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Evaluación Técnica/Equipos

Adaptation and analytical evaluation of urinary ammonium measurement using an automated method for plasma ammonium quantification

Automatización y evaluación del rendimiento de un método adaptado para la determinación del amonio urinario

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ABSTRACT

Urinary ammonium quantification provides valuable information to evaluate the renal response to metabolic acidosis. It is useful in differential diagnosis between pathological states of metabolic or renal origin improving the clinical management of these patients. In this study, we evaluate the adaptation of a direct enzymatic assay to measure ammonium in urine samples using the Beckman Coulter DXC700AU analyzer. Intra-day and inter-day imprecision were < 1.0 % and < 7.5 % respectively. Trueness was < 3.5 % and < 8 %, mean SE = 5.64 %. As so, these tests were acceptable. The instrument responded adequately to the standard addition of the analyte. Dilution effect was negligible, and recoveries obtained ranged from 112-150 %. The method showed to be linear between 10-600 µmol/L. The instrument responded adequately to the addition of the standard addition analyte. Dilution effect from diluted urine samples was negligible (< 15 %) for overall concentrations tested. The adaptation and validation of an automated method for plasma ammonium quantification to measure ammonium in urine samples in the laboratory provides a simple, fast, precise and accurate method, avoids the handling of toxic substances, and reduces occupational risks.

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Palabras clave:
Amonio urinario. Ensayo enzimático. Acidosis metabólica.

RESUMEN

La cuantificación de amonio en orina proporciona una valiosa información para evaluar la respuesta renal a la acidosis metabólica. Es útil en el diagnóstico diferencial entre estados patológicos de origen metabólico o renal, ya que mejora el manejo clínico de estos pacientes. En este estudio, evaluamos la adaptación de un ensayo enzimático directo para medir el amonio en muestras de orina utilizando el analizador Beckman Coulter DXC700AU. Las imprecisiones intra- e interdiarias fueron de < 1,0 % y < 7,5 %, respectivamente. La veracidad fue < 3,5 % y < 8 %, con una media de SE = 5,64 %. Por lo tanto, estas pruebas fueron aceptables. El instrumento respondió adecuadamente a la adición estándar del analito. El efecto de dilución fue insignificante y las recuperaciones obtenidas oscilaron entre 112 y 150 %. El método demostró ser lineal entre 10-600 µmol/L. El instrumento respondió adecuadamente a la adición del analito de adición estándar. El efecto de dilución de las muestras de orina diluidas fue insignificante (< 15 %) para todas las concentraciones probadas. La adaptación y la validación de un método automatizado de cuantificación de amonio en plasma para medir el amonio en muestras de orina en el laboratorio proporcionan un método sencillo, rápido, preciso y exacto, evitan la manipulación de sustancias tóxicas y reducen los riesgos laborales.

INTRODUCTION

Ammonium production and excretion are key steps in the renal regulation of acid-base balance. Under basal conditions, urinary ammonium excretion accounts for 50-70 % of net acid excretion, but when endogenous acid production is enhanced, it increases up to 80-90 % (1). Furthermore, decreased urinary ammonium excretion is associated with an excessive accumulation of acid, resulting in progressive metabolic acidosis, which is commonly developed in advanced stages of chronic kidney disease, associated to other undesirable outcomes (2). For this reason, and as it allows to distinguish between different pathological states of metabolic or renal origin, urinary ammonium quantification provides valuable information in the evaluation of renal response to metabolic acidosis, helping in the management of patients suffering from this pathology (2-4).

Over the years, numerous methodologies have been used for the determination of ammonium in urine samples, including formol tritation, Conway microdiffusion technique, Berthelot reaction, Nessler method, fluorometry, ammonia ion-selective electrodes, high-performance liquid chromatography and coulometric flow titration (5). Although these methods directly and accurately measure the ammonium concentration in urine samples, most of them are manual, time-consuming and/or require handling hazardous and toxic substances or expensive instrumentation, so they are not available in most clinical laboratories (6). Additionally, some indirect estimations of urinary ammonium excretion have been used based on the urinary anion gap or the osmolal gap calculation (7), but these are not considered accurate enough to be a reliable alternative to the direct measurement of urinary ammonium (6).

To overcome these limitations, several authors have implemented the urinary ammonium determination in biochemistry autoanalyzer's, by using methods originally developed to quantify plasma ammonium concentrations, with satisfactory results (6,8-10). In this study, we evaluated the urinary ammonium measurement in single urine samples by adapting an automated method for plasma ammonium quantification.

MATERIALS AND METHODS

In this scientific study, we describe a method validation, for whose calculations and analysis of the data we used Microsoft Excel.

The Infinity™ Ammonia assay is a direct enzymatic procedure based on glutamate dehydrogenase reaction for the quantitative determination of ammonium concentrations in human plasma. We used this automated method to quantify urinary ammonium in the DXC700AU analyzer (Beckman Coulter, USA).

Precision and trueness

To assess the analytical specifications (imprecision, trueness and total error), the following materials were used: a pool of urine samples collected in tubes without additives, Liquichek Urine Chemistry Control (Bio-Rad Laboratories) and two standard solutions (10 and 40 mmol/L, NH_4I in Type 1 Ultrapure Water).

Since the ammonium concentration is approximately 1000 times higher in urine than in plasma, urine samples, quality control and ammonium standard solutions were diluted prior analysis with Milli-Q water (1:100).

Reproducibility of the assay was evaluated by measuring the urine pool, Liquichek quality control and both ammonium standard solutions. Repeatability was calculated using 20 replicates of urine pool under the same analytical conditions. Reproducibility was assessed by comparing the mean concentrations of the four test samples obtained on 20 different days. Analytical trueness was also evaluated using the ammonium standard solutions. Trueness was calculated as the difference between the measured ammonium concentrations and the expected concentrations. Acceptance criteria were established according to the "desirable" quality specifications for biological variation (coefficient of variation [CV] = 12.4 %, systematic error [SE] = 9.2 %, total error [TE] = 29.6 %) (11).

Linearity

Linearity was verified by analysing serial dilutions of 1 mmol/L (1000 µmol/L) NH4I in MilliQ Water, with concentrations ranging from 10 to 600 µmol/L. Two replicates were measured for each dilution level. The results were plotted against the expected values. Linearity was assumed when the correlation coefficient was > 0.95.

Recovery

The standard additions method corrects for the matrix effect. To calculate the recovery, we compared the results from both, the usual procedure and the standard additions method.

Two pools of urine were diluted with MilliQ water (1:20), and a 50-uL volume of a standard solution of NH4I (50 µmol/L) was added. The volume in each aliquot was completed with water up to 250 µL, maintaining the same sample dilution (1:100). A simple linear regression was performed to verify the linearity of the assay. For the evaluation of recovery, we analysed the slope of the analytical curve obtained. Dilution effect was considered to be negligible if the obtained results deviated < 10 % from the expected ones.

RESULTS

Precision and trueness

Intra-day imprecision was < 1.0 % at medium ammonium concentrations evaluated in the urine pool (31.8 mmol/L). Inter-day imprecision was < 7.5 % at all evaluated concentrations (ranging from 6.3-44.2 mmol/L), mean CV = 4.97 %. Trueness was < 3.5 % at low (13.4 mmol/L) and < 8 % at medium (44.2 mmol/L) ammonium concentrations, mean SE = 5.64 % (Table I). Precision and trueness tests were acceptable as the allowable CV and SE were both 12.4 % and 9.2 %. Moreover, the modified method keeps the specific performance characteristics as described for plasma samples by Beckman Coulter (CV < 5 %).

Linearity

The regression equation of modified assay was:

Measured ammonium concentration = 3.31 + 1.112·expected ammonium concentration (1).

Measured results were plotted against expected values (Fig. 1). The method showed to be linear between 10-600 µmol/L, with an excellent coefficient of determination (R^2 = 0.9998).

Recovery

Measured ammonium concentrations plotted against increasing volumes of stock solution are defined by the following equation:

Measured concentration = 225.2 + 0.298·expected concentration (R^2 = 0.96) (2).

Table I.
Precision and accuracy results

	Urine pool		Liquichek Control	NH_4I 10 M	NH_4I 40 M
	Repeatability	Reproducibility	Reproducibility	Reproducibility	Reproducibility
Mean (mmol/L)	31.8	31.7	6.3	13.4	44.2
SD (mmol/L)	0.18	0.88	0.35	0.99	1.84
CV (%)	0.58	2.77	5.54	7.40	4.16
SE (%)				3.44	7.84
TE (%)				15.65	14.70

M: mmol/L; SD: standard deviation; CV: coefficient of variation; SE: standard error; TE: total error.

