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# Development of a liquid chromatography–mass spectrometry method for the high-accuracy determination of creatinine in serum

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**Abstract**

An LC–MS method for the high-accuracy determination of creatinine in serum has been developed and used to provide results for an international measurement evaluation programme (IMEP) and the Comité Consultatif pour la Quantité de Matière (CCQM) international inter-laboratory studies. An assessment of different sample preparation methods, including ion-exchange chromatography, solid-phase extraction, plasma ultrafiltration and ethanol protein precipitation, revealed that no bias or reduced precision was associated with the quicker less extensive clean-up methods, when using liquid chromatography–isotope dilution mass spectrometry (LC–IDMS) for quