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Measurement of urinary F_2-isoprostanes by gas chromatography-mass spectrometry is confounded by interfering substances

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Abstract
Analysis of F_2-isoprostanes in urine using gas chromatography-mass spectrometry is confounded by the presence of endogenous compounds interfering with the internal standard, 15-F_{2t}-IsoP-d_4 (m/z 573). Previous efforts to resolve the 15-F_{2t}-IsoP-d_4 from co-eluting peaks with different solid phase extractions were unsuccessful. This study has now used a highly-deuterated, d_9-analogue of the derivatization agent N,O-Bis(trimethyl-d_9-silyl) trifluoroacetamide (BSTFA-d_9) yielding trimethylsilyl ethers, but this was not successful in resolving the 15-F_{2t}-IsoP-d_4 from co-eluting peaks. It was hypothesized that interfering peaks at m/z 573 could be the tetrahydro analogue of 15-F_{2t}-IsoP. However, using an authentic standard showed the interfering peaks are not due to this metabolite. In subsequent experiments good resolution was shown of the 15-F_{2t}-IsoP peak using 8-F_{2t}-IsoP-d_4 (m/z 573) as the internal standard. These data show that care must be taken when using GC-MS for quantitation of F_2-IsoPs to prevent interfering substances affecting the results.

Keywords: *Isoprostanes; GC-MS; 15-F_{2t}-IsoP.*

Introduction

Morrow et al. [1] reported that F_2-Isoprostanes (F_2-IsoPs), a complex group of prostaglandin $F_{2\alpha}$-like compounds, were produced *in vivo* by non-enzymatic free radical peroxidation of arachidonic acid (20:4 ω–6, AA). Four regioisomers differing in the nature of their side chains are formed and eight isomers can be produced from each of these regioisomers, leading to formation of 64 theoretical F_2-IsoPs [2–4]. F_2-IsoPs are thought to be formed from esterified arachidonate in phospholipids and are released as free acids by phospholipases [5,6].

The validity of F_2-IsoPs as markers of *in vivo* lipid peroxidation was demonstrated in a multi-laboratory validation study 'Biomarkers of Oxidative Stress Study (BOSS)' that compared different markers of oxidative stress using acute CCl_4 poisoning in a rodent model of oxidative stress [7]. The results in plasma and urine showed that the most accurate method to assess *in vivo* oxidant stress status was the quantification of F_2-IsoPs.

Various analytical methods have been developed to quantify F_2-IsoPs including enzyme immunoassay (EIA) [8], radioimmunoassay (RIA) [9], gas chromatography-mass spectrometry (GC-MS) [10–12], gas chromatography-tandem mass spectrometry (GC-MS-MS) [13], liquid chromatography-mass spectrometry (LC-MS) [14,15] and LC-tandem mass spectrometry (LC-MS-MS) [16]. To date the MS-based methods appear the most robust. Although immunoassays are commercially available, it was shown that results obtained by GC-MS and EIA are not equivalent [17].

The laboratory developed a gas chromatography-negative ion chemical ionization-mass spectrometry (GC-NICI-MS) method to analyse F_2-IsoPs [12]. However, the authors and others have reported that in urine, endogenous compounds co-eluting with the

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internal standard (IS) 15-F_{2t}-IsoP-d_4 at *m/z* 573 potentially confound quantitative analyses [11]. The authors also showed the use of different GC conditions and different solid phase extraction procedures (SPE) did not resolve the peak representing 15-F_{2t}-IsoP-d_4 from the interfering peaks [11].

The aims of this study were to (i) resolve the internal standard from the co-eluting peaks using a highly-deuterated analogue of the derivatization agent *N,O*-Bis(trimethyl-d_9-silyl) trifluoroacetamide (BSTFA-d_9) yielding the corresponding trimethylsilyl (TMS) ethers, (ii) examine whether the use of the 8-F_{2t}-IsoP-d_4 (IS) produced reproducible results for urinary F_2-IsoPs and (iii) identify whether the interfering peaks at *m/z* 573 could be the tetrahydro analogue of the15-F_{2t}-IsoP.

Materials and methods

Chemicals, reagents

15-F_{2t}-IsoP-d_4 and 8-F_{2t}-IsoP-d_4 were purchased from Cayman Chemicals (Ann Arbor, MI) and used without further purification. Pentafluorobenzylbromide (PFBBr) and *N,N*-diisopropylethylamine (DIPEA) were purchased from Sigma Chemicals (St Louis, MO). The silylating agent *N,O-bis*-(trimethylsilyl)trifluoroacetamide (Mw 257.4) with 1% trimethylchlorosilane (BSTFA-TMCS, 99:1) (BSTFA) was purchased from Pierce Chemicals (Rockford, IL); the silylating agent *N,O*-Bis(trimethyl-d_9-silyl) trifluoroacetamide (BSTFA-d_9, Mw 275.51) was from C/D/N Isotopes Inc. (Pointe-Claire, Quebec). Certify II cartridges were from Varian (Lake Forrest, CA). All solvents were of HPLC grade.

Urine collection

A pooled 24-h urine sample from healthy volunteers was collected and aliquots were stored frozen at −80°C until analysis without preservative.

