

Measurement of Aldosterone in Human Plasma by Semiautomated HPLC–Tandem Mass Spectrometry

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BACKGROUND: Reliable measurement of aldosterone with less interlaboratory variation than RIA would help standardize testing for primary aldosteronism. We set out to validate a high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method for aldosterone in human plasma.

METHODS: We prepared samples (EDTA plasma, lithium heparin plasma, and serum from separator and plain clot tubes) and measured aldosterone using on-line HPLC–MS/MS with d₇-aldosterone as internal standard. We also analyzed EDTA plasma samples by immunoassay. We established a reference range for HPLC–MS/MS aldosterone by analyzing blood collected midmorning from 97 normotensive seated subjects.

RESULTS: The linear range was 69.4–5548.0 pmol/L (2.5–200 ng/dL) ($r^2 > 0.994$, $n = 14$). Inter- and intraday analytical recovery and imprecision for quality control samples of 166.4, 1109.6, and 4161.0 pmol/L (6.0, 40.0, and 150.0 ng/dL) were 92.2%–102.0% and <6.3%, respectively ($n = 5$). The lower limit of quantification was 69.4 pmol/L (2.5 ng/dL), with inter- and intraday analytical recovery and imprecision of 91.4%–94.5% and <9.5% ($n = 5$). No interferences were observed in plasma from Addison's disease patients ($n = 5$). Comparison of collection tubes, using EDTA as the reference, revealed similar aldosterone results. Comparison of HPLC–MS/MS with immunoassay gave an acceptable mean bias (0.83%) but wide range (–44.8% to 39.7%) of differences. HPLC–MS/MS aldosterone concentrations in normotensive subjects ranged from <69.4 to 635.2 pmol/L (<2.5 to 22.9 ng/dL).

CONCLUSIONS: This first reported aldosterone method using online HPLC–MS/MS is precise across the clinically relevant range, not influenced by collection tube

type, and offers semiautomated sample preparation and high throughput.

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Primary aldosteronism (PAL)⁴ is a specifically treatable and potentially curable form of hypertension. The accurate measurement of circulating aldosterone concentrations is essential for the correct diagnosis of endocrine disorders such as PAL. The importance of aldosterone measurement has greatly increased with the recent recognition that PAL is a more frequent cause of hypertension than previously reported, accounting for at least 5% of hypertensive patients (1). The current mainstay for measuring aldosterone is by antibody-based methods. These methods can be direct or require initial extraction of plasma or serum using liquid-liquid extraction, solid-phase extraction, or chromatographic methods (2–6). Overall, immunoassays have problems with varying selectivity and poor interlaboratory reproducibility, requiring each laboratory to establish its own reference range for the diagnosis of PAL (7). For example, Schirpenbach et al. (8) recently reported a 2- to 3-fold difference in aldosterone concentrations measured by 4 currently used methods. Such discrepancies in aldosterone measurement between laboratories suggest a need for improved aldosterone measurement for both screening and confirmation of PAL (9, 10).

GC–MS has been used to measure aldosterone in biological fluids (11–13). Although this technique is considered a reference method that provides both accurate results and excellent specificity, these methods in general require extensive sample preparation including chemical derivatization. The lack of automation and complexity of sample preparation has relegated GC–MS to specialty clinical laboratories and is not used in routine clinical services.

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⁴ Nonstandard abbreviations: PAL, primary aldosteronism; HPLC–MS/MS, high-performance liquid chromatography–tandem mass spectrometry; FST, fludrocortisone suppression testing; CAH, congenital adrenal hyperplasia.

High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) is a powerful analytical technique that is becoming increasingly used in the clinical setting (14). HPLC-MS/MS offers the opportunity to provide more reliable measurement of aldosterone than immunoassays (15). Recently it has been shown that online solid-phase extraction coupled to HPLC-MS/MS was suited to plasma free metanephrine analysis and the diagnosis of pheochromocytoma (16). Using this general approach, we report the development and validation of an HPLC-MS/MS method of measuring aldosterone that uses online semiautomated sample preparation.

