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Determination of serum uric acid using high-performance liquid chromatography (HPLC)/isotope dilution mass spectrometry (ID-MS) as a candidate reference method

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Abstract

Uric acid is an important diagnostic marker of catabolism of the purine nucleosides, and accurate measurements of serum uric acid are necessary for proper diagnosis of gout or renal disease appearance. A candidate reference method involving isotope dilution coupled with liquid chromatography/mass spectrometry (LC/MS) has been described. An isotopically labeled internal standard, [1,3-$^{15}N_2$] uric acid, was added to serum, followed by equilibration and protein removal clean up to prepare samples for liquid chromatography/mass spectrometry electrospray ionization (LC/MS-ESI) analyses. $(M-H)^-$ ions at *m/z* 167 and 169 for uric acid and its labeled internal standard were monitored for LC/MS. The accuracy of the measurement was evaluated by a comparison of results of this candidate reference method on lyophilized human serum reference materials for uric acid (Standard Reference Materials SRM909b) with the certified values determined by gas chromatography/mass spectrometry reference methods and by a recovery study for the added uric acid. The method performed well against the established reference method of ion-exchange followed by derivatization isotope dilution (ID) gas chromatography mass spectrometry (ID-GC/MS). The results of this method for uric acid agreed well with the certified values and were within 0.10%. The amounts of uric acid recovered and added were in good agreement for the three concentrations. This method was applied to determine uric acid in samples of frozen serum pools. Excellent precision was obtained with within-set CVs of 0.08–0.18% and between-set CVs of 0.02–0.07% for LC/MS analyses. Liquid chromatography/tandem mass spectrometry electrospray ionization (LC/MS/MS-ESI) analysis was also performed. The LC/MS and LC/MS/MS results were in very good agreement (within 0.14%). This LC/MS method, which demonstrates good accuracy and precision, and is in the speed of analysis without the need for a derivatization stage, qualifies as a candidate reference method. This method can be used as an alternative reference method to provide an accuracy base to which the routine methods can be compared.

Keywords: Uric acid; Serum; LC/ID-MS; Candidate reference method

1. Introduction

Uric acid (2,6,8-trihydroxypurine, UA) is the final product of catabolization of the purine nucleosides [1]. Its determination in serum is a powerful indicator of metabolic alterations or disease appearance [2]. Furthermore, one of the biggest problems about the uric acid metabolism is gout [3], which can be caused by an increase in uric acid production and a decrease in elimination of uric acid by the kidneys. Uric acid, like creatinine, is also a marker for renal failure, as well as toxicity. Therefore, serum uric acid concentration is one of the most frequently performed clinical measurements.

Many routine clinical methods for serum uric acid, primarily based on the enzymatic conversion principle by uricase, are constantly biased as compared to mass spectrometric methods [4–9]. There is a need for a critically evaluated reference method for uric acid to provide an accuracy base to which routine methods can be traceable. Some ID-GC/MS definitive and reference methods have been published for the determination of uric acid in serum [10,11]. A lyophilized human serum is reference material for uric acid [10]. Standard Reference Materials (SRM909b) (from National Institute of Standards and Technology (NIST)) [10] was certified using the GC/MS reference methods for traceability assessment.

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Recent developments of highly sensitive liquid chromatography (LC)/MS instrumentation with robust interfaces such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) make it possible to develop highly reproducible, selective, and sensitive LC/MS methods for low-concentration analytes [12,13]. LC/MS methods offer a powerful tool for the determination of nonvolatile and thermally labile compounds without the need for derivatization (in contrast to the usual situation with GC/MS for many clinical analytes). A LC/MS method using ESI has been reported for the determination of uric acid in urine [14]. However, the CVs of this method ranged from 0.9 to 3.0%, and the results deviated from the target concentrations of external quality control materials (no labeled internal standard was used). This method is a good routine clinical method due to its short chromatographic run time, high throughput, and higher specificity than the routine enzymatic method, but does not have sufficient precision and accuracy to be used as a reference method. To the best of our knowledge, no LC/MS or LC/MS/MS reference methods have been published for serum uric acid with the precision and accuracy comparable to GC/MS reference methods. In this work, we have developed a candidate reference method for uric acid in serum involving ID coupled with LC/MS-ESI. The accuracy of the measurement was evaluated by a comparison of results of this candidate reference method on SRM909b with the certified values determined by GC/MS reference methods and by a recovery study for the added uric acid. This candidate reference method was found to be free from interferences by testing the same serum sample using LC/MS/MS method. The results of LC/MS and LC/MS/MS measurements for serum uric acid were compared. This LC/MS-based method was applied to the determination of uric acid in samples of frozen serum pools.

