Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 861 (2008) 145–150
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of urinary phytoestrogens by HPLC–MS/MS: A comparison of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI)

Michael E. Rybak [a,*], Daniel L. Parker [a,b], Christine M. Pfeiffer [a]

[a] *Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta, GA 30341, USA*
[b] *Battelle Memorial Institute, Columbus, OH 43201, USA*

Received 17 August 2007; accepted 8 November 2007
Available online 21 November 2007

Abstract

A comparison of the analytical performance of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) for the quantitative determination of six urinary phytoestrogens (daidzein, *O*-desmethylangolensin, equol, enterodiol, enterolactone and genistein) by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) is presented here. Both APCI and ESI were suitable for the analysis of these compounds; however, ESI did improve measurement imprecision and sensitivity in certain cases. Method imprecision (between-run coefficients of variation [CVs] from duplicate analysis of three quality control [QC] urine pools across 20 runs) was 5.6–12% for ESI, as opposed to 5.3–30% for APCI. At low concentrations (3–60 ng/mL, analyte dependent) imprecision was lower with ESI, whereas both techniques were generally commensurate at high concentrations (200–1000 ng/mL, analyte dependent). Method accuracy (spiked analyte recovery from the QC pools) was comparable between techniques: 86–114% for ESI; 95–105% for APCI. Limits of detection (LODs) were equivalent or better with ESI compared to APCI, with the most significant LOD improvement observed for equol (ESI: 0.3 ng/mL; APCI: 2.7 ng/mL). This translated into a substantial increase in equol detection frequency (% of sample results above LOD) within a random patient sample subset (98% for ESI, compared to 81% for APCI, $n = 378$). Correlation (Pearson) and agreement (Deming regression, Bland-Altman bias) between ESI and APCI results in the patient subset was better in cases where imprecision and sensitivity was similar for both techniques (daidzein, enterolactone, genistein: $r = 0.993$–0.998; slope = 0.98–1.03; bias = −4.2 to −0.8%); correlation and/or agreement was poorer for analytes, where APCI imprecision and sensitivity were inferior (equol, *O*-desmethylangolensin, enterodiol). Baring significant factors arising from differences in ionization source design, these observations suggest that ESI is more appropriate for urinary biomonitoring of these compounds by LC–MS/MS.
Published by Elsevier B.V.

Keywords: Phytoestrogens; High-performance liquid chromatography; Tandem mass spectrometry; Electrospray ionization; Atmospheric pressure chemical ionization

1. Introduction

Phytoestrogens are plant-derived polyphenolic compounds, such as isoflavones, lignans, coumestans and stilbenes that bear structural similarities to endogenous estrogens and are capable of estrogen-receptor binding. Their endocrine activity, as well as their potential influence on other biologic pathways, has led to considerable interest in phytoestrogens from an epidemiological standpoint [1]. The consumption of diets high in phytoestrogen-rich foods has been associated with lower rates of such hormone-dependent cancers as breast [2–4] and prostate [3,4] cancer, with improved bone health [5,6], with post-menopausal symptoms [4], and with cardiovascular disease [7]. Whether phytoestrogens are indeed the active components responsible for these benefits, however, has come under scrutiny [1,8], and the significance of their purported health benefits has been challenged [9]. Individual studies and meta analyses have often resulted in apparently conflicting findings, such as whether phytoestrogens do [10] or do not [11] significantly reduce the frequency and intensity of menopausal hot flushes. Potential toxic effects associated with phytoestrogen exposure have also been identified [8].

Regardless of possible efficacy and safety concerns, increased consumer awareness and marketing of the potential health ben-

* Corresponding author. Tel.: +1 770 488 7394; fax: +1 404 638 5431.
E-mail address: MRybak@cdc.gov (M.E. Rybak).

1570-0232/$ – see front matter Published by Elsevier B.V.
doi:10.1016/j.jchromb.2007.11.013

efits of phytoestrogens has led to augmented intake in Western diets of foods and dietary supplements rich in these compounds. The US Centers for Disease Control and Prevention (CDC) has included several urinary isoflavones (daidzein, genistein, equol, *O*-desmethylangolensin) and lignans (enterodiol, enterolactone) in the National Health and Nutrition Examination Survey (NHANES) for the purposes of monitoring phytoestrogen exposure in the US population [12]. A variety of analytical techniques have been developed for quantifying phytoestrogens in biologic samples for epidemiological purposes [13], as well as other matrices and applications [14,15]. Techniques based on high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) detection have been of particular interest and popularity, primarily because of the inherent selectivity and sensitivity of MS/MS detection [16]. HPLC–MS/MS analyses are performed most commonly by using either atmospheric pressure chemical ionization (APCI) [17,18] or electrospray ionization (ESI) [19–22]. The view is generally held that APCI can be used for relatively non-polar compounds that can undergo acid–base reactions in the gas phase, whereas ESI is more suitable for polar compounds that can be ionized in solution [23]. CDC has historically used APCI for its HPLC–MS/MS analyses of the above-named urinary phytoestrogens for NHANES [24] and other biomonitoring studies, but this approach has resulted in a significant number of non-detects, in some groups upwards of 35% [25]. The purpose of this study was to evaluate ESI as an alternative for urinary phytoestrogen biomonitoring.

2. Methods

The sample preparation and analysis performed in this study were based on methods previously developed and used at CDC for phytoestrogen biomonitoring [17,18], with modifications. Because the changes made are quite numerous and they span the entire sample preparation and analysis procedure, the method we used in this study is described in its entirety below.

HPLC-grade methanol, ethanol, acetonitrile, and dimethylsulfoxide (Burdick & Jackson, Muskegon, MI, USA) and 0.45 μm filtered distilled deionized water (Aqua Solutions, Jasper, GA, USA) were used in the preparation of all standards, samples, and mobile phases. HPLC-grade ammonium acetate (Sigma, St. Louis, MO) was used to prepare mobile phases. All other chemicals used were reagent grade or higher. Individual standard stock solutions of daidzein, genistein (Indofine Chemical Company, Somerville, NJ, USA), enterolactone, enterodiol, equol (Sigma, St. Louis, MO, USA), and *O*-desmethylangolensin (laboratory of Dr. Nigel Botting, University of St. Andrews, St. Andrews, Scotland) were prepared by dissolving 3–5 mg of each solid material in 0.2 mL of dimethylsulfoxide and diluting with ethanol to a final concentration of 120–200 μg/mL. Nine mixed working standards containing all six analytes were prepared in ethanol and stored in 50 μL aliquots at −80 °C, from which calibrators were prepared. The nine calibrators ranged in concentration from 0.3 ng/mL to 2800 ng/mL, depending on the analyte. $^{2}H_{8}$-enterodiol, $^{2}H_{6}$-enterolactone, $^{2}H_{4}$-genistein, $^{2}H_{3}$-daidzein, $^{2}H_{4}$-equol and $^{2}H_{5}$-*O*-desmethylangolensin (Laboratory of Prof. Kristiina Wähälä, University of Helsinki, Helsinki, Finland) were used for internal standardization of the analyte signals studied. A mixed standard solution of deuterated compounds was prepared in water from single-compound stock solutions dissolved in ethanol and stored in 1 mL aliquots at −80 °C. Internal standard concentrations in this solution were 100–960 μg/mL, depending on the analyte. A 2 mg/mL solution of β-glucuronidase/sulfatase from *Helix pomatia* H1 (Sigma) in 1 mol/L ammonium acetate buffer (pH 5.0) was prepared daily and used to enzymatically deconjugate the analytes of glucuronide and sulfate moieties during sample preparation. A deconjugation internal standard solution containing 24 μg/mL 4-methylumbelliferyl glucuronide (Sigma) and 20 μg/mL 4-methylumbelliferyl sulfate in ethanol for monitoring the extent of the deconjugation reaction was prepared and stored in 1 mL aliquots at −80 °C. Patient urine specimens ($n = 378$) for the method comparison were selected at random from the current 2003–2004 NHANES, and as such these specimens are not representative of the US population.

For each patient specimen, 50 μL of the mixed deuterated internal standards solution, 10 μL of the deconjugation internal standard solution, and 1 mL of urine were combined in a 16 × 100 mm screw-top glass culture tube and vortex-mixed. A 500 μL aliquot of the β-glucuronidase/sulfatase solution was added to the mixture, and the tube was capped and gently rocked several times. Samples were incubated for at least 17 h at 37 °C. Following incubation, analytes were extracted by use of 3 mL solid phase extraction (SPE) cartridges containing 60 mg of an *m*-divinylbenzene/*N*-vinylpyrrolidone copolymer stationary phase (Waters Oasis HLB, Waters Corporation, Milford, MA, USA). The SPE process was performed by use of an automated, positive-displacement, positive-pressure SPE system (Gilson 215, Gilson Inc. Madison, WI, USA). SPE cartridges were conditioned successively with 2 mL of methanol and 2 mL of water prior to sample loading. A 1 mL aliquot of water was added to each patient sample and mixed. The entire contents were then loaded onto the SPE cartridge. Each cartridge was then successively washed with 1 mL of water and 2 mL of a 1:1 methanol/water solution. Analytes were eluted from the cartridges by use of 3 mL of methanol and collected in 12 × 75 mm glass culture tubes. Samples were then evaporated to dryness for 1.5 h under vacuum at 55 °C (SpeedVac SPD 131DDA, Thermo Scientific, Milford, MA, USA) and reconstituted in 100 μL of 15:15:70 methanol/acetonitrile/10 mM ammonium acetate buffer (pH 6.5). Reconstituted samples were then transferred to autosampler vials with reduced volume (300 μL) inserts. Calibrators were prepared by first adding 50 μL of the mixed deuterated internal standards solution directly to each of the nine mixed working standard aliquots. Each working standard was then vortex-mixed and its contents transferred to a 12 × 75 mm glass culture tube. Calibrators were then evaporated to dryness, reconstituted, and transferred to autosampler vials, as described above for patient samples.

Chromatographic separations were performed on an HPLC system (Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) consisting of a binary pump and degasser (model G1312A), autosampler (model G1313A) and thermostatted

column compartment (model G1316A). Compounds were separated by use of a 50 × 3 mm, 5 μm particle size C_{18} column (Betasil C_{18}, Thermo Scientific) and a 4 × 2 mm guard column (SecurityGuard C_{18}, Phenomenex, Torrance CA, USA). Mobile phases were 10 mM ammonium acetate buffer, pH 6.5 (solvent A), and 1:1 methanol/acetonitrile (solvent B). The separation gradient consisted of the following linear steps (expressed as % solvent B): 0 min, 35%; 2 min, 35%; 4 min, 50%; 5 min, 55%; 7 min, 95%; 7.5 min, 35%. The HPLC system was re-equilibrated for 1.5 min prior to the next sample injection. For all samples, a 25 μL injection volume was used. The HPLC system was interfaced to a triple quadrupole mass spectrometer (API 4000, Applied Biosystems, Foster City, CA, USA) capable of both ESI and APCI (Turbo V Ion Source). All analyses were performed by use of a negative ion mode and unit mass resolution. Ionization interface temperatures (ESI: 550 °C; APCI: 500 °C), needle voltages (ESI: −3500 V; APCI: −3 V), and various gas settings were optimized to maximize peak height signal-to-noise ratio (S/N). A summary of mass spectrometric conditions used is given in Table 1. All HPLC–MS/MS components were controlled, and data analysis was performed by use of Analyst software (version 1.3.1, Applied Biosystems). Analytes were quantified interpolating peak area ratios for the MS/MS transitions against a calibration curve obtained from aqueous calibrators (1/*x* weighting).

3. Results and discussion

Method sensitivity was quantitatively assessed by calculation of limits of detection (LODs) as proposed by Taylor [26] for both APCI and ESI, using a zero-concentration standard deviation estimate (σ_0) obtained by extrapolation from repeat analysis ($n = 9$) of the "low" urine QC pool and four dilutions thereof (1:1, 1:3, 1:7, and 1:15). In general, LODs ($3\sigma_0$) for ESI were either commensurate with or showed improvement over their respective APCI counterparts. Improvements in LOD also resulted in improvements in detection frequency, defined here as the percentage of results from the patient subset at or above the calculated LOD (Table 2). With ESI, detection frequency was ≥97% for all analytes, with the greatest improvement observed for equol. The lower detection frequency observed for equol with APCI in the patient subset is consistent with that observed in past biomonitoring studies [25] where APCI was also used, and it is assumed that a similar improvement would be observed if ESI were used as an alternative to biomonitoring. The ability to detect equol is of significant

Table 2
Limits of detection

Analyte	Limit of detection (ng/mL)[a]		Detection frequency (%)[b]	
	APCI	ESI	APCI	ESI
Daidzein	0.3	0.3	99	99
O-Desmethylangolensin	0.05	0.05	87	97
Equol	2.7	0.3	81	98
Enterodiol	0.2	0.06	93	97
Enterolactone	0.3	0.4	98	98
Genistein	0.3	0.06	99	99

[a] Calculated according to [27]. See text for details.
[b] Defined as percentage of results ≥LOD from patient subset. See text for details.

Table 1
MS/MS analysis parameters

Analyte	Retention time (min)	Calibration range (ng/mL)[a]	MRM transitions (*m/z*)		Dwell time (ms)	Mass spectrometer potentials (V)[b]			
			Molecular ion	Product ion		DP	EP	CE	CXP
Daidzein	2.4	1–500	253.1	223.1	50	−110	−10	−47	−15
		>500–1400	253.1	117.1	50	−110	−10	−47	−15
		IS	256.0	226.0	50	−110	−10	−47	−15
O-Desmethylangolensin	5.6	0.4–55	257.1	108.1	50	−25	−6	−40	−5
		>55–250	257.1	148.0	50	−25	−6	−40	−5
		IS	261.0	111.0	50	−70	−10	−33	−7
Equol	4.5	0.3–100	241.1	119.1	50	−100	−10	−30	−10
		IS	245.0	123.0	50	−70	−10	−24	−5
Enterolactone	5.0	2–750	297.3	107.1	50	−50	−11	−45	−7
		>750–2800	297.3	93.0	50	−50	−11	−45	−7
		IS	303.0	110.0	50	−70	−10	−60	−15
Enterodiol	3.1	0.3–300 (ESI)	301.3	253.2	50	−10	−9	−35	−6
		0.3–300 (APCI)	301.3	241.1	50	−10	−9	−35	−6
		IS	304.0	255.7	50	−75	−10	−32	−5
Genistein	4.5	0.4–150	269.1	133.0	50	−80	−10	−45	−13
		>150–600	269.1	224.1	50	−80	−10	−40	−13
		IS	273.0	137.0	50	−70	−10	−47	−7
Umbelliferone	1.5	IS	174.7	133.0	50	−46	−3	−35	−20

[a] IS, internal standard.
[b] DP, declustering potential; EP, collision cell entrance potential; CE, collision cell excitation potential; CXP, collision cell exit potential.

Table 3
Method imprecision

Analyte	Urine QC pool	Concentration (ng/mL)[a]	Between-run coefficient of variation (CV, %)	
			APCI	ESI
Daidzein	Low	21.6	23	6.1
	Medium	189	7.6	6.1
	High	985	5.3	6.5
O-Desmethylangolensin	Low	2.80	24	8.0
	Medium	97.6	7.5	12
	High	183	8.1	7.6
Equol	Low	4.78	30	6.4
	Medium	13.4	14	6.4
	High	29.5	6.6	5.9
Enterodiol	Low	20.2	28	7.7
	Medium	63.3	26	8.9
	High	69.77	21	8.9
Enterolactone	Low	57.9	13	9.2
	Medium	141	9.9	11
	High	1075	6.4	7.0
Genistein	Low	19.8	13	4.4
	Medium	90.7	5.3	7.0
	High	540	5.4	5.6

[a] Mean concentration using ESI.

epidemiological interest; equol is formed by the biotransformation of daidzin and daidzein by intestinal bacteria, and the existence of equol "producing" and "non-producing" phenotypes among various populations has been identified and studied [27,28]. The ability to detect equol with increased sensitivity will greatly assist in the ability to identify these specific groups reliably.

