane EIA assay (Cayman Chemical Company). The plate was developed for 60 min (B_0 ranged from 0.5 to 0.8 AU) and absorbance measurements were made at a wavelength of 405 nm using a SpectraMax Plus microplate reader (Molecular Devices, Sunnyvale, CA). The 15(S)-8-iso-PGF 2α antibody used in the EIA kit measures the free form of 8-iso-PGF 2α and exhibits cross-reactivity with 8-isoprostaglandin F 3α , (20.6%), 2,3-dinor-8-isoprostaglandin F 2α (4%), 8-isoprostaglandin E 2 (1.84%), 2,3-dinor-8-isoprostaglandin F 1α (1.7%) and 8-isoprostaglandin E 1 (1.56%), and smaller than 1% for other isoforms.

Creatinine analysis

Creatinine concentration in urine samples was measured on the COBAS (Roche, Mannheim, Germany) instrument following the CREA Jaffé method (27).

Assessment of analytical performance

Initially, the EIA assay performance was validated by analysis of 8-iso-PGF2 α standards at concentration levels from 0.8 to 500 pg/mL (eight-point calibration). Intra-assay and inter-assay analytical variation values were calculated by the assessment of replicates within one assay and multiple assays, respectively.

Analytical performance of all three sample preparation methods (Procedures I–III) were assessed by the analysis of unspiked and spiked (5 ng/mL) plasma and urine samples purified by these methods, and by determination of corresponding spiked recovery values and reproducibility values. The spiked recovery of 8-iso-PGF2 α in percentage was calculated using the following equation: $[(X_1 - B)/X_0] \times 100$, where the 8-iso-PGF2 α amount measured in unspiked and spiked samples are denoted by B and X_1 , respectively, whereas the actual amount of 8-iso-PGF2 α used to spike is X_0 .

For the IAP method, further method performance verification tests were conducted. Extended analysis of recovery values was conducted by spiking 200 μ L aliquots of pooled urine or plasma samples over a range of 8-iso-PGF2 α concentrations (0.25, 0.5, 1 and 5 ng/mL), followed by analysis using the IAP–EIA method. In addition, pooled plasma or urine samples purified by IAP were post-spiked with 0.8, 2, 12.8, 32, 200 and 500 pg/mL concentrations of 8-iso-PGF2 α and calibration curves were constructed to determine r^2 values ($N=3$ for each concentration). Pooled plasma and urine aliquots clarified by the IAP method were spiked with 8-iso-PGF2 α at levels of 5 and 80 pg/mL, which were not used in the construction of the calibration curve, and analyzed to determine the accuracy of the IAP–EIA method. Additional plasma and urine samples were analyzed for 8-iso-PGF2 α levels using the optimized IAP–EIA method in triplicate by two analysts to test inter-analyst variation. Furthermore, three different batches of assay kits were tested to determine the impact of Cayman EIA assay kit batch-to-batch variation on the overall IAP–EIA method.

Analysis of plasma and urine samples from healthy individuals

Aliquots (200 μ L) of plasma or urine samples from five healthy individuals (commercially obtained from Innovative Research, Inc.) were analyzed using the IAP–EIA method. Each sample was analyzed in triplicate.

Results and Discussion

Oxidative stress has been implicated in several pathologies and high risk physiologies, such as cardiovascular, pulmonary and neurological disorders. Biochemical changes associated with oxidative stress can be followed by an assessment of oxidative modification of proteins, lipids and DNA. Measurement of 8-iso-PGF2 α is one way to investigate oxidative modification of lipids. This molecule is considered to be formed as the result

of a reaction between arachidonic moiety of membrane phospholipids and free radicals such as superoxide anion (28).

Although there are several reports on the measurement of free 8-iso-PGF2 α in biological fluids, they are labor intensive or costly, and thus are not compatible with high-throughput screening of large number of biological matrices. The purpose of developing this new IAP method was to offer a valid alternative to other purification methods available for 8-iso-PGF2 α analysis in plasma and urine samples. Initially, the Cayman EIA assay was validated. The detection limit of the EIA assay was 0.8 pg/mL. The practical quantitation limit was 4 pg/mL. Linear performance of the assay was $r^2 = 0.98$ in the range of 0.8–500 pg/mL. The intra-assay and inter-assay coefficients of variation were 4.9 and 13.4%, respectively.

Results from the three sample purification procedures used in this work were then compared for 8-iso-PGF2 α recovery following the analysis of spiked (5 ng/mL) and unspiked plasma and urine samples (Table I). Analysis of 8-iso-PGF2 α following the IAP method (Procedure I) was associated with analytical precision values ($\pm 5\%$) and yielded the best spiked recovery values for plasma (99.8%). For urine sample analysis, the analytical precision value was $\pm 15\%$ and spiked recovery was 54.1%. The Cayman affinity purification method (Procedure II) and the SPE–C18 method (Procedure III) overestimated the amount of 8-iso-PGF2 α both in plasma and urine, as revealed by the spiked recovery values, which were generally $>120\%$ (Table I). This can be attributed to experimental artefacts caused by cross-reactivity due to other co-contaminants such as isoforms of 8-iso-PGF2 α (22). The unspiked plasma 8-iso-PGF2 α level associated with Procedure III is in the same order of magnitude as the levels reported by Vassalle *et al.* (8), using the same procedure.