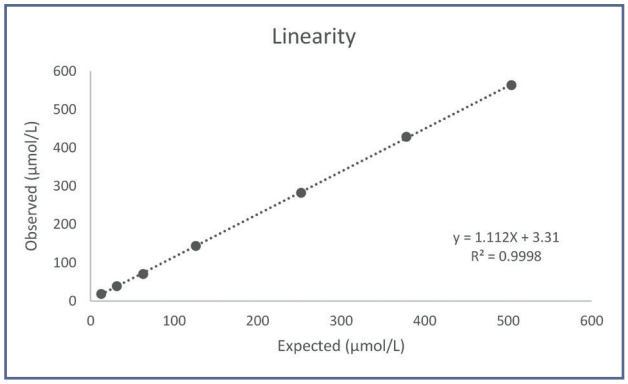

Figure 1

The simple linear regression analysis describes a slope = 0.298 and a y-intercept = 225.2. The instrument responds adequately to the standard addition of the analyte. Dilution effect from diluted urine samples was negligible (< 15 %) for overall concentrations tested by the modified assay (Fig. 2), and recoveries obtained ranged from 112-150 %, (mean ± standard deviation: 119 ± 9 %).

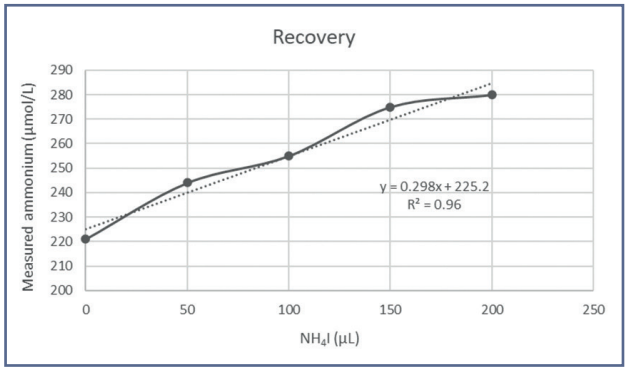

Figure 2

DISCUSSION AND CONCLUSION

In this study, the adaptation of an automated method for plasma ammonium quantification to measure ammonium in urine samples using the Beckman Coulter DXC700AU analyser is reported for the first time. This provides a simple, fast, precise and accurate method that has been validated for assessing the urinary ammonium concentration in routine laboratory work. The obtained results meet the quality specifications established by our laboratory and are consistent with method validation results on other analysers (9,13). In addition, the reported linearity as a function of dilution of the plasma technique is 10-600 µmol/L, which is consistent with our results.

In the process of adapting or modifying analytical methods, their validation is a fundamental step to ensure the reliability of the results. However, results obtained by different methods often differ, especially when laboratories using manual techniques switch to automated ones. For this reason, it is advisable to carry out a correlation study between methods to evaluate their agreement. When they differ, laboratory reports should be modified by introducing new reference values.

One of the main advantages that supports the use of automated systems in clinical laboratories is the analytes' quantification in a more simple, fast, accurate, economical and standardized way compared to manual techniques, which are more complex, time-consuming and sometimes risky (8-10). In the case of Nessler method, previously used in our laboratory, potassium tetraiodomercuriate (II) (K_2HgI_4) and potassium hydroxide (KOH) solutions were highly toxic by inhalation and skin contact and have a risk of cumulative effects, severe burns and negative environmental impact (12), entailing an additional hazard for laboratory staff. According to the Commission Directive 2000/39/EC and its subsequent adaptation in Spanish law (Real Decreto 374/2001, de 6 de abril), it is mandatory to avoid the handling of toxic substances whether it is technically possible. As so, when automatic methods are available and have been validated, the use of manual techniques and toxic reagents should be abandoned.

Despite its usefulness in the diagnosis of renal tubular acidosis, most providers do not include urine as a validated sample for ammonium quantification. This specimen generally involves additional considerations. Firstly, urinary ammonium concentrations are approximately 1000-fold higher than those in plasma. Consequently, urine samples and daily quality controls must be diluted after centrifugation and supernatant separation prior analysis. Secondly, ureolytic bacteria growth must be prevented by refrigerating the samples, as it can produce significant false positives in urinary ammonium levels (6). Finally, it is recommended to collect urine samples under paraffin to avoid contamination and spurious elevations of ammonium levels from precursors such as glutamine (13). Recent studies have demonstrated that ammonium concentrations are stable if urine samples are collected in no additive tubes and stored < 24 hours at room-temperature or < 7 days at 4 °C (6,9). As so, it seems the trueness of ammonium measurement is not adversely influenced neither for the method of collection nor for the storage.

In conclusion, in this study, the automatic diluted urine ammonium assay, adapted from the ammonia assay on an DXC700AU analyser demonstrated its operational validity. This fully automated technique is simple and fast and can be used routinely. The main interest of the ammonia assay is to have precise values of the acid load elimination during urine acidification tests in order to make the diagnosis of renal tubular acidosis. Estimation by calculation of various indices is not sufficient to show minor variations, so assay is essential. A simple, rapid and accurate assay helps to reduce the workload of laboratory technicians and to simplify and improve patient care.

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