Materials and Methods

REAGENTS

We purchased aldosterone and d₇-aldosterone (internal standard) from Sigma-Aldrich and IsoSciences, respectively. The internal standard was found to be stable under conditions used (i.e., no deuterium exchange) and contained no observable unlabeled aldosterone. We prepared stock solutions of aldosterone and internal standard in methanol (Merck) and the calibrators and quality controls in aldosterone-free EDTA plasma, obtained from patients with Addison's disease (17). The final calibrator concentrations were 69.4, 138.7, 277.4, 693.5, 1387.0, 2774.0, and 5548.0 pmol/L (2.5, 5.0, 10, 25, 50, 100, and 200 ng/dL). The concentrations of the quality control samples were 69.4, 166.4, 1109.6, 4161.0, and 5548.0 pmol/L (2.5, 6.0, 40, 150, and 200 ng/dL). We used a quality control sample with a concentration outside the analytical range (13 870.0 pmol/L; 500 ng/dL) for 1:5 and 1:10 dilution studies and prepared a precipitation reagent with 0.3 mol/L zinc sulfate:methanol (1:5, vol:vol) containing the internal standard (8183.3 pmol/L; 295 ng/dL).

SAMPLE PREPARATION AND ON-LINE EXTRACTION

Plasma calibrators, quality controls and patient samples (200 μ L) were added to 1.5-mL polypropylene centrifuge tubes, pretreated with 200 μ L precipitation reagent, vortex-mixed (10 s), and centrifuged (20 200g, 5 min). The supernatant was transferred to a Symbiosis HPLC-online solid-phase extraction system (Spark Holland). A Hysphere C18 HD extraction cartridge (10 by 2 mm, 7 μ m) was preconditioned with 1 mL acetonitrile followed by 1 mL water and loaded with 250 μ L supernatant using 1 mL water. The cartridge was sequentially washed with 1 mL of 10% acetonitrile in 0.1% ammonium hydroxide, 1 mL of 10% acetonitrile in 0.1% formic acid, and 1 mL of 10% acetonitrile in water. The analytes were eluted from the cartridge under the initial chromatographic conditions (shown below) for 45 s before the cartridge was taken offline. The

processing of samples was performed in parallel (i.e., while one sample is being extracted the previous is eluted). A more detailed description of the operation of the Symbiosis system has been published by de Jong et al. (16).

HPLC-MS/MS

We performed chromatography using a Waters Sunfire C18 analytical column (50 by 3.0 mm, 3 μ m) at ambient temperature, under isocratic conditions (35% acetonitrile/water) with a flow rate of 0.3 mL/min. For the first minute of the analysis, the eluate was diverted to waste. At 2.5 min after injection, a column wash with 100% acetonitrile at 1 mL/min was performed for 2 min. The column was then reequilibrated at starting conditions for 2.5 min, giving a total chromatographic analysis time of 7 min.

Under negative electrospray ionization conditions (–2500 V), the analytes were predominantly in the deprotonated form, $[M-H]^-$. Mass spectrometric detection was undertaken by selected reaction monitoring (aldosterone m/z 358.9 \rightarrow 330.9; internal standard m/z 365.9 \rightarrow 337.9) on a Quattro Premier tandem quadrupole mass spectrometer (Waters Corp.). The dwell times for aldosterone and the internal standard were 500 and 200 ms, respectively. The compound-specific operating parameters of cone voltage and collision energy were –30 V and –17 eV, respectively. The HPLC-MS/MS including the Symbiosis was controlled and data processed using MassLynx version 4.1 (Waters Corp.).