In all experiments, plasma and urine samples were treated with antioxidants to prevent postmortem oxidative changes during sample handling that could contribute to experimental artefacts. Pre-clean-up such as deproteinization and the choice of an antibody with comparably less cross-reactivity with the other isoforms of 8-iso-PGF2 α could have led to a better performance of the IAP method. However, the results revealed that the inclusion of a deproteinization step in the urine sample preparation procedure did not improve spiked recovery values, and thus a deproteinization step was not included in urine sample preparations. Deproteinization for urine matrix may not have significantly altered the recovery values because urine contains comparably less protein than the plasma matrix. Also, extraction of the urine samples with ethyl acetate did not improve the recovery of 8-iso-PGF2 α and was therefore not further pursued. Although urinary 8-iso-PGF2 α analysis by IAP was associated with lower spiked recovery values, it was consistent through repeated analyses (Tables I and II).

Extended analytical validation tests were conducted for the IAP–EIA method to assess the robustness of this method. Recovery values over a range of spiked concentrations (0.25, 0.5, 1 and 5 ng/mL) of 8-iso-PGF2 α were determined for both plasma and urine matrices (Table II). Mean recovery values for 8-iso-PGF2 α in plasma and urine were $90.5 \pm 8\%$ and $52.6 \pm 1.5\%$, respectively, and were independent of 8-iso-PGF2 α levels in these biological matrices. The calibration curve [EIA response expressed as $\text{logit}(B/B_0)$ versus concentration expressed as pg/mL, where B is the sample bound and B_0 is

Table IComparison of Analytical Performance of Three Sample Preparation Methods Used in the Analysis of 8-iso-PGF2 α in Human Plasma and Urine*

Procedure	Plasma			Urine		
	Measured amount in unspiked samples (pg/mL)	Measured amount in the spike [†] (pg/mL)	Spiked recovery [‡] (%)	Measured amount in unspiked samples (pg/mL)	Measured amount in the spike [†] (pg/mL)	Spiked recovery [‡] (%)
I	33.8 \pm 1.5	5,023 \pm 312	99.8	83.0 \pm 11.1	2,789 \pm 929	54.1
II	178.1 \pm 40.1	7,606 \pm 257	148.6	809.9 \pm 61.7	8,103 \pm 850	145.9
III	190.7 \pm 34.2	7,118 \pm 730	138.6	2,090.2 \pm 375.7	8,331 \pm 671	124.8

*Note: Results are presented as mean \pm SD, $n = 3$.[†]Amount of 8-iso-PGF2 α used to spike (X_0) = 5,000 pg/mL.[‡]Concentration in the spike = Measured amount in spiked samples (X_1) – Measured amount in unspiked samples (B); Spiked recovery = (Concentration in the spike/Actual amount used to spike) \times 100 = $[(X_1 - B)/X_0] \times 100$.**Table II**Percent Recoveries for a Range of Spiked Concentrations of 8-iso-PGF2 α in Plasma and Urine Samples Using the Optimized IAP–EIA Method*

Spiked amount (pg/mL)	Plasma		Urine	
	Concentration in the spike [†] (pg/mL)	Spiked recovery [‡] (%)	Concentration in the spike [†] (pg/mL)	Spiked recovery [‡] (%)
250	207.7 \pm 41.7	83.1	131.1 \pm 94.4	53.5
500	478.6 \pm 191.2	95.7	252.3 \pm 6.0	50.5
1,000	833.6 \pm 35.9	83.4	534.7 \pm 203.5	52.4
5,000	4,990 \pm 312	99.8	2,706 \pm 929	54.1

*Note: Results are presented as mean \pm SD, $n = 3$.[†]Concentration in the spike = Measured amount in spiked samples (X_1) – Measured amount in unspiked samples (B); B for plasma = 34 \pm 1.5 pg/mL, urine = 83 \pm 11 pg/mL.[‡]Spiked recovery = (Concentration in the spike/Actual amount used to spike) \times 100 = $[(X_1 - B)/X_0] \times 100$.

the maximum binding of tracer] for the plasma matrix exhibited a correlation coefficient of $r^2 = 0.993$, a slope of -0.9091 and an intercept of 2.4027. For the urine matrix, $r^2 = 0.978$, and the slope was -0.6451 and intercept was 1.1859. Accuracy values associated with this method for 8-iso-PGF2 α levels of 5 and 80 pg/mL in plasma matrix were $86 \pm 14\%$ and $84 \pm 7\%$, respectively, and $73 \pm 8\%$ and $77 \pm 7\%$, respectively, for the urine matrix. The inter-analyst variation based on the results from triplicate analysis of the pooled samples by two analysts was determined at less than $\pm 10\%$ for plasma and $\pm 27\%$ for urine. Similarly, three different batches of EIA assay kits were tested in triplicate and the assay kit batch-to-batch variation was less than $\pm 14\%$ for plasma and $\pm 30\%$ for urine.