Measurement of F_2-isoprostanes

Free F_2-isoprostanes were measured by GC-NICI-MS in a modification of a previously reported method [12]. 8-F_{2t}-IsoP-d_4 and 15-F_{2t}-IsoP-d_4 were added to urine (0.20 ml). After acidification (pH 4.5–4.7) with 1 M HCl, samples were applied to SPE cartridges and washed with methanol/water and hexane/ethyl acetate. F_2-isoprostanes were eluted with ethyl acetate/methanol, dried and derivatized.

Derivatization

The F_2-isoprostane fraction was treated with a mixture of PFBBr (40 µl) and DIPEA (20 µl). The sample was dried under nitrogen and then treated with BSTFA + TMCS (99:1, 20 µL) or with BSTFA-d_9 (20 µL) and anhydrous pyridine (10 µL) at 45°C for 20 min to yield the trimethylsilylethers that were dried under nitrogen and reconstituted in isooctane.

Accuracy. Accuracy, expressed as a percentage, was determined as described by Tsikas et al. [18]. Accuracy of the method was calculated from the equation: (mean of spiked samples – mean of unspiked samples)/expected amount. 15-F_{2t}-IsoP at different concentrations (0–400 pg/mL) and 15-F_{2t}-IsoP-d4 and 8-F_{2t}-IsoP-d4 (5 ng) were added to samples prior to extraction. Five determinations per concentration level were realized.

Within- and between-day precision. Within-day precision was calculated from repeated analysis (n = 5) of urines spiked with three different concentrations (0, 500 and 1000 pg/mL) of 15-F_{2t}-IsoP during 1 working day, by the same operator. Between-day precision was calculated from analysis (n = 5) of urines spiked at the same concentrations (0, 500 and 1000 pg/mL) of 15-F_{2t}-IsoP. One analysis was performed each day.

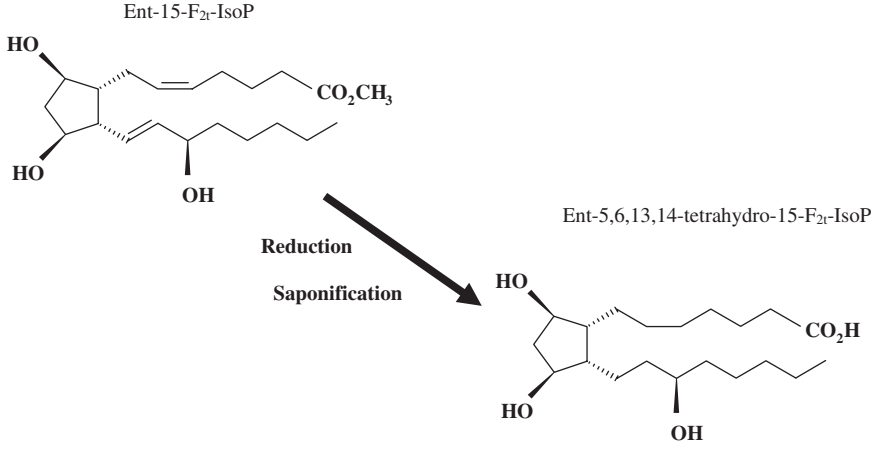

Figure 1. Reduction of ent-15-F_{2t}-IsoP methyl ester to ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP.