2. Experimental

2.1. Materials and instruments

All chemicals were of analytical-reagent grade. Natural uric acid with a certified purity of $99.8 \pm 0.3\%$ was provided from China (GBW09202, NRCCRM). [1,3-$^{15}N_2$] uric acid with an isotopic purity of >99 atom% was obtained from Cambridge Isotope Laboratories, Co., USA. Acetonitrile was purchased from Fisher Scientific Inc. (HPLC, USA). Lyophilized human serum SRM909b was purchased from NIST. Solutions were prepared with Milli-Q (18.2 MΩ cm) distilled deionized water and filtered through 0.22 μm pore filters from Sugelabor S.A. (Madrid, Spain). Serum samples were kindly provided by National Center of Clinical Laboratory (Beijing, China), which were received frozen and stored at −80 °C until required. These serum samples were collected by some patients and volunteers. Because the amount of serum of individual is limited and the concentrations of uric acid are not representative, we mixed several people's serum to obtain the three sets of serum pools. We tried to obtain an equal number of specimens approaching the high and the low limit of the reference interval of normal people. In addition, we selected a set of specimens where the uric acid values went beyond the reference value. A balance: Mettler ME22 (Max = 2.1 g, $d = 1.0$ μg), made by Mettler Co. Another balance: ME 235S (capacity: 230 g; $d = 0.01$ mg), made by Sartorius Co. A Finnigan LTQ LC–MS (Linear Ion Trap) which equipped with ESI ion source, a Surveyor LC system, and Xcalibur data system was used for LC–MS and LC–MS/MS analysis. An autosampler (Finnigan Surveyor) was used.

2.2. "Stock" standard and working standard solution preparation

Two independently weighed "stock" standard solution containing 0.2 mg/g of natural uric acid (0.002 mol/L ammonium hydroxide as the solvent) were prepared. Twenty milligrams of uric acid reference compound for each stock solution was accurately weighed on an analytical balance (Mettler ME22), and dissolved in 0.002 mol/L ammonium hydroxide [15]. The balance was calibrated and demonstrated to be accurate to 1 μg. These solutions were stored in a refrigerator at −20 °C in a well-stoppered brown all-glass container. And these stock standard solutions are stable for at least 3 months. "Working" standards containing specified concentration of uric acid may be prepared by diluting specified amount of the stock standard solution with ammonium hydroxide. The concentrations of each "working" standard solution approached the concentrations of uric acid in serum samples. These "working" standard solutions should be prepared daily. The labeled uric acid stock standard solution containing 0.2 mg/g of [1,3-$^{15}N_2$] uric acid (dissolved in 0.002 mol/L ammonium hydroxide) was prepared as the unlabeled uric acid.

2.3. Sample preparation

2.3.1. Preparation of lyophilized serum for the confirmatory measurement

Lyophilized samples were reconstituted by adding about 10 g of deionized water (supplied by NIST) to each vial of the freeze-dried material, using a calibrated Class A pipette. Individual vials were reconstituted on separate days. The mass of added water was determined by weight rather than by volume. For example, the weight of water added to the levels I and II lyophilized serum are 10.05376 and 10.04988 g, respectively. The serum was gently mixed at regular intervals for 2 h until fully reconstituted. About 0.3 g of each serum sample was placed in separate amber vials (5 mL). Each sample was spiked with a known amount of isotopically labeled uric acid (to give an ∼1:1 ratio of analyte to internal standard for each spiked serum sample). Each spiked sample was mixed by gently swirling with a vortex mixer immediately after addition of the labeled uric acid. The spiked samples were then allowed to equilibrate for 4 h at 4 °C prior to extraction and analysis.

2.3.2. Preparation of frozen serum pools

Samples of frozen serum pools were prepared in three different sets (each set on different days). Each set consisted of three vials each of the three concentrations of 22, 77 and 117 mg/kg for concentrations 1, 2 and 3, respectively. These frozen samples were thawed and left to equilibrate to room temperature for half

an hour prior to analysis. The same procedure was used to add internal standard and equilibrate for the frozen serum as for the lyophilized serum.