METHOD VALIDATION

Although aldosterone is an endogenous compound, patients with Addison's disease do not have circulating aldosterone. Six plasma samples from patients with previously diagnosed Addison's disease were analyzed to test the specificity of the method (i.e., to investigate potential interference from other endogenous compounds). We tested linearity over the range of 69.4–5548.0 pmol/L (2.5–200 ng/dL) on 14 days. Inter- and intraassay recovery and imprecision were determined by measuring quality control samples. Interassay parameters were based on a single analysis on each of 5 days. Intraassay parameters were determined from 5 measurements performed on 1 day. We assessed the lower limit of quantification by preparing a quality control at the concentration of the lowest calibrator 69.4 pmol/L (2.5 ng/dL) and analyzing by the protocol described above for quality controls. The lower limit of quantification acceptance criteria for recovery and imprecision were 80% to 120% and <20%, respectively. The suitability of 1:5 and 1:10 dilution studies, in terms of inter- and intraassay recovery and imprecision, were

performed by the protocol for quality controls described above.

Inter- and intraassay recovery and imprecision were further assessed by measuring 5 external quality controls obtained from the German Society for Clinical Chemistry and Laboratory Medicine (www.dgkl-rfb.de). Interday parameters were based on a single analysis on each of 5 days, and intraday parameters were determined from 5 measurements performed on 1 day. Recovery (accuracy) was determined by comparing results obtained for this current method against the reference method, GC-MS, used by the proficiency scheme (11, 12).

We determined the influence of matrix effects on absolute signal by comparing the internal standard peak area obtained for water-based standards ($n = 14$) with that obtained for patient samples ($n = 12$). This comparison was performed for all 4 types of collection tubes, and matrix effects were expressed in terms of process efficiency. Process efficiency represents the combination of matrix effects and recovery of the analyte from the sample by the extraction process (18, 19).

PATIENT SAMPLES

We validated the current method using EDTA plasma as the sample matrix. To assess if other collection tubes would provide equivalent results, we obtained a series of samples from 14 patients undergoing fludrocortisone suppression testing (FST) (20) and analyzed them for aldosterone. Of these 14 patients, 12 with hypertension who had screened positive for PAL by aldosterone/renin ratio testing (20) underwent FST to definitively confirm or exclude the diagnosis; FST confirmed PAL in each case. The remaining 2 patients, who had undergone unilateral adrenalectomy as treatment for previously confirmed unilateral PAL, underwent FST to determine whether the condition had been cured from a biochemical perspective; cure was confirmed in both individuals. Inclusion of these patient samples allowed for a wide range of peripheral blood aldosterone concentrations, highest in those with confirmed, florid, untreated PAL and lowest in those for whom PAL had been surgically cured. These studies were undertaken with the Center's Institutional Review Board approval.

During FST, samples were collected at 0700 h (after overnight recumbency) and again at 1000 h (after sitting, standing or walking) basally (prefludrocortisone treatment) and after 3 and 4 days of administration of fludrocortisone acetate (0.1 mg/6 h) and slow-release sodium chloride administration (slow sodium 30 mmol 3 times a day with meals), with sufficient potassium supplementation (given per 6 h as Slow K) to maintain plasma potassium concentrations as close as possible to 4.0 mmol/L. Under these conditions, a day-4 upright (1000 h) serum aldosterone of >166.4

pmol/L (>6.0 ng/dL) was considered diagnostic of PAL. At each time point, samples were obtained from 4 different collection tubes (EDTA plasma, lithium heparin plasma, serum from separator tubes, and plain clot serum). Eleven of the 14 patients had all samples at all time points collected for HPLC-MS/MS aldosterone measurement. In total, we analyzed 72 time points in this study with EDTA plasma calibrators used to determine all results. Using the results obtained for EDTA plasma as the reference, we compared aldosterone concentrations in different tube types by Deming regression and Bland-Altman plots.

COMPARISON OF ALDOSTERONE RESULTS OBTAINED BY THE HPLC-MS/MS METHOD AND AN IMMUNOASSAY

The EDTA plasma patient samples described in the previous section ($n = 72$) were analyzed for aldosterone by an immunoassay as part of routine clinical care (DPC Coat-a-Count™ aldosterone kit; Diagnostic Products Corp.). Analyses were performed in numerous batches over a period of several months. We compared aldosterone results obtained by the two methods were compared by Deming regression and Bland-Altman plots.