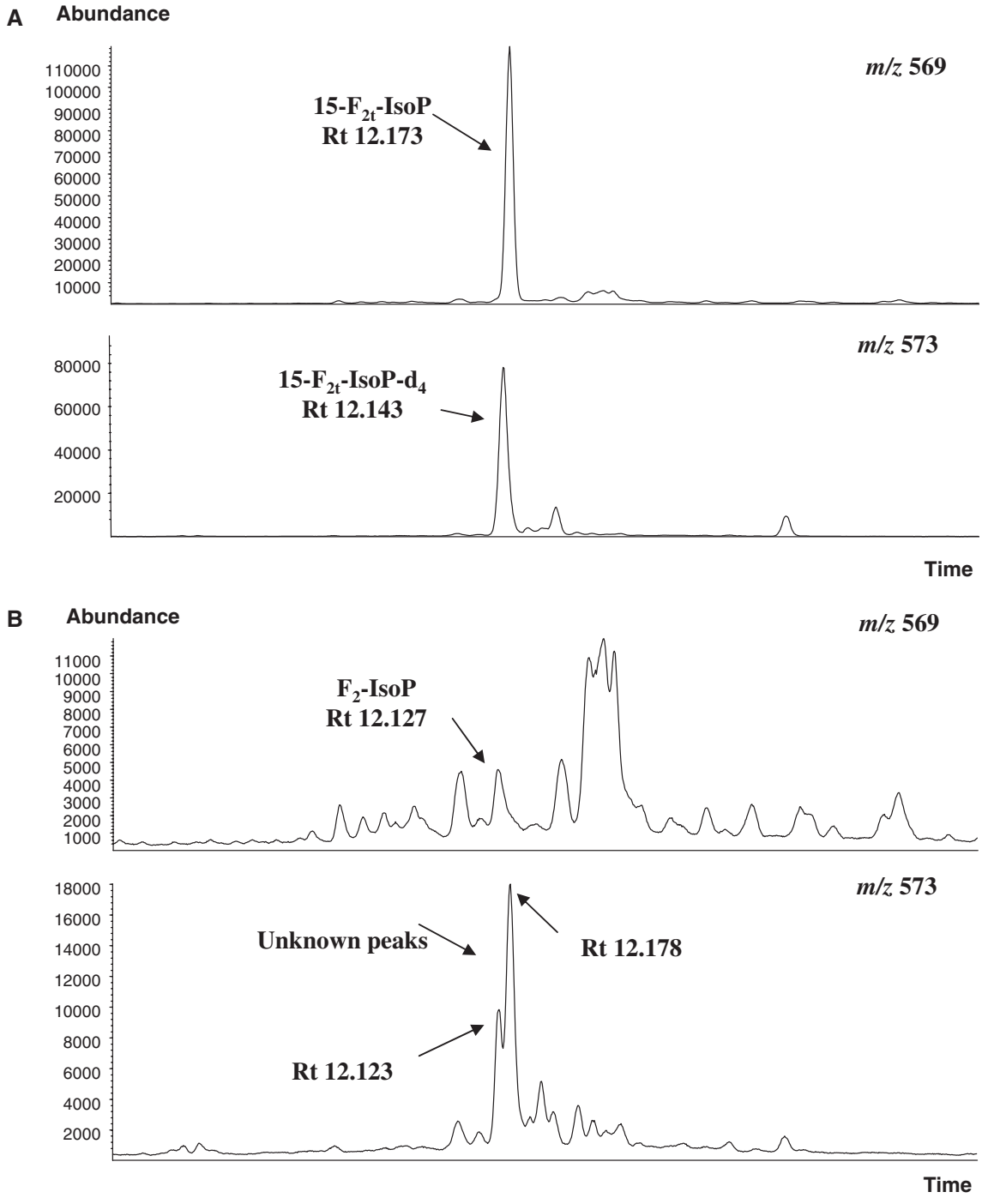

Figure 2. Partial ion chromatograms from GC-NICI-MS analysis of urinary sample (1 mL) derivatized with BSTFA. (A) Urine spiked with 15-F_{2t}-IsoP (2 ng) and 15-F_{2t}-IsoP-d_4 (5 ng) (*m/z* 569 and *m/z* 573); (B) Non-spiked urine (*m/z* 569 and *m/z* 573).

In both, 15-F_{2t}-IsoP-d_4 and 8-F_{2t}-IsoP-d_4 (5 ng) were added prior to SPE extraction.

Chemical synthesis of the tetrahydro analogue of 15-F_{2t}-IsoP

Ent-15-F_{2t}-IsoP was synthesized in the laboratory as previously described [19]. Ent-15-F_{2t}-IsoP methyl ester was reduced to ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP (Figure 1) by hydrogenation and saponification of methyl ester. Briefly, a solution of the methyl ester (10 mg, 0.027 mmol) in ethanol and 10% palladium on carbon (4 mg) in ethanol (1.5 mL) was hydrogenated at atmospheric pressure for 20 min. The mixture was filtered through Celite and concentrated to provide the hydrogenated ester as a colourless oil (10.1 mg, 100%). To the solution of the crude ester (10 mg, 0.027 mmol) in THF (tetrahydrofuran)/H_2O (1:1, 1 mL) was added monohydrated lithium hydroxide (7.2 mg,