Method imprecision was assessed by replicate measurement of the three QC urine pools in duplicate across 20 runs conducted over a period of 2 months, using both APCI and ESI (Table 3). With the exception of the use of APCI for enterodiol, between-run coefficients of variation (CVs) observed for the high QC pool were comparable in both techniques for all six analytes, covering a collective range of 5.3–8.9%. The CVs observed at intermediate and low concentrations were markedly better with ESI than with APCI in all cases. For example, the CVs observed with ESI across all analytes for the low QC pool were 4.4–9.2%, as compared to 13–30% with APCI. The greatest overall improvements in between-run CVs were observed for *O*-desmethylangolensin, equol, and enterodiol, analytes that coincidentally demonstrated sensitivity (i.e., LOD) and detection frequency enhancements when ESI was used, as opposed to APCI.

Method accuracy was evaluated by amending each of the three urine QC pools with each of the analytes in concentrations approximately equal to their endogenous concentrations and by calculating the recovery of the added amount from replicate determinations ($n = 3$). Analyte recoveries were very similar for both techniques, ranging from 95% to 105%, with a mean recovery (95% confidence limits) of 99% (95%, 103%) for APCI, and with a range from 86% to 114% with a mean recovery of 100% (92%, 108%) for ESI. For enterodiol, the recovery obtained with the 301.3/253.2 (m/z) transition by use of APCI was negative, and it varied greatly between pools. This suggested the presence of an interference in this transition and necessitated the use of 301.3/241.1 (m/z) as an alternative transition (Table 1). The 301.3/253.2 transition, however, was suitable for ESI, with a recovery of 96% (88%, 104%).

Correlation and agreement between APCI and ESI were assessed by analysis of a random subset of urine samples ($n = 378$), using both techniques. The results from this subset showed concentrations that spanned three to four orders of magnitude, with the distribution of these results being right-skewed. A $\log_{10}$ transformation of the data corrected for the skewness and resulted in values that were more normally distributed; consequentially, a $\log_{10}$-transformed dataset was used in all statistical comparisons, except for regression analysis. Regression analysis was performed on untransformed data, so that the calculated slopes and intercepts could be used as indicators of proportional and absolute bias, respectively.

Pearson correlation tests showed a high degree of correlation between results obtained with ESI and APCI for all analytes (Table 4). The highest degree of correlation was observed for daidzein, *O*-desmethylangolensin, enterolactone and genistein ($r = 0.986$–0.998), whereas correlation coefficients for equol (0.897) and enterodiol (0.867) were lower. Deming regression was used for testing agreement between the two techniques. Deming regression slopes (y = ESI, x = APCI) for daidzein, enterolactone, and genistein for the random sample

Table 4
Correlation and agreement statistics[a]

Analyte	Pearson correlation (r)	Deming regression		Bland-Altman bias analysis (%)		
		Slope	Intercept	Bias	Limits of agreement	
					Lower 95%	Upper 95%
Daidzein	0.9983 (0.9979, 0.9986)	1.004 (0.991, 1.016)	−0.857 (−4.952, 3.237)	−0.8 (−1.6, 0.1)	−15.2 (−16.4, −14.0)	16.1 (14.5, 17.8)
O-Desmethyl-angolensin	0.9860 (0.9826, 0.988)	1.114 (1.092, 1.136)	0.514, (−0.565, 1.594)	26.1 (21.8, 30.6)	−32.2 (−36.1, −28.0)	134.5 120.9, 148.9)
Equol	0.8973 (0.8726, 0.9174)	0.797 (0.749, 0.846)	−0.728 (−1.989, 0.533)	−25.7 (−29.2, −22.0)	−67.6 (−70.2, −64.9)	70.7 (57.2, 85.4)
Enterodiol	0.8637 (0.8328, 0.8892)	1.089 (1.030, 1.148)	5.046 (0.173, 9.918)	26.3 (18.4, 34.6)	−59.6 (−63.8, −54.9)	294.5 (253.7, 340.0)
Enterolactone	0.9932 (0.9916, 0.9945)	0.982 (0.959, 1.005)	−8.676 (−28.583, 11.230)	−4.2 (−5.9, −2.5)	−30.9 (−32.9, −28.8)	32.6 (28.7, 36.7)
Genistein	0.9966 (0.9958, 0.9972)	1.030 (1.015, 1.045)	−1.538 (−3.679, 0.603)	−1.3 (−2.3, −0.2)	−19.1 (−20.5, −17.6)	20.4 (18.3, 22.6)