As a further assessment of assay selectivity, we also analyzed plasma from 2 patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH) by HPLC-MS/MS and DPC immunoassay.

MEASUREMENT OF PLASMA ALDOSTERONE IN AN UPRIGHT NORMOTENSIVE POPULATION

We collected blood samples midmorning from 97 normotensive, seated patients. This cohort consisted of 54 males and 43 females with a mean (SD) age of 53.6 (12.5) (range 16–75) years and 58.2 (11.4) (range 32–81) years, respectively. This study was approved by the Princess Alexandra Hospital Ethics Committee and conducted in accordance with the guidelines of the Declaration of Helsinki. These samples were analyzed for aldosterone using HPLC-MS/MS.

Results

We investigated both negative and positive electrospray ionization modes in the development of this method. Aldosterone was ionized to 2 major forms in positive mode, the protonated and sodiated species. The protonated species was found to fragment through a series of nonspecific water losses (data not shown). Using negative ionization mode, aldosterone formed 1 predominant precursor ion, $[M-H]^-$. Fragmentation of this precursor ion gave a predominant ion at m/z 330.9, which corresponded to a loss of CO (-28 amu). Thus negative electrospray ionization was considered the better option of the 2 modes. No substantial cross-

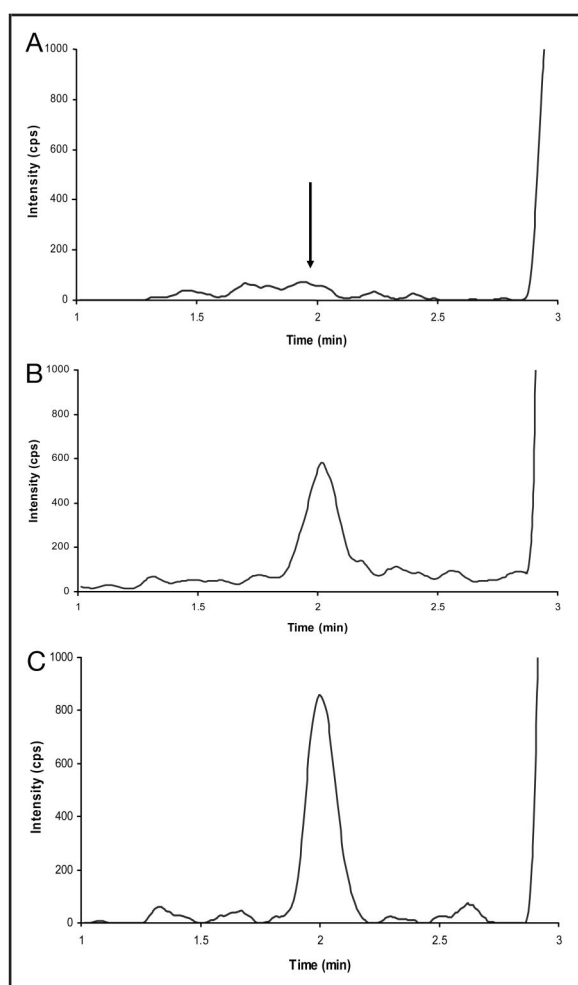


Fig. 1. Representative chromatograms for the aldosterone mass transition (m/z 358.9→330.9) of plasma extracts from a patient with Addison's disease before (A) and after (B) addition of aldosterone (69.4 pmol/L; 2.5 ng/dL).

(C), Sample collected midmorning from a seated normotensive subject (147.0 pmol/L; 5.3 ng/dL). The arrow represents the retention time of aldosterone.

talk was observed between the mass transitions used for aldosterone and the internal standard.