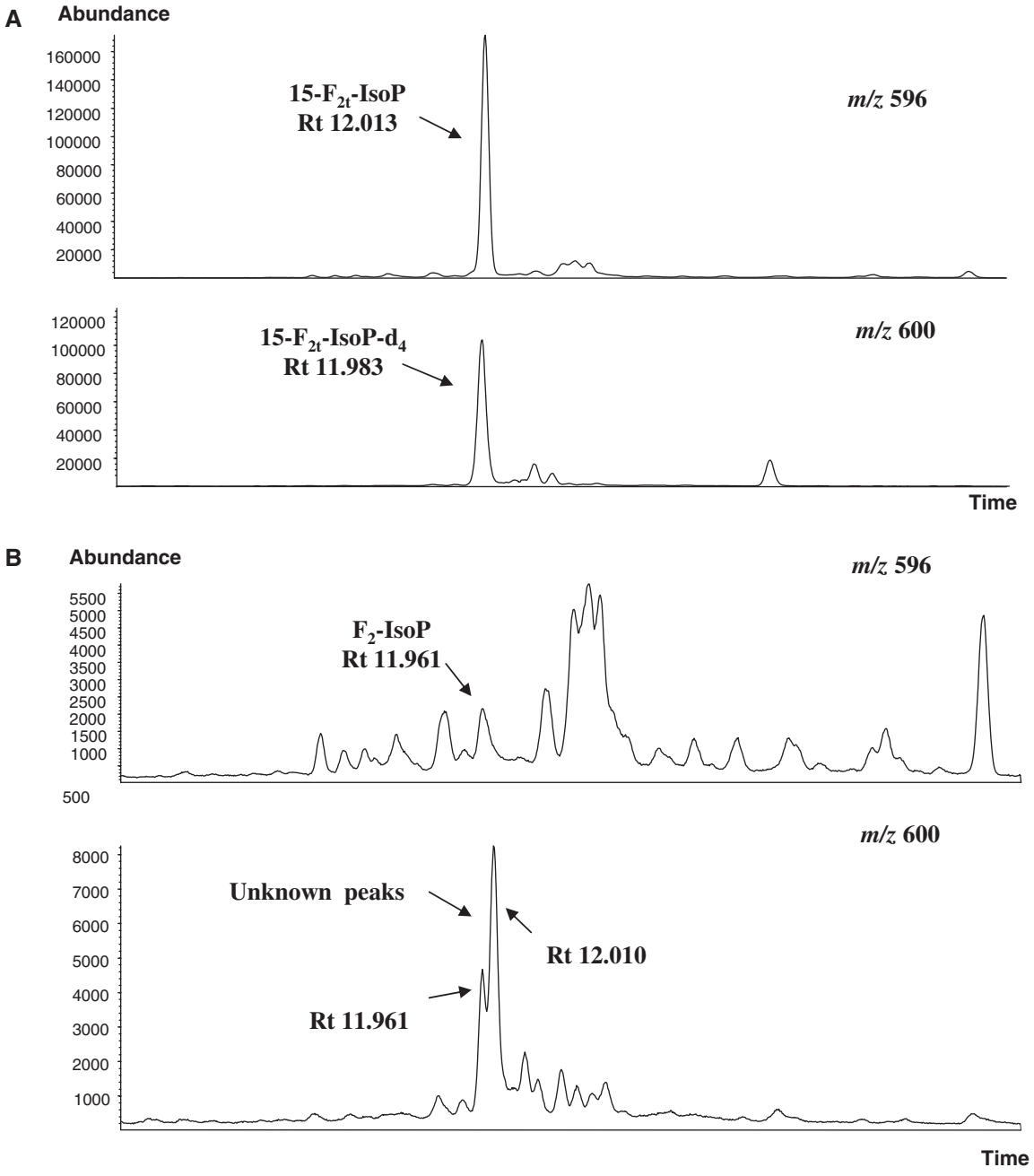

Figure 3. Partial ion chromatograms from GC-NICI-MS analysis of urinary sample (1 mL) derivatized with BSTFA-d_9. (A) Urine spiked with 15-F_{2t}-IsoP (2 ng) and 15-F_{2t}-IsoP-d_4 (5ng) (*m/z* 596 and *m/z* 600); (B) Non-spiked urine (*m/z* 596 and *m/z* 600).

0.172 mmol, 6.4 eq) and the reaction was stirred at room temperature for 4 h. The reaction mixture was acidified with 1 M HCl and extracted with ethyl acetate. The combined organic layer was washed with brine, dried with $MgSO_4$, concentrated and purified by flash chromatography (9:1 ethyl acetate/methanol) to afford the free acid (8.7 mg, 92% yield). The chemical structure was confirmed by 1 D and 2 D NMR (Nuclear magnetic resonance) and HRMS (high-resolution mass spectrometry) techniques.

Results

Derivatization with BSTFA

GC-MS analysis of urine spiked with 15-F_{2t}-IsoP and the d_4-analogue monitored ions at *m/z* 569 for 15-F_{2t}-IsoPs and *m/z* 573 for 15-F_{2t}-IsoP-d_4 (IS) following derivatization with PFBBr and BSTFA (Figure 2A). Figure 2B shows a chromatogram of human urine monitoring ions at *m/z* 569 for endogenous urinary F_2-IsoPs (Rt 12.127 min) and monitoring ions at *m/z* 573 shows the unknown peaks