[a] 95% confidence limits appear in parentheses.

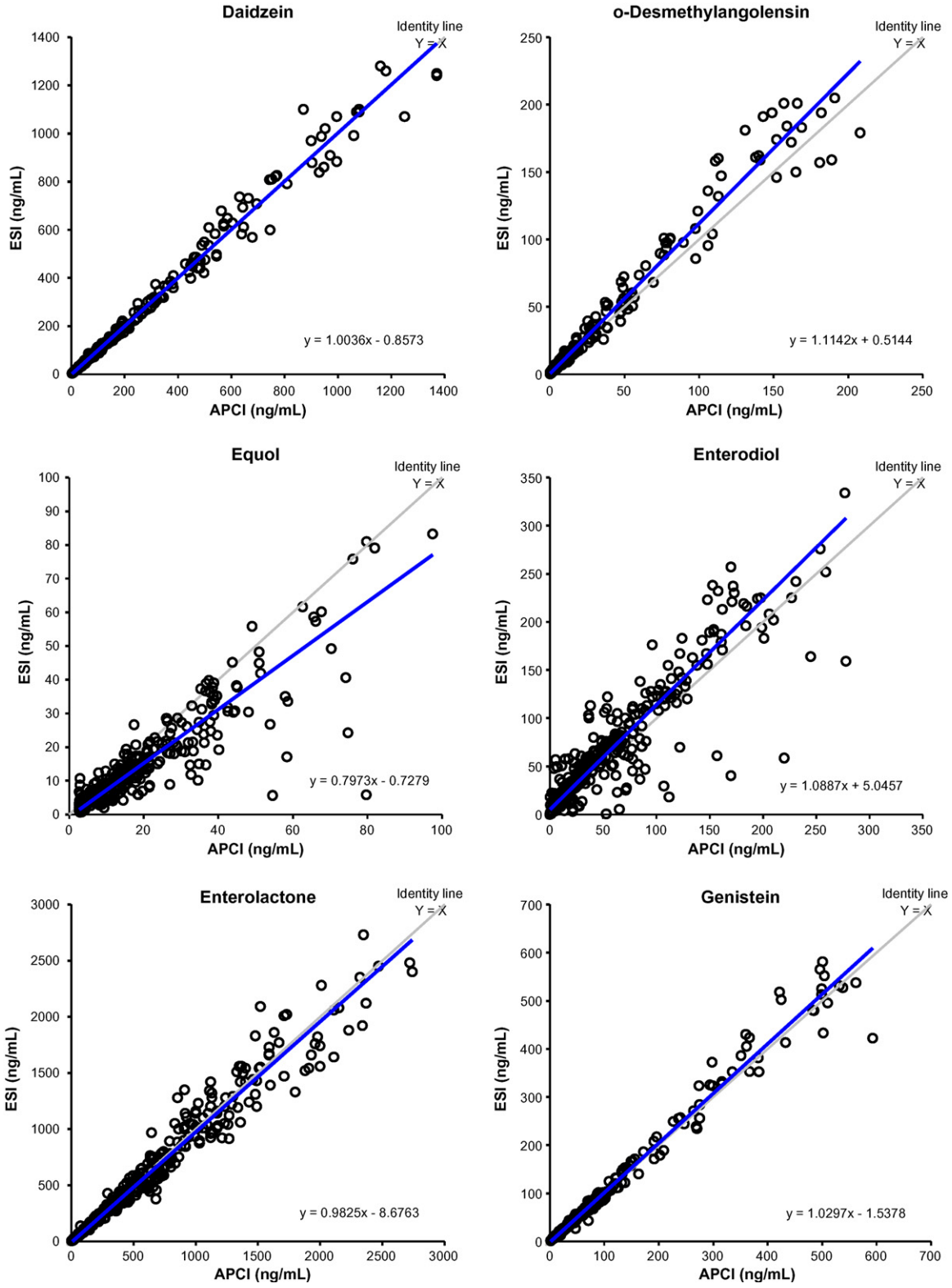

Fig. 1. Deming regression plots of ESI and APCI results for a random patient urine sample subset ($n = 378$).

subset ranged from 0.98 to 1.03, demonstrating good agreement between the two techniques (Table 4 and Fig. 1). In contrast, the regression slopes for equol (0.80), enterodiol (1.09) and *O*-desmethylangolensin (1.11) all deviated from an ideal value of unity for the same set of samples (Table 4 and Fig. 1). Bland-Altman tests for bias were also performed, using the log-transformed ESI and APCI data to compensate for concentration-dependent differences [29,30]. When this

approach was used for the Bland-Altman bias analysis (y = ESI, x = APCI), no significant bias (95% CI) was observed for ESI measurements of daidzein, and only a marginal bias of −1.3% was observed for genistein (Table 4). A negative bias of −4.2% was observed for enterolactone measurements by ESI, whereas equol, enterodiol and *O*-desmethylangolensin measurements by ESI had apparent biases that were larger (Table 4).

The apparent discordance between the ESI and APCI results for equol, enterodiol, and *O*-desmethylangolensin raises the issue of whether the observed differences are true methodological biases or measurement artifacts related to imprecision. From the data presented earlier (Table 3), it is clear that the APCI measurements are considerably less precise than the ESI measurements for equol and enterodiol. As for *O*-desmethylangolensin, even though only the low QC pool showed a relatively high CV