Under the described chromatographic conditions, the retention time of aldosterone and the internal standard was 2.0 min. To illustrate the selectivity of the method, no interferences were observed when analyzing plasma from patients with Addison's disease ($n = 5$). Fig. 1A shows an example chromatogram of the aldosterone mass transition from one of these patients with no interference observed at the retention time of aldosterone. Similar results were observed for the internal standard (data not shown). Plasma obtained from these patients was subsequently used to prepare calibrators and quality controls.

The HPLC-MS/MS method was found to be linear over the analytical range of 69.4–5548.0 pmol/L (2.5–200 ng/dL) ($r^2 > 0.994$, $n = 14$). The mean (SD) slope and intercept were 0.000671 (0.0000241) and 0.000436 (0.00213), respectively. The 95% confidence limits for each calibrator are shown in Table 1. Excellent recovery (97.4% to 102%) and imprecision ($<4.7\%$) was obtained across the concentration range for these calibrators. A summary of these results is shown in Table 1. Table 2 shows that the inter- and intraassay recovery and imprecision for quality control samples (166.4, 1109.6, 4161.0 pmol/L; 6.0, 40, 150 ng/dL) were 92.2% to 102% and $<6.3\%$, respectively ($n = 5$). The lower limit of quantification was 69.4 pmol/L (2.5 ng/dL) (Fig. 1B). The inter- and intraassay recovery and imprecision of 91.4%–94.5% and $<9.5\%$ ($n = 5$), respectively, were well within the defined acceptance criteria. Further, the method was validated so that samples with measured concentrations up to 13 870 pmol/L (500 ng/dL) could be diluted 1:5 or 1:10 (Table 2). The inter- and intraassay recovery and imprecision for the dilution studies were within acceptable limits as set down by current regulatory opinion (21).

The aldosterone results obtained for the 5 external quality controls measured by this current method compared favorably against the values obtained by the reference method used in this proficiency scheme (Table 3). The range of inter- and intraassay recovery was

Table 1. Interassay recovery and imprecision of aldosterone calibration samples measured by HPLC-MS/MS ($n = 14$).

Parameter	Nominal aldosterone concentration, pmol/L (ng/dL)						
	69.4 (2.5)	138.7 (5.0)	277.4 (10)	693.5 (25)	1387.0 (50)	2774.0 (100)	5548.0 (200)
Analytical recovery, %	101	97.4	101	102	98.8	99.0	100
95% Confidence limits, pmol/L (ng/dL)	69.07–70.74 (2.49–2.55)	131.77–138.70 (4.75–5.00)	274.63–288.50 (9.90–10.4)	699.1–718.5 (25.2–25.9)	1350.9–1389.8 (48.7–50.1)	2715.8–2774.0 (97.9–100)	5492.5–5631.2 (198–203)
Imprecision, %	1.8	4.6	4.3	2.3	2.6	2.0	2.5

Table 2. Inter- and intraassay recovery and imprecision of aldosterone quality control samples measured by HPLC-MS/MS (n = 5).

Parameter	Nominal aldosterone concentration, pmol/L (ng/dL)						
	69.4 (2.5)	138.7 (6.0)	1109.6 (40)	4161.0 (150)	5548.0 (200)	13 870 (500) ^a	13 870 (500) ^b
Interday							
Analytical recovery, %	94.5	96.4	96.4	97.7	99.5	102	102
95% Confidence limits, pmol/L (ng/dL)	57.98–73.23 (2.09–2.64)	150.63–170.32 (5.43–6.14)	1034.70–1104.05 (37.3–39.8)	3966.8–4161.0 (143–150)	5270.6–5742.2 (190–207)	13 592.6–14 563.5 (490–525)	13 426.2–14 757.7 (484–532)
Imprecision, %	9.4	5.0	2.7	2.1	3.4	2.8	3.8
Intraday							
Analytical recovery, %	91.4	94.0	92.2	102	90.1	105	100
95% Confidence limits, pmol/L (ng/dL)	57.42–69.35 (2.07–2.50)	144.25–168.66 (5.20–6.08)	1004.19–1040.25 (36.2–37.5)	4105.5–4355.2 (148–157)	4965.5–5048.7 (179–182)	14 313.8–14 729.9 (516–531)	13 564.9–14 258.4 (489–514)
Imprecision, %	7.6	6.3	1.5	2.2	0.74	1.1	2.0

^a 1:5 dilution.^b 1:10 dilution.