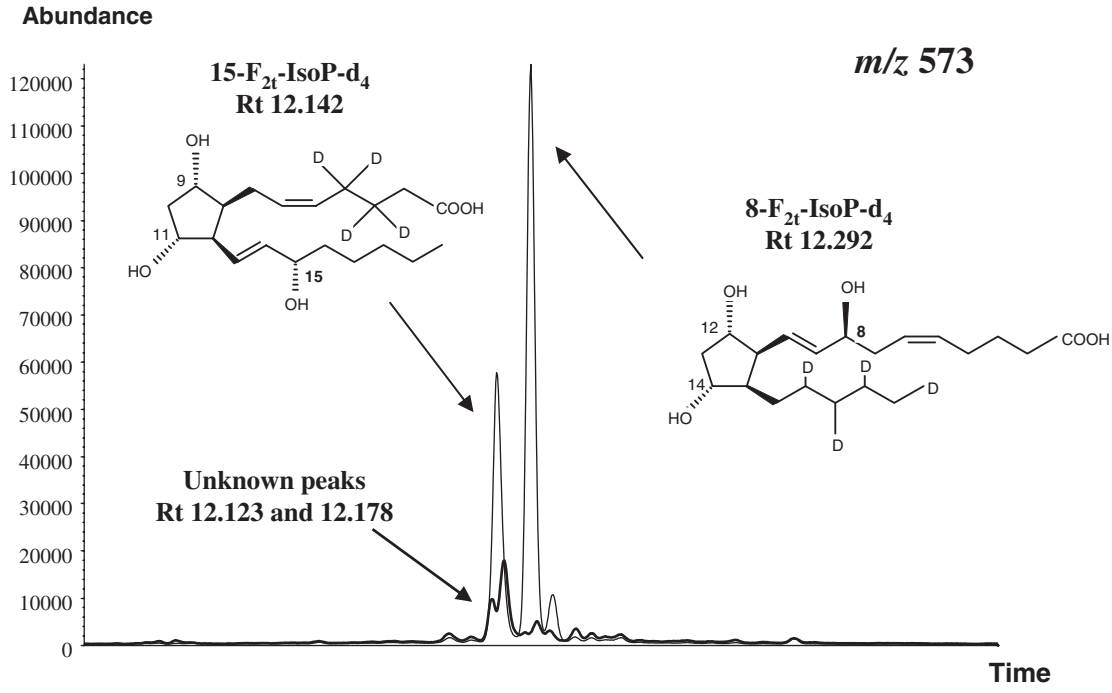

Figure 4. Overlay chromatograms of urine spiked with 15-F_{2t}-IsoP-d_4 and 8-F_{2t}-IsoP-d_4 (5 ng) with chromatogram of urine blank at *m/z* 573 derivatized with BSTFA.

(Rt 12.123 and 12.178 min), near the retention time (Rt 12.143 min) of 15-F_{2t}-IsoP-d_4 (5 ng).

Derivatization with BSTFA-d_9

In a first attempt to resolve the peak representing 15-F_{2t}-IsoP-d_4 from the two interfering peaks we derivatized the samples with BSTFA-d_9. Silylation with BSTFA-d_9 increased the mass of 15-F_{2t}-IsoP and 15-F_{2t}-IsoP-d_4 by the expected 27 Da (3 × (CD3)3Si-) for the three hydroxyl groups. The ions monitored are *m/z* 596 for F_2-IsoPs and *m/z* 600 for 15-F_{2t}-IsoP-d_4 (IS).

Using standards of 15-F_{2t}-IsoP (2 ng) and 15-F_{2t}-IsoP-d_4 (5 ng) we established the retention times of the BSTFA-d_9 analogues as 12.013 min and 11.983, respectively (Figure 3A).

BSTFA-d_9 adducts of 15-F_{2t}-IsoP and 15-F_{2t}-IsoP-d_4 eluted earlier than the BSTFA derivatives. Urine without IS and monitoring at *m/z* 600 demonstrated two peaks at 11.961 and 12.010 min (Figure 3B), near the retention time (Rt 11.983 min) of 15-F_{2t}-IsoP-d_4 (5 ng) (Figure 3A).

Using 8-F_{2t}-IsoP-d_4 as internal standard

Figure 4 shows overlay chromatograms at *m/z* 573 of non-spiked urine and urine spiked with 15-F_{2t}-IsoP-d_4 (5 ng) and 8-F_{2t}-IsoP-d_4 (5 ng). We observed that the unknown peaks (Rt 12.123 and 12.178 min) and 8-F_{2t}-IsoP-d_4 (12.292 min) were fully resolved and that 8-F_{2t}-IsoP-d_4 could be used as an internal standard for the assay.

Table I. Precision and accuracy of the method using 8-F_{2t}-IsoP-d_4. Analyses were performed in five measurements. The concentration of internal standard 8-F_{2t}-IsoP-d_4 is 5 ng/mL.

	F_{2t}-IsoP measured (pg/ml)			
15-F_{2t}-IsoP added (pg/ml)	Measured mean	SD	Accuracy (%)	Precision (RSD, %)
0	375.74	19.28	N. A.	5.18
50	411.38	10.5	71.27	2.57
100	456.74	16.74	80.99	3.69
150	500.60	12.66	83.24	2.55
200	527.18	9.36	75.72	1.79
400	682.94	16.92	76.8	2.49

Table II. Precision and accuracy of the method using 15-F_{2t}-IsoP-d_4. Analyses were performed in five measurements. The concentration of internal standard 15-F_{2t}-IsoP-d_4 is 5 ng/mL.

	F_{2t}-IsoP measured (pg/ml)			
15-F_{2t}-IsoP added (pg/ml)	Measured mean	SD	Accuracy (%)	Precision (RSD, %)
0	240.84	9.52	N. A.	3.95
50	260.16	7.01	38.64	2.70
100	289.71	12.43	48.87	4.29
150	308.23	12.03	44.93	3.90
200	318.46	12.83	38.81	4.03
400	419.48	6.38	44.66	1.52

Table III. Within- and between-day precision of 15-F_{2t}-IsoP in human urine with 8-F_{2t}-IsoP-d_4. Analyses were performed in five measurements. The concentration of internal standard 8-F_{2t}-IsoP-d_4 is 5 ng/mL.

	Between day			Within day		
15-F_{2t}-IsoP pg/ml added	*M*	SD	RSD %	*M*	SD	RSD %
0	352.72	41.78	11.85	374.71	19.34	5.16
500	765.51	74.87	9.78	787.28	52.13	6.62
1000	1187.86	69.36	5.84	1230.28	33.42	2.72

Accuracy. Table I shows accuracy obtained with 8-F_{2t}-IsoP-d_4 (5 ng). Mean F_2-IsoP in urine was 375.74 ± 19.28 pg/mL, RSD, 5.18% at baseline. Externally added 15-F_{2t}-IsoP was recovered from urine quantitatively with high accuracy (77.60% ± 4.68%) and low imprecision (RSD relative standard deviation, 3.04%) at each concentration, indicating the validity of the method over the concentration range tested.