The IAP method was then used to analyze commercially obtained human (female) plasma and urine samples for 8-iso-PGF2 α (Table III). The mean basal plasma levels of 8-iso-PGF2 α were determined to be 31.8 ± 5.5 pg/mL [mean \pm standard deviation (SD)]. Interestingly, this value is in line with the values (ranging from 35 to 42 pg/mL) reported by Milne *et al.* (23) and Ohashi *et al.* (24) using LC–ESI-MS and GC–MS analyses. This study's results indicated that urine 8-iso-PGF2 α levels exhibited more biological variability between individuals, ranging from 0.72 to 5.92 ng/mg of creatinine with a mean value of 2.9 ± 2.0 ng/mg creatinine (Table III). This is within the range of previously reported concentrations of 8-iso-PGF2 α in urine from healthy human volunteers determined by mass spectrometric methods (GC–MS, LC–MS and LC–MS-MS), which were 0.22 to 10.6 ng/mg creatinine (2, 22–26)

Table IIIUnspiked (Basal) Levels of 8-iso-PGF2 α Concentration in Human Plasma and Urine Samples

Plasma		Urine	
Modified IAP–EIA Procedure I (mean \pm SD) ($N = 5$)	Literature values (mean \pm SD)	Modified IAP–EIA Procedure I (mean \pm SD) ($N = 5$)	Literature values
31.8 \pm 5.5 pg/mL	35 \pm 6 pg/mL (23)	2.9 \pm 2.0 ng/mg creatinine	0.41–10.6 ng/mg creatinine (2)
	42 \pm 2 pg/mL (24)		0.22 \pm 0.14 ng/mg creatinine (22)
			1.6 \pm 0.60 ng/mg creatinine (23)
			0.43 \pm 0.10 ng/mg creatinine (24)
			1.11 \pm 0.45 ng/mg creatinine (25)
			0.8 \pm 0.54 ng/mg creatinine (26)

(Table III). Therefore, urine 8-iso-PGF2 α levels determined by the IAP–EIA method are in line with the values reported in the literature. Devaraj *et al.* (2) assessed this analyte using a GC–MS method and observed a larger variation in urinary 8-iso-PGF2 α levels between subjects compared to plasma levels. In addition, the larger inter-individual variation observed with 8-iso-PGF2 α levels in urine compared to plasma is reflected by the reported values in Table III. Increased biological variability associated with 8-iso-PGF2 α levels in urine can probably be affected by diets of individuals (26). Diets rich in antioxidants by consumption of fruits and vegetables have been reported by Thompson *et al.* (26) to be associated with a reduction in urinary concentration of 8-iso-PGF2 α .

The IAP method reported here employed only 200 μ L of a plasma or urine sample. By comparison, GC-based and LC-based analyses of healthy human plasma and urine levels for 8-iso-PGF2 α usually require sample volumes of 1 to 5 mL (22, 30). This can probably be accounted for by the sensitivity of detection associated with these different types of detection methods and sample preparation procedures that can negatively impact recovery values (22, 24, 30). The specificity of the IAP–EIA method, offered by using an antibody against 8-iso-PGF2 α , minimizes clean-up steps, which can be advantageous over other methods employing extensive sample manipulations, thus resulting in comparably fewer experimental artefacts.

Typical sample preparation methods used in the analysis of 8-iso-PGF2 α have been reported to involve anywhere between

6 h and 2 days (18, 29, 30). For the IAP method reported here, the time taken for purification of a batch of 36 samples is approximately 6 h. The time has been estimated to be ≥ 20 h for the analysis of 8-iso-PGF 2α by GC-MS or LC-MS-MS methods (instrument preparation, blanks and standard runs and data analysis; $N=36$ samples), based on the information provided in the literature (18, 29, 30). For similar analysis by the EIA method, the time involved is approximately 19 h. Therefore, the time for sample preparation and analysis of 8-iso-PGF 2α by the IAP-EIA method is either less or comparable to previously reported methods for sample preparation. Meanwhile, the cost associated with the IAP-EIA method for 8-iso-PGF 2α analysis is approximately one third of that associated with GC-MS or LC-MS-MS methods. Additionally, the GC-MS or LC-MS-MS methods of analysis require specialized personnel, whereas the IAP-EIA method does not. Furthermore, the IAP-EIA method reported here is also amenable to automation.

In conclusion, the IAP-EIA method is a valid alternative method for 8-iso-PGF 2α purification and exhibits good analytical performance for 8-iso-PGF 2α analysis in both plasma and urine matrices. The IAP-EIA method is simple, sensitive, less costly and requires smaller sample volume than other reported methods, and is therefore amenable to high-throughput screening of large number of samples for 8-iso-PGF 2α in biological matrices.

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