99.0%–102% and 102%–105%, respectively. The imprecision obtained for these external quality controls of <12% for interassay and <5.8% for intraassay compared favorably with the data obtained for the in-house-prepared quality controls (shown above). The agreement between HPLC-MS/MS and the reference method suggests there is little or no interference from other analytes present in these external quality control samples and further illustrates the selectivity of the current method. These analytes, at various physiological concentrations, include cortisol, estradiol-17 β , unconjugated estriol, progesterone, testosterone, 17 OH-progesterone, and thyroxine.

The evaluation of 3 collection tubes (lithium heparin, serum plain clot, and serum separator tube), using EDTA collection tubes as the reference, revealed excellent agreement for lithium heparin tubes but slightly poorer agreement for serum plain clot and serum separator tubes (Supplemental Table 1, which accompanies the online version of the article at <http://www.clinchem.org/content/vol55/issue6>). As an example, comparison of aldosterone results obtained from lithium heparin vs EDTA collection tubes (online Supplemental Fig. 1) gave the following Deming regression equation: $y = 1.01x - 0.133$ ($n = 72$). The 95% CIs for the slope and intercept were 1.00 to 1.02 and -0.393 to 0.127 , respectively. The Bland–Altman plot revealed a mean bias of 1.886 pmol/L (0.068 ng/dL), with lower and upper 95% limits of agreement of -45.22 and 49.10 pmol/L (-1.63 and 1.77 ng/dL), respectively.

Process efficiency was observed to differ between the 4 collection tubes evaluated. Process efficiency was found to be similar for EDTA and lithium heparin samples (87.1% and 87.4%, respectively) but was much lower in the plain clot (51.3%) and serum separator (42.2%) samples.

For a series of 69 samples from patients undergoing FST, comparison of the aldosterone results obtained by the HPLC-MS/MS method with those from the DPC immunoassay is shown in Fig. 2. Three samples, all from 1 patient, with aldosterone concentrations >1664.0 pmol/L (>60 ng/dL) by HPLC-MS/MS were omitted from this analysis. These data showed approximately 45% underestimation by the DPC immunoassay. Further studies with a larger number of samples from a variety of patients in this higher concentration range are required to understand these results. For the remaining 69 samples, the Deming regression analysis of these data gave the following equation: $y = 1.03x - 0.258$. The 95% CIs for the slope and intercept were 0.979 to 1.08 and -1.09 to 0.572 , respectively. The Bland–Altman plot revealed a mean bias of 3.329 pmol/L (0.120 ng/dL), with lower and upper 95% limits of agreement of -110.405 and

Table 3. Inter- and intraassay recovery and imprecision of aldosterone external quality controls measured by HPLC-MS/MS (n = 5).

Parameter	Aldosterone reference method value, pmol/L (ng/dL)				
	226.91 (8.18)	388.4 (14.0)	671.3 (24.2)	743.4 (26.8)	1439.7 (51.9)
Interday					
Analytical recovery, % ^a	101	100	102	101	99.0
95% Confidence limits, pmol/L (ng/dL)	196.95–261.31 (7.10–9.42)	357.8–421.6 (12.9–15.2)	663.0–704.6 (23.9–25.4)	726.8–779.5 (26.2–28.1)	1398.1–1450.8 (50.4–52.3)
Imprecision, %	11.3	6.5	2.5	2.9	1.5
Intraday					
Analytical recovery, %	105	103	104	103	102
95% Confidence limits, pmol/L (ng/dL)	221.64–254.93 (7.99–9.19)	368.94–427.20 (13.3–15.4)	676.9–726.8 (24.4–26.2)	737.9–790.6 (26.6–28.5)	1412.0–1520.2 (50.9–54.8)
Imprecision, %	5.6	5.8	2.9	2.7	2.9

^a Analytical recovery was calculated against the aldosterone reference method values supplied by the external proficiency testing scheme.