Table II shows accuracy obtained with 15-F_{2t}-IsoP-d_4 (5 ng). Mean F_2-IsoP in urine was 240.84 ± 9.52 pg/mL, RSD, 3.95% at baseline. Externally added 15-F_{2t}-IsoP was recovered from urine quantitatively with 45.29% ± 4.62% accuracy and low imprecision (RSD relative standard deviation, 3.35%).

F_2-IsoP measured with 8-F_{2t}-IsoP-d_4 (375.74 ± 19.28 pg/mL, RSD, 5.18%) were significantly different from F_2-IsoP measured with 15-F_{2t}-IsoP-d_4 (240.84 ± 9.52 pg/mL, RSD, 3.95%) ($p < 0.0001$).

Linearity. Linear regression analysis between measured (y) and added (x) F_2-IsoPs concentrations resulted in the regression equation for the 8-F_{2t}-IsoP-d_4, $y = 0.77x + 377$, $R^2 = 0.998$, and for the 15-F_{2t}-IsoP-d_4, $y = 0.44x + 239$, $R^2 = 0.993$.

Within- and between-day precision. Using 8-F_{2t}-IsoP-d_4 (5 ng, $n = 5$) showed within-day variation ranged between 2.72–6.62% and between-day variation ranged between 5.84–11.85% (Table III).

Using 15-F_{2t}-IsoP-d_4 (5 ng, $n = 5$) showed within-day variation ranged between 2.10–17.24% and between-day variation ranged between 5.97–12.26% (Table IV).

Identification of interfering compounds

We proposed that the co-eluting interfering peaks at *m/z* 573 could be the tetrahydro analogue of the 15-F_{2t}-IsoP; 5,6,13,14-tetrahydro-15-F_{2t}-IsoP (Figure 1). However, we observed that the ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP eluted at 12 741 min which is significantly different from the 15-F_{2t}-IsoP-d_4 (12 143 min). A comparison of the retention times of the unknown compounds (Rt 12.123 and 12.178 min) and ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP (Rt 12.741 min) indicates they are not the same.

Discussion

We have shown herein that accurate and reproducible quantitative analysis of urinary F_2-IsoP can be achieved using 8-F_{2t}-IsoP-d_4 as internal standard in conjunction with SPE and GC-MS.

Previous attempts by our group to resolve 15-F_{2t}-IsoP-d_4 from co-eluting compounds at *m/z* 573 examined different GC conditions [11]. However, we were unable to separate the co-eluting compounds from the 15-F_{2t}-IsoP-d_4 and suggested the use of 4(*RS*)-F_{4t}-neuroprostane as the internal standard for quantification of urinary IsoPs [11].

Previous reports have noted that a limitation in the analysis of urinary F_2-IsoP by GC-MS is the presence of peaks in the *m/z* 573 spectrum co-eluting with the 15-F_{2t}-IsoP-d_4. Consequently the true concentration of urinary F_2-IsoP could be underestimated. For example we previously reported using 15-F_{2t}-IsoP-d_4 that urine F_2-IsoP concentration in healthy males was ~ 1.1 ng/mg creatinine [12]. In contrast, urinary excretion of F_2-IsoP was ~ 2.1 ng/mg creatinine in healthy men and women using 8-F_{2t}-IsoP-d_4 as internal standard [20]. Another limitation

Table IV. Within- and between-day precision of 15-F_{2t}-IsoP in human urine with 15-F_{2t}-IsoP-d_4. Analyses were performed in five measurements. The concentration of internal standard 15-F_{2t}-IsoP-d_4 is 5 ng/mL.

	Between day			Within day		
15-F_{2t}-IsoP pg/ml added	*M*	SD	RSD %	*M*	SD	RSD %
0	199.09	24.40	12.26	213.03	36.73	17.24
500	442.80	28.99	6.55	441.09	57.60	13.06
1000	695.44	41.52	5.97	739.12	15.54	2.10

when using 15-F_{2t}-IsoP-d_4 as internal standard for the quantitation of F_2-IsoP is that the peak areas of the co-eluting impurities can be highly variable between urine samples.

Analysis of F_2-IsoP by GC-MS requires derivatization to the strong electron-capturing PFB esters using PFB and DIPEA as a catalyst [12,21–25]. As a rule, esterification of the carboxyl group precedes the silylation of the hydroxyl groups because silylating agents such as BSTFA readily convert the carboxyl group to its TMS ester. Etherification of the hydroxyl groups is carried out with BSTFA-TMCS yielding TMS ethers.

BSTFA-d_9 was previously used by Waugh et al. [26], Fessel et al. [27] and Song et al. [28] to characterize isoprostanes, isofurans and neurofurans, respectively, by stable isotope dilution GC/electron capture/negative ionization as the pentafluorobenzyl ester, trimethylsilyl ether derivative. In this regard, BSTFA-d_9 was used to determined the number of the hydroxyl groups. Recently, VanRollins et al. [29] also used the BSTFA-d_9 to identify unesterified adrenate products (F_2-Dihomo-Isoprostanes) using authentic 17-F_{4c}-NeuroP as standard [30].