2.3.3. Preparation of calibrators

At the same time that the serum sample was spiked with the labeled internal standard solution, a work standard solution of known concentration of uric acid (GBW09202) was prepared. And the calibrators were spiked with the same labeled uric acid solution used to spike the serum samples. The ratio of the natural uric acid/labeled uric acid approached 1:1 in these calibrators. The concentrations of the calibrators are almost equimolar to that of the natural uric acid in each set of serum samples. For example, in Table 2 the standard solution concentration is 45.58 mg/kg for level I and 118.86 mg/kg for level II sample. Each spiked standard solution was mixed by gently swirling with a vortex mixer immediately after addition of the labeled uric acid. These calibrators must be prepared daily and placed in a well-stoppered brown all-glass container at 4 °C prior to analysis.

2.3.4. Extraction methodology-protein precipitation

Different extraction and sample clean-up protocols were investigated for the analysis of uric acid in human serum [16,17]. In these references, the authors had applied several methods to remove serum protein. Two of these methods are found to be superior to the others for metabolites analysis. Protein removal by ultrafiltration and precipitation using organic solvent worked well. In this paper, when we selected ultrafiltration to remove protein the HPLC column back pressure increased after several injections and it was a costly method of extraction due to the price of the ultrafiltration tubes and the guard column. So we use acetonitrile method for serum protein removal prior to analysis by LC–MS because the efficient of precipitation is high and the prepared samples fit for the LC–MS analysis (as can be seen in Figs. 1 and 2). The following procedures were used to deproteinize serum samples: About 0.6 g of spiked sample was added to the void created by gently vortex mixing acetonitrile (0.6 mL) in a 5 mL glass amber vial. The solutions were allowed to stand for 5 min before being centrifuged at 4000 rpm for 10 min. The supernatant liquid was transferred to an amber vial (5 mL) taking care not to remove any particulate matter. The extract was then evaporated at room temperature to dryness under nitrogen before being reconstituted in ammonium hydroxide (0.002 mol/L). Finally the reconstituted extract was passed through a 0.22 μm membrane before being presented to the LC–MS system for analysis.

2.4. Analytical recovery of the spiked uric acid

Vials of frozen serum samples were combined, and eleven 2 mL sera were taken for a study of the accuracy of the method. A known amount of unlabeled uric acid was added to 9 of the 11 sera at 3 concentrations, 3 each with 7.9, 32.9 and 59.0 mg/kg uric acid. No uric acid was added to the other two sera. Different amounts of [1,3-$^{15}N_2$] uric acid were added to each serum (the ratio of unlabeled/labeled about 1:1 in each serum sample), and

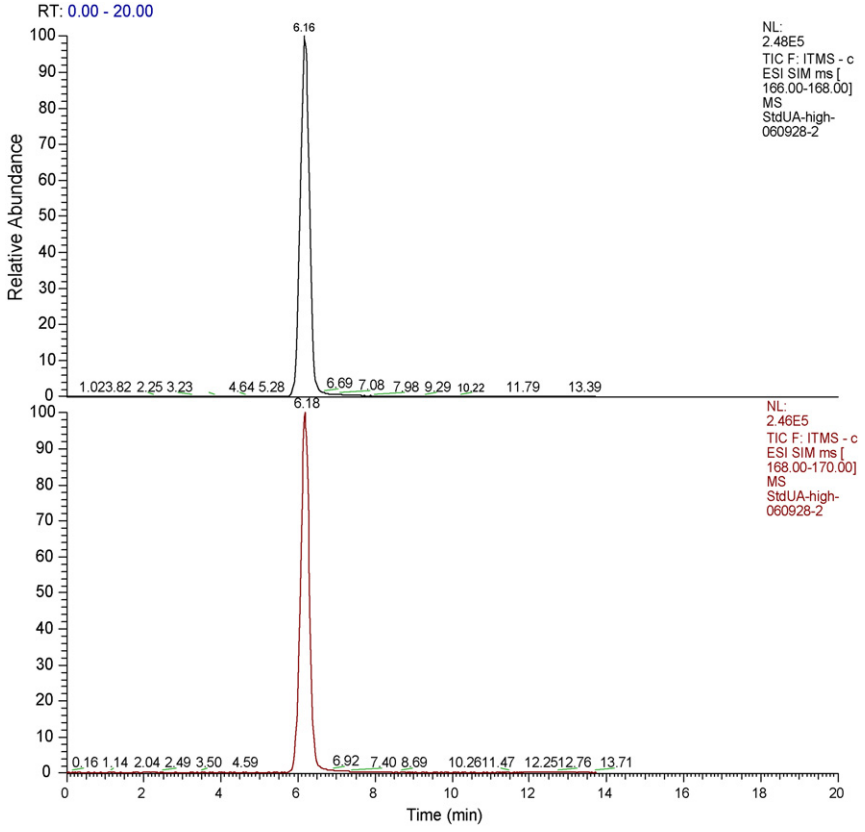

Fig. 1. Ion chromatograms by LC/MS for uric acid and [1,3-$^{15}N_2$] uric acid from a standard solution.

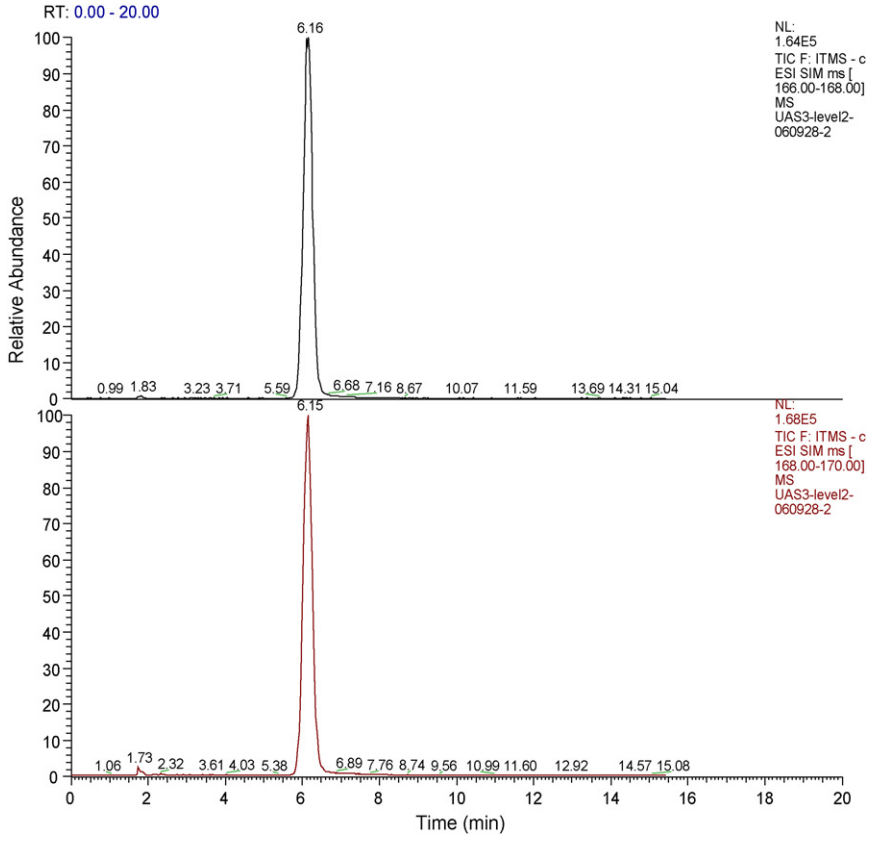

Fig. 2. Ion chromatograms by LC/MS for uric acid and [1,3-$^{15}N_2$] uric acid from a serum sample.

the sera were then processed as described above for extraction and analysis.

2.5. Equilibration

To test for complete equilibration of labeled uric acid and natural uric acid in serum, vials of frozen serum samples were combined, and five 2.0 mL sera were taken for the equilibration study. A given amount of [1,3-$^{15}N_2$] uric acid was added to each sample after mixing, and the samples were equilibrated at 4 °C for various times (0.5, 1, 2, 3, 4 h). The samples were processed as described above for LC/MS analysis.

2.6. Standards cross-check

We prepared two independent sets of standards and test one standard by single-point calibration method [18] with standard from the other set. The weight ratio determined by the IDMS measurements is then compared with the weighed-in weight ratio for that standard.

2.7. Linearity

A group of work standard solutions of natural uric acid was prepared by diluting the stock solution using ammonium hydroxide. A special concentration solution of labeled uric acid was prepared by dilution of the stock labeled uric acid solution. Then this labeled uric acid solution with the same amount were added to the above described serial work standard solutions. So a group of spiked standard solutions was prepared which contained the designed different concentrations of natural uric acid and the same concentration of labeled uric acid.