with APCI (24%), stratification of these results revealed that approximately 35% of the subset samples (n = 131) had *O*-desmethylangolensin concentrations at or below the concentration found in the low QC pool. Method accuracy is inevitably linked to method precision [31], and we hypothesize that the relatively high imprecision observed in the APCI measurements for equol, enterodiol, and *O*-desmethylangolensin is in part influencing the observed bias when measurements are made with ESI.

In summary, we have shown that the quantitative determination of selected urinary phytoestrogens by LC–MS/MS is amenable to both ESI and APCI. Method imprecision with ESI was typically superior to that obtained by APCI, and good agreement was found between results obtained by both methods for the analysis of a subset of random patient urine samples when method imprecision and sensitivity were not an overwhelming factor. Analyte sensitivity was either equivalent or better with ESI. The most significant sensitivity improvement was observed for equol, for which detection frequency has been a challenge in the past. On the basis of these observations, and in the absence of any significant influences arising from differences in ionization source designs encountered in other LC–MS/MS systems, it is our recommendation that ESI be considered for the biomonitoring of urinary daidzein, *O*-desmethylangolensin, equol, enterodiol, enterolactone, and genistein.

Acknowledgement

The authors would like to thank Ram B. Jain (CDC) for collegial discussions regarding the statistics presented in this work.