We found that as expected silylation with BSTFA-d_9 compared to BSTFA increased the mass of the derivatives by 27 Da: 15-F_{2t}-IsoP (*m/z* 569 → 596) and 15-F_{2t}-IsoP-d_4 (*m/z* 573 → 600). Using this approach, however, we were still unable to resolve the 15-F_{2t}-IsoP-d_4 from the co-eluting interfering compounds.

We have calculated the accuracy of the method using 15-F_{2t}-IsoP-d_4 or 8-F_{2t}-IsoP-d_4. The use of 15-F_{2t}-IsoP-d_4 as an internal standard resulted in poor accuracy compared with 8-F_{2t}-IsoP-d_4 (45.29% ± 4.62% and 77.60% ± 4.68%, respectively) and under-estimated the concentrations of F_2-IsoP. Using 8-F_{2t}-IsoP-d_4 also showed better within-day variation than using 15-F_{2t}-IsoP-d_4.

It is noteworthy that using different extraction methods, the interfering peaks are observed in urine but not in plasma, cerebrospinal fluid (CSF) or in tissue extracts. In our hands plasma F_2-IsoP measured using 8-F_{2t}-IsoP-d_4 or 15-F_{2t}-IsoP-d_4 gave similar results (207.10 ± 7.78 pg/mL and 207.36 ± 10.36 pg/mL, respectively). Our data are in contrast to the report by Lee et al. [31] that showed 8-F_{2t}-IsoP-d_4 and 15-F_{2t}-IsoP-d_4 have equal response.

We hypothesized that the unknown compounds detected at *m/z* 573 could be the tetrahydro analogue of 15-F_{2t}-IsoP: ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP. However, ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP was shown to elute at a longer retention time than F_2-IsoPs. Therefore, ent-5,6,13,14-tetrahydro-15-F_{2t}-IsoP is not the peak co-eluting with 15-F_{2t}-IsoP-d_4 at *m/z* 573.

In conclusion, we suggest that the use of 8-F_{2t}-IsoP-d_4 as an internal standard provides more accurate and reproducible quantification of urinary F_2-IsoPs than 15-F_{2t}-IsoP-d_4 which under-estimates the true concentration of urinary isoprostanes due to the presence of unknown compounds at *m/z* 573 that have a retention time similar to this internal standard.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Morrow JD, Awad JA, Kato T, Takahashi K, Badr KF, Roberts LJ, 2nd, Burk RF. Formation of novel non-cyclooxygenase-derived prostanoids (F2-isoprostanes) in carbon tetrachloride hepatotoxicity. An animal model of lipid peroxidation. J Clin Invest 1992;90:2502–2507.

[2] Jahn U, Galano JM, Durand T. Beyond prostaglandins—chemistry and biology of cyclic oxygenated metabolites formed by free-radical pathways from polyunsaturated fatty acids. Angew Chem Int Ed Engl 2008;47:5894–5955.

[3] Klein T, Reutter F, Schweer H, Seyberth HW, Nusing RM. Generation of the isoprostane 8-epi-prostaglandin F2alpha *in vitro* and *in vivo* via the cyclooxygenases. J Pharmacol Exp Ther 1997;282:1658–1665.

[4] Montuschi P, Barnes PJ, Roberts LJ, 2nd. Isoprostanes: markers and mediators of oxidative stress. Faseb J 2004;18:1791–1800.

[5] Morrow JD, Awad JA, Boss HJ, Blair IA, Roberts LJ, 2nd. Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed *in situ* on phospholipids. Proc Natl Acad Sci USA 1992;89:10721–10725.

[6] Basu S, Helmersson J. Factors regulating isoprostane formation *in vivo*. Antioxid Redox Signal 2005;7:221–235.

[7] Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, Fitzgerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts LJ, 2nd, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP, Barrett JC. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 2005;38:698–710.

[8] Wang Z, Ciabattoni G, Creminon C, Lawson J, Fitzgerald GA, Patrono C, Maclouf J. Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. J Pharmacol Exp Ther 1995;275:94–100.

[9] Helmersson J, Basu S. F2-isoprostane excretion rate and diurnal variation in human urine. Prostaglandins Leukot Essent Fatty Acids 1999;61:203–205.

[10] Bessard J, Cracowski JL, Stanke-Labesque F, Bessard G. Determination of isoprostaglandin F2alpha type III in human urine by gas chromatography-electronic impact mass spectrometry. Comparison with enzyme immunoassay. J Chromatogr B Biomed Sci Appl 2001;754:333–343.

[11] Mas E, Michel F, Guy A, Bultel V, Falquet Y, Chardon P, Rossi JC, Cristol JP, Durand T. Quantification of urinary F(2)-isoprostanes with 4(RS)-F(4t)-neuroprostane as an internal standard using gas chromatography-mass spectrometry application to polytraumatized patients. J Chromatogr B Analyt Technol Biomed Life Sci 2008;872:133–140.

[12] Mori TA, Croft KD, Puddey IB, Beilin LJ. An improved method for the measurement of urinary and plasma F2-isoprostanes using gas chromatography-mass spectrometry. Anal Biochem 1999;268:117–125.

[13] Tsikas D, Schwedhelm E, Fauler J, Gutzki FM, Mayatepek E, Frolich JC. Specific and rapid quantification of 8-iso-prostaglandin F2alpha in urine of healthy humans and patients with Zellweger syndrome by gas chromatography-tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 1998;716:7–17.