2.8. Chromatographic separation

Two microliters of calibrators or sample extracts was analyzed by LC coupled with an electrospray ionization MS system (Finnigan, LTQ). Chromatographic separations were performed at 25 °C on an Inertsil ODS-SP column (150 mm × 2.1 mm i.d.), 5 μm particle size (supplied by G&L Technologies, Japan). The mobile phase (100% ammonium acetate 10 mM at pH 4.5) was delivered at a flow rate of 0.2 mL/min. Mass spectral identification of uric acid was carried out with an electrospray ionization interface and an ion-trap mass analyzer. The mass spectrometer was operated in the negative ion mode. $(M-H)^-$ ions at *m/z* 167.1 and 169.1 were monitored for uric acid and [1,3-$^{15}N_2$] uric acid, respectively. The nitrogen drying gas temperature was set at 350 °C. The sheath gas flow rate was 25 and auxiliary gas flow rate was 10. Spray voltage was 3.5 kV.

2.9. Calibration methods

To determine the concentration of a given serum, 2 mL serum were divided into three independent samples, and spiked and extracted, respectively. Three repeat injections were performed for each sample extract. The results of the ID-MS determinations

of uric acid are calculated from peak area ratios measured by the extracted ion monitoring technique. The single-point calibration method [18] is used to calculate the uric acid concentration in mg/kg of serum. The order of measurements was done by injecting the standard, followed by the sample and then by the standard and the sample and standard. Each sample injection was bracketed by an injection of the spiked standard of almost equal concentration and isotope ratio to that of the sample. Four calibration injections are used for the calculation of a sample result.

2.10. Interference testing

The LC/MS/MS method is used to test the interferences in serum sample. LC/MS/MS-ESI analysis was performed on the same LC/MS instrument. Standards and sample extracts were separated by LC on the same column (ODS-SP) with the mobile phase consisting of 10 mmol/L ammonium acetate at pH 4.5. The flow rate was 0.20 mL/min. The column temperature was set at 25 °C. ESI in the negative ion and select reaction monitoring (SRM) modes were used for LC/MS/MS. The transitions at *m/z* 167 → 124 and 169 → 125 were monitored for uric acid and labeled uric acid, respectively. The collision gas was argon, and the collision energy was 40 eV. The dwell time was 0.2 s for SRM.

3. Results and discussion

3.1. Evaluation and verification of the LC/MS method

3.1.1. Equilibration

Complete equilibration of uric acid in serum with the added internal standard is necessary for accurate measurement; therefore, we investigated the length of time required to achieve this. Under our conditions, equilibration was complete in 2 h and the ratio of labeled to unlabeled uric acid was unchanged for at least 24 h at 4 °C. Four hours was chosen as the time allowed to reach equilibrium.

3.1.2. Standards cross-check

The accuracy of results obtained for serum samples depends on the accuracy of the standard mixtures used for calibration. We prepared two independent sets of standards and tested each standard by single-point calibration with a standard from the other set. The weight ratio determined by the IDMS measurements was compared with the weighed-in weight ratio for that standard. The biases were 0.04 and −0.03% for the two stock standard solutions.

3.1.3. Linearity and LOD

A linear regression line was generated for each set of calibrators. Excellent linearity was obtained with the correlation coefficients (R) of all linear regression lines for the ratio of peak area versus mass concentration ranging from 0.99982 to 0.99994. Using this LC/MS method, the concentration of UA is linear from 6.0 to 200 mg/kg. The limits of detection (LOD) were evaluated at a signal-to-noise ratio of 3. The LOD of uric acid was 0.032 ng. Thus, taking into account the injected volume (2 μL), the limit of detection was 16 μg/L, corresponding to the injected solution. This low LOD permits sample dilution to avoid matrix effects. The limit of quantification (calculated at a signal-to-noise ratio of 10) was 0.120 ng.

Table 1
Recovery of added uric acid

Added (mg/kg)	Expected (mg/kg)	Measured (mg/kg)	CV (%)	Recovery (%)
0		34.23	0.15	
7.89	42.12	42.34	0.10	100.52
32.93	67.16	67.46	0.15	100.45
59.01	93.24	94.23	0.14	101.06

3.1.4. Recovery of the spiked uric acid

The level of recovery by the proposed method was evaluated by assaying serum samples spiked with a standard uric acid solution. The observed value for the un-spiked sample was subtracted from that of the spiked sample. Table 1 shows the percentage recovery of uric acid from serum spiked with three different concentrations of standards. This method of protein removal had recovery levels of 100.52, 100.45 and 101.06% for 42, 67 and 93 mg/kg spiked samples, respectively. The serum pools contained 34.23 mg/kg of uric acid.