117.063 pmol/L (–3.98 ng/dL and 4.22 ng/dL), respectively. These data were further analyzed by comparing the percentage difference between the two methods against the mean of these methods. These data showed a mean difference of 0.83% (range –44.8% to 39.7%).

Aldosterone concentrations in plasma samples obtained from 2 patients with CAH were measured by both HPLC-MS/MS and DPC immunoassay. Results of these analyses were 255.2 pmol/L (9.2 ng/dL) (HPLC-MS/MS) and 1925.2 pmol/L (69.4 ng/dL) (DPC immunoassay) for case 1 and 188.6 pmol/L (6.8 ng/dL) (HPLC-MS/MS) and 840.5 pmol/L (30.3 ng/dL) (DPC immunoassay) for case 2.

The results of analyzing a series of samples obtained from upright (seated) normotensive control subjects are shown in online Supplemental Fig. 2. HPLC-MS/MS aldosterone concentrations ranged from <69.4 to 635.2 pmol/L, mean 205.3 (130.4) pmol/L [<2.5–22.9 ng/dL, mean 7.4 (4.7) ng/dL]. A chromatogram for a patient from this group with a measured concentration of 147.0 pmol/L (5.3 ng/dL) is shown in Fig. 1C.

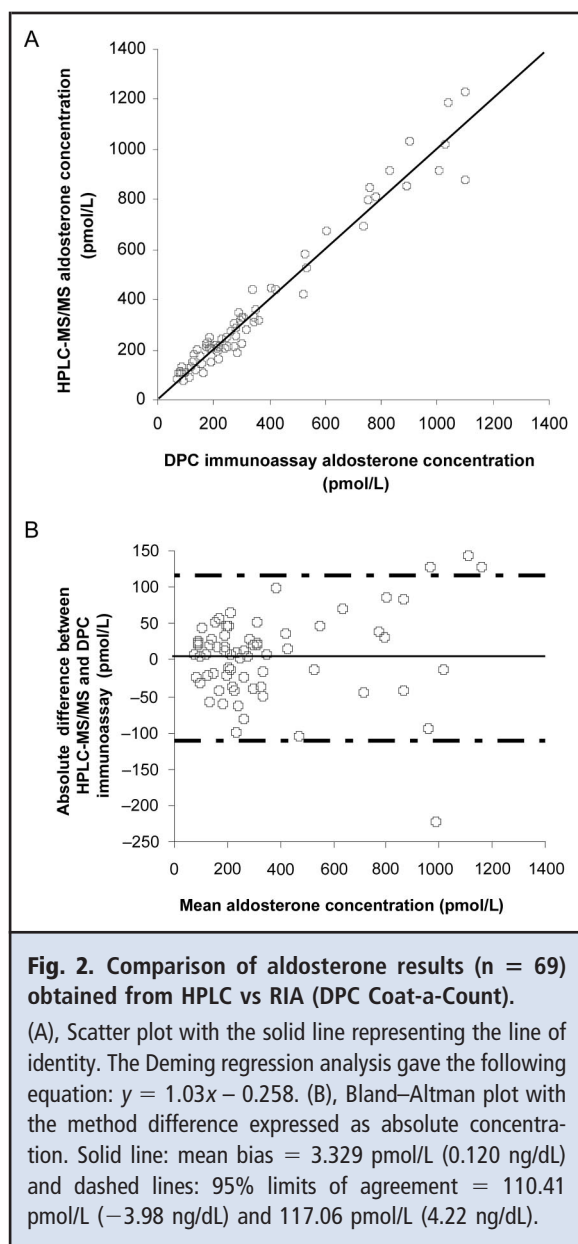
Discussion

Currently the most important clinical application of aldosterone measurement is in the detection and subsequent diagnostic workup of PAL. Screening includes measurement of the plasma aldosterone-to-renin ratio. Although it is recognized that the ratio is particularly sensitive to changes in renin (22), it is clearly nevertheless dependent (albeit to a lesser degree) on aldosterone, and hence accurate measurement of aldo-