[14] Li H, Lawson JA, Reilly M, Adiyaman M, Hwang SW, Rokach J, FitzGerald GA. Quantitative high performance liquid chromatography/tandem mass spectrometric analysis of the four classes of F(2)-isoprostanes in human urine. Proc Natl Acad Sci USA 1999;96:13381–13386.

[15] Bohnstedt KC, Karlberg B, Basun H, Schmidt S. Porous graphitic carbon chromatography-tandem mass spectrometry for the study of isoprostanes in human cerebrospinal fluid. J Chromatogr B Analyt Technol Biomed Life Sci 2005;827: 39–43.

[16] Davies SS, Zackert W, Luo Y, Cunningham CC, Frisard M, Roberts LJ, 2nd. Quantification of dinor,dihydro metabolites of F(2)-isoprostanes in urine by liquid chromatography/ tandem mass spectrometry. Anal Biochem 2006;348:185–191.

[17] Proudfoot J, Barden A, Mori TA, Burke V, Croft KD, Beilin LJ, Puddey IB. Measurement of urinary F(2)-isoprostanes as markers of *in vivo* lipid peroxidation—A comparison of enzyme immunoassay with gas chromatography/mass spectrometry. Anal Biochem 1999;272:209–215.

[18] Tsikas D, Schwedhelm E, Suchy MT, Niemann J, Gutzki FM, Erpenbeck VJ, Hohlfeld JM, Surdacki A, Frolich JC. Divergence in urinary 8-iso-PGF(2alpha) (iPF(2alpha)-III, 15-F(2t)-IsoP) levels from gas chromatography-tandem mass spectrometry quantification after thin-layer chromatography and immunoaffinity column chromatography reveals heterogeneity of 8-iso-PGF(2alpha). Possible methodological, mechanistic and clinical implications. J Chromatogr B Analyt Technol Biomed Life Sci 2003;794:237–255.

[19] Oger C, Brinkmann Y, Bouazzaoui S, Durand T, Galano JM. Stereocontrolled access to isoprostanes via a bicyclo[3.3.0] octene framework. Org Lett 2008;10:5087–5090.

[20] Tsai IJ, Croft KD, Mori TA, Falck JR, Beilin LJ, Puddey IB, Barden AE. 20-HETE and F2-isoprostanes in the metabolic syndrome: the effect of weight reduction. Free Radic Biol Med 2009;46:263–270.

[21] Milne GL, Yin H, Brooks JD, Sanchez S, Jackson Roberts L, 2nd, Morrow JD. Quantification of F2-isoprostanes in biological fluids and tissues as a measure of oxidant stress. Methods Enzymol 2007;433:113–126.

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[22] Morrow JD, Roberts LJ, 2nd. Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress. Methods Enzymol 1999;300:3–12.

[23] Nourooz-Zadeh J, Gopaul NK, Barrow S, Mallet AI, Anggard EE. Analysis of F2-isoprostanes as indicators of non-enzymatic lipid peroxidation *in vivo* by gas chromatography-mass spectrometry: development of a solid-phase extraction procedure. J Chromatogr B Biomed Appl 1995;667:199–208.

[24] Lawson JA, Li H, Rokach J, Adiyaman M, Hwang SW, Khanapure SP, FitzGerald GA. Identification of two major F2 isoprostanes, 8,12-iso- and 5-epi-8, 12-iso-isoprostane F2alpha-VI, in human urine. J Biol Chem 1998;273: 29295–29301.

[25] Tsikas D. Application of gas chromatography-mass spectrometry and gas chromatography-tandem mass spectrometry to assess *in vivo* synthesis of prostaglandins, thromboxane, leukotrienes, isoprostanes and related compounds in humans. J Chromatogr B Biomed Sci Appl 1998;717:201–245.

[26] Waugh RJ, Morrow JD, Roberts LJ, 2nd, Murphy RC. Identification and relative quantitation of F2-isoprostane regioisomers formed *in vivo* in the rat. Free Radic Biol Med 1997;23:943–954.

[27] Fessel JP, Porter NA, Moore KP, Sheller JR, Roberts LJ, 2nd. Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension. Proc Natl Acad Sci USA 2002;99:16713–16718.

[28] Song WL, Lawson JA, Reilly D, Rokach J, Chang CT, Giasson B, FitzGerald GA. Neurofurans, novel indices of oxidant stress derived from docosahexaenoic acid. J Biol Chem 2008;283:6–16.

[29] VanRollins M, Woltjer RL, Yin H, Morrow JD, Montine TJ. F2-dihomo-isoprostanes arise from free radical attack on adrenic acid. J Lipid Res 2008;49:995–1005.

[30] Musiek ES, Cha JK, Yin H, Zackert WE, Terry ES, Porter NA, Montine TJ, Morrow JD. Quantification of F-ring isoprostane-like compounds (F4-neuroprostanes) derived from docosahexaenoic acid *in vivo* in humans by a stable isotope dilution mass spectrometric assay. J Chromatogr B Analyt Technol Biomed Life Sci 2004;799:95–102.

[31] Lee CY, Jenner AM, Halliwell B. Rapid preparation of human urine and plasma samples for analysis of F2-isoprostanes by gas chromatography-mass spectrometry. Biochem Biophys Res Commun 2004;320:696–702.