3.1.5. Confirmatory measurement using SRM909b

As another assessment of the accuracy of the candidate reference method, samples of SRM909b were analyzed as described in Section 2 and the results were compared to the certified values determined using the GC/MS reference method [10]. The concentrations of uric acid in NIST SRM909b determined by LC–MS are given in Table 2. The values obtained agree well with the certified value and are within 0.02% for the low concentration samples (CV = 0.13%) and 0.10% for the high-concentration samples (CV = 0.11%). The calculated uncertainty for the GC–MS reference method and this LC–MS method was almost equivalent, which is an important result: Conventionally, LC–MS has been considered less satisfactory for quantitative analysis than GC–MS methods, due to the inferior ion signal stability observed in LC–MS. Much of the uncertainty for the GC–MS method comes from the sample preparation steps (i.e. the separation of uric acid and derivatization), which are not used in the LC–MS method.

3.1.6. Measurement of frozen serum materials

The LC/MS method for the determination of uric acid was applied to samples of frozen serum pools containing uric acid at 22, 77 and 117 mg/kg. Samples were prepared and analyzed as described in Section 2, and the results are given in Table 3. Excellent reproducibility was obtained for all three concentrations: Within-set CV ranged from 0.08 to 0.18%, and between-set CV ranged from 0.02 to 0.07%. The data given in Tables 1 and 3 show the good repeatability and reproducibility of this LC/MS method.

Table 2
LC/MS measurements of uric acid in SRM909b (mg/kg)

Certified concentration	Set	Certified uncertainty (%)	Measured mean (mg/kg)	SD (mg/kg)	CV (%)	Overall mean (mg/kg)	Overall SD (mg/kg)	Overall CV (%)	Relative error (%) compared with the certified value by NIST
(a) Level I									
SRM909b (I) 45.56	1	4.33	45.64	0.06	0.13	45.57	0.06	0.13	0.02
SRM909b (I) 45.56	2	4.33	45.53	0.04	0.09				
SRM909b (I) 45.56	3	4.33	45.55	0.04	0.09				
SRM909b (I) 45.56	4	4.33	45.51	0.08	0.17	45.55	0.04	0.09	0.02
SRM909b (I) 45.56	5	4.33	45.54	0.03	0.07				
SRM909b (I) 45.56	6	4.33	45.59	0.07	0.15				
Certified concentration	Set	Certified uncertainty (%)	Measured mean (mg/kg)	SD (mg/kg)	RSD (%)	Overall mean (mg/kg)	Overall SD (mg/kg)	Overall CV (%)	Relative error (%) compared with the certified value by NIST
(b) Level II									
SRM909b (II) 118.83	1	3.14	118.69	0.22	0.19	118.79	0.13	0.11	0.03
SRM909b (II) 118.83	2	3.14	118.94	0.14	0.12				
SRM909b (II) 118.83	3	3.14	118.75	0.17	0.14				
SRM909b (II) 118.83	4	3.14	118.96	0.11	0.09	118.95	0.09	0.08	0.10
SRM909b (II) 118.83	5	3.14	118.85	0.04	0.03				
SRM909b (II) 118.83	6	3.14	119.04	0.15	0.13				

The two "overall means" represent the means of sets 1–3 and 4–6, respectively.

3.1.7. Interferences

The same sample extracts were analyzed by LC/MS and LC/MS/MS. The chromatograms for unlabeled and labeled uric acid are shown in Fig. 2 for LC/MS and in Fig. 3 for LC/MS/MS. The transitions of uric acid *m/z* 167 → 124 and 169 → 125 were monitored for unlabeled and labeled uric acid, respectively. Interference is unlikely because of the high resolution of the new type of HPLC column (ODS-SP column, G&L Corp., Japan) and the selectivity of the MS determination. No co-elution peak can be seen in the LC/MS/MS chromatogram (Fig. 3). The results obtained by LC/MS and LC/MS/MS are given in Table 4. Excellent reproducibility was obtained for this concentration: Within-set CV ranged from 0.06 to 0.10% for LC/MS, and from 0.12 to 0.15% for LC/MS/MS. Between-set CVs were 0.14% for LC/MS and 0.17% for LC/MS/MS. The LC/MS and LC/MS/MS results were in very good agreement (within 0.14%). In addition, normal and patient's sera were tested and no interference peak appeared. The results are given in Table 3.