sterone is a critical factor in reliable screening. Accurate measurement of aldosterone is essential for reliable interpretation of results obtained during suppression testing (used to definitively confirm or exclude PAL) and adrenal venous sampling (used to differentiate unilateral, surgically correctable forms from bilateral forms that are usually treated with specific medications that antagonize aldosterone action) (20).

The HPLC-MS/MS method described here was found to be accurate and precise across the clinically important range (69.4–5548.0 pmol/L; 2.5–200 ng/dL). This range includes aldosterone concentrations that would be expected to occur among normotensive controls and patients with aldosterone excess states. Whereas the method was validated using EDTA plasma as the sample matrix, we found that results were not affected significantly by collection tube type. Interestingly, process efficiency was much lower in the serum samples than in the plasma samples. The lower response obtained for serum samples may be due to poorer extraction or greater ion suppression than that of plasma samples. Presumably, the use of the stable isotope-labeled aldosterone as an internal standard adequately compensated for any variability in extraction efficiency or signal (18, 23).

It is likely that the currently reported HPLC-MS/MS method provides a higher degree of specificity than antibody-based methods. No interferences were observed when analyzing plasma from patients with Addison's disease; conversely, in 2 patients with CAH, aldosterone concentrations obtained using the DPC immunoassay were frankly increased and much higher than those measured by HPLC-MS/MS, which were in



the low-normal range. This might be explained by cross-reactivity with 21-deoxyaldosterone and other steroids, which are present in excess in this condition (17). CAH in these patients was caused by 21-hydroxylase deficiency and is characterized by high circulating concentrations of steroids and precursors such as 21-deoxyaldosterone (24), pregnenolone, progesterone, 17 α -hydroxyprogesterone, and androgens that may cross-react with the antibody used in the immunoassay and thus interfere with the method. The results obtained for the external quality controls further confirm the analytical recovery, reproducibility, and specificity of the HPLC-MS/MS method.

The wide spread of differences (ranging from −44.8% to 39.7%) between HPLC-MS/MS and the DPC immunoassay observed among 69 patient samples (3 outliers excluded) may possibly be attributable to the imprecision of the immunoassay [approximately 15% at 166.4 pmol/L (6.0 ng/dL)] and/or nonspecificity of the antibody. Some caution should be taken when interpreting these results, as these 72 samples were from a limited cohort of 14 patients. Three samples, all from one patient, with aldosterone concentrations >1664.0 pmol/L (>60 ng/dL) (by HPLC-MS/MS) would appear to be outliers with >50% higher results obtained by HPLC-MS/MS compared to the mean of both methods. Further long-term studies in larger cohorts of patients are required to obtain a fuller understanding of the differences between these methods in terms of analytical performance and clinical decision making.

There is a substantial capital outlay for an HPLC-MS/MS system coupled with online sample preparation. This compares unfavorably with immunoassays that can be established with little initial capital outlay. Thus the most cost-effective approach will be the use of HPLC-MS/MS in tertiary referral centers or large private pathology laboratories where sample numbers will be large. Although HPLC-MS/MS aldosterone methods have been reported by us (15) and other groups (25, 26), the method described in detail here offers the benefits of semiautomated sample preparation and relatively high throughput of approximately 8 min/sample, making it highly clinically applicable. The nature of this approach would be expected to facilitate greater reproducibility across laboratories than antibody-based methods, but this needs to be confirmed with multicenter studies. Further studies are also required to establish reference intervals for both plasma aldosterone and aldosterone/renin ratios among normotensive and hypertensive populations.

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