References

[1] T. Cornwell, W. Cohick, I. Raskin, Phytochemistry 65 (2004) 995.
[2] P.H.M. Peeters, L. Keinan-Boker, Y.T. van der Schouw, D.E. Grobbee, Breast Cancer Res. Treat. 77 (2003) 171.
[3] H. Adlercreutz, Lancet Oncol. 3 (2002) 364.
[4] S.R. Davis, F.S. Dalais, E.R. Simpson, A.L. Murkies, Recent Prog. Hormone Res. 54 (1999) 185.
[5] D.-F. Ma, L.-Q. Qin, P.-Y. Wang, R. Katoh, Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials. Eur. J. Clin. Nutr. (2007), doi:10.1038/sj.ejcn.1602748, in press.
[6] V. Coxam, Brit. J. Nutr. 89 (2003) S75.
[7] I. Flight, P. Clifton, Eur. J. Clin. Nutr. 60 (2006) 1145.
[8] C.R. Sirtori, A. Arnoldi, S.K. Johnson, Ann. Med. 37 (2005) 423.
[9] F.M. Sacks, A. Lichtenstein, L. Van Horn, W. Harris, P. Kris-Etherton, M. Winston, Circulation 113 (2006) 1034.
[10] L.G. Howes, J.B. Howes, D.C. Knight, Maturitas 55 (2006) 203.
[11] H.D. Nelson, K.K. Vesco, E. Haney, R. Fu, N. Nedrow, J. Miller, C. Nicolaidis, M. Walker, L. Humphrey, JAMA 295 (2006) 2057–2071.
[12] Centers for Disease Control and Prevention, National Health and Nutrition Examination Survey, US Department of Health and Human Services, Atlanta. Website: http://www.cdc.gov/nchs/nhanes.htm.
[13] A.A. Hoikkalal, E. Schiavoni, K. Wähälä, Brit. J. Nutr. 89 (2003) S5.
[14] Q.L. Wu, M.F. Wang, J.E. Simon, J. Chromatogr. B 812 (2004) 325.
[15] C.C. Wang, J.K. Prasain, S. Barnes, J. Chromatogr. B 777 (2002) 3.
[16] J.K. Prasain, C.C. Wang, S. Barnes, Free Rad. Biol. Med. 37 (2004) 1324.
[17] Z. Kuklenyik, X.Y. Ye, J.A. Reich, L.L. Needham, A.M. Calafat, J. Chromatogr. Sci. 42 (2004) 495.
[18] L. Valentín-Blasini, B.C. Blount, H.S. Rogers, L.L. Needham, J. Expo. Anal. Environ. Epidemiol. 10 (2000) 799.
[19] D.B. Clarke, A.S. Lloyd, N.P. Botting, M.F. Oldfield, P.W. Needs, H. Wiseman, Anal. Biochem. 309 (2002) 158.
[20] A.A. Franke, L.J. Custer, L.R. Wilkens, L. Le Marchand, A.M.Y. Nomura, M.T. Goodman, L.N. Kolonel, J. Chromatogr. B 777 (2002) 45.
[21] M. Satterfield, D.M. Black, J.S. Brodbelt, J. Chromatogr. B 759 (2001) 33.
[22] S. Barnes, L. Coward, M. Kirk, J. Sfakianos, Proc. Soc. Exp. Biol. Med. 217 (1998) 254.
[23] M.C. McMaster, LC/MS: A Practical User's Guide, John Wiley & Sons, Hoboken, NJ, USA, 2005, 51–53.
[24] Centers for Disease Control and Prevention, Third National Report on Human Exposure to Environmental Chemicals, US Department of Health and Human Services, Atlanta, GA, USA, 2005, pp. 285–305.
[25] L. Valentín-Blasini, M.A. Sadowski, D. Walden, L. Caltabiano, L.L. Needham, D.B. Barr, J. Expo. Anal. Environ. Epidemiol. 15 (2005) 509.
[26] J.K. Taylor, Quality Assurance of Chemical Measurements, Lewis Publishers, Boca Raton, FL, USA, 1987, 79–82.
[27] K.D.R. Setchell, S.J. Cole, J. Nutr. 136 (2006) 2188.
[28] K.D.R. Setchell, N.M. Brown, E. Lydeking-Olsen, J. Nutr. 132 (2002) 3577.
[29] J.M. Bland, D.G. Altman, Lancet 1 (1986) 307.
[30] M.A. Pollock, S.G. Jefferson, J.W. Kane, K. Lomax, G. Mackinnon, C.B. Winnard, Ann. Clin. Biochem. 29 (1992) 556.
[31] D. Stöckl, K. Dewitte, L.M. Thienpont, Clin. Chem. 44 (1998) 2340.