3.2. Uncertainty analysis

Uncertainty in the standards would contribute to uncertainties in the determination of uric acid levels in serum. The cross-checking of standard sets ensured the absence of significant bias. Furthermore, the large numbers of measurements made on each serum pool, using standards from different sets, would reduce any effect of random bias.

Although the uncertainty of the method is small and there is no evidence of significant bias in the measurement process, an analysis of possible sources of uncertainty was made.

A variety of analytical steps can be a source of analytical uncertainty. Pipetting uncertainties were eliminated by weighing all volumes. A balance (Mettler micro balance model ME22) was used to weigh the uric acid (GBW09202) and the labeled uric acid, and all amounts were adjusted to give masses ≥20 mg, so that weighing uncertainties before dilution were kept <0.005%. Uncertainty during weighing can arise from weights of sam-

Table 3
LC/MS measurements of uric acid in sera (mg/kg)

Concentration	Set	Mean (mg/kg)	SD[a] (mg/kg)	CV (%)	Overall mean (mg/kg)	Overall SD[b] (mg/kg)	Overall CV (%)
1	1	22.27	0.04	0.18	22.27	0.01	0.07
1	2	22.28	0.03	0.13			
1	3	22.25	0.02	0.09			
2	1	77.03	0.10	0.13	77.02	0.04	0.05
2	2	76.98	0.11	0.14			
2	3	77.05	0.06	0.08			
3	1	117.41	0.14	0.12	117.41	0.03	0.02
3	2	117.44	0.12	0.10			
3	3	117.39	0.16	0.14			

[a] Standard deviation (SD) of a single measurement within a set.
[b] SD of the mean for that level.

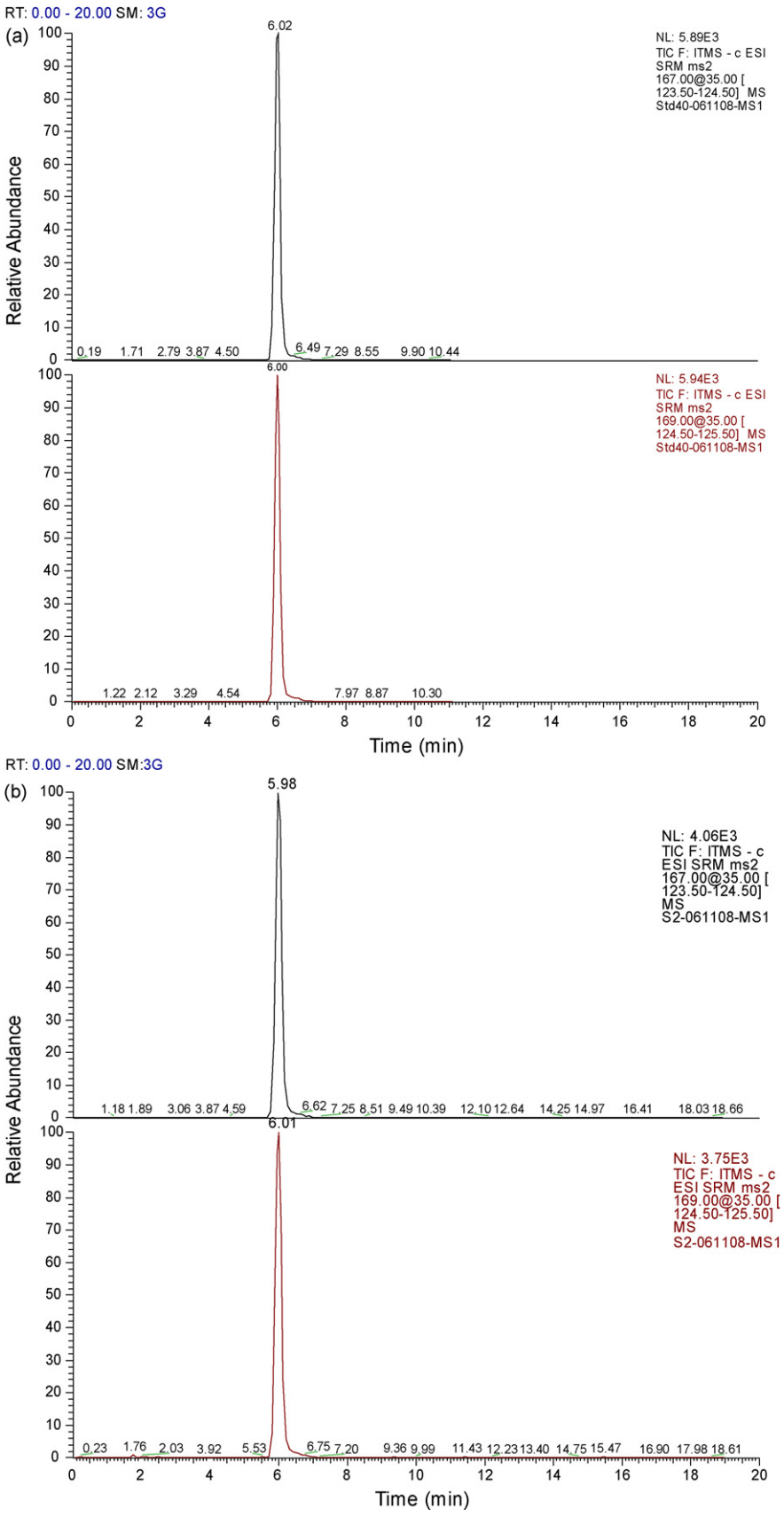

Fig. 3. Ion chromatograms by LC/MS/MS for uric acid and [1,3-$^{15}N_2$] uric acid from: (a) a standard solution and (b) a serum sample.

Table 4
The comparison LC/MS with LC/MS/MS method in the same serum

	Set	Mean (mg/kg)	SD[a] (mg/kg)	CV (%)	Overall mean (mg/kg)	Overall SD[b] (mg/kg)	Overall CV (%)	The RSD of the two methods (%)
LC/MS								
1	1	40.75	0.04	0.10	40.82	0.06	0.14	
1	2	40.84	0.04	0.09				
1	3	40.86	0.02	0.06				
LC/MS/MS								0.14
2	1	40.82	0.05	0.12	40.87	0.07	0.17	
2	2	40.85	0.05	0.13				
2	3	40.95	0.06	0.15				

[a] Standard deviation (SD) of a single measurement within a set.
[b] SD of the mean for that level.

Table 5
Expanded uncertainties for serum uric acid by LC/MS measurements

Uncertainty table	Concentration 1	Concentration 2	Concentration 3
Method precision[#] (%)	0.07	0.05	0.02
Calibration solution (%)	0.17	0.17	0.17
Weighings (%)	0.04	0.03	0.02
Combined uncertainty (%)	0.19	0.18	0.17
K factor	2	2	2
Relative expanded uncertainty[a] (%)	0.38	0.36	0.34
Expanded uncertainty (mg/kg)	0.14	0.19	0.27

[a] Uncertainty of 95% confidence interval.

ples and work standard solutions. Another balance (Sartorius model ME 235S) was used, and all amounts were adjusted to give masses ≥300 mg, so that the weighing uncertainties were kept <0.017%. Uncertainty during weighing can arise from evaporation of liquid during pipetting the serum and the internal standards. The weighing time was as short as possible, the temperature was always 22–24 °C, and the solvent was nonvolatile. Concentration/deterioration effects of the primary standards or samples can occur during storage and handling, but these effects were minimized because of the utmost care taken in those steps; e.g. all frozen solutions were discarded after analysis. The SRM of GBW09202, which determines the theoretically achievable accuracy, is certified with an uncertainty of ±0.3%. In summary, the uncertainty associated with the final measured concentration was calculated by combining the relative standard uncertainty for the precision of the method as a whole with the uncertainties associated with weighing and the concentration of the natural standard solution. The total uncertainty of the described method is estimated to be <0.5% within a typical 95% confidence interval, as shown in Table 5.

4. Conclusions

A high-accuracy LC–MS method has been developed that is much faster than the conventional GC–MS method used for the determination of uric acid in serum. A simple clean-up to remove protein was shown to provide a suitable sample for LC–MS analysis. This compares favorably with the more involved selective clean-up necessary when preparing samples for GC–MS, which also required a derivatization step. The combination of high precision and the lack of significant bias qualify this method as a candidate reference method for the measurement of uric acid in serum. This method can be used to provide an accuracy base to which routine methods can be compared, and can be used to certify the concentration of uric acid in a new standard reference material in frozen serum pools, which is intended to provide a means for clinical laboratories to test the accuracy of their methods and calibrators, and to demonstrate traceability of the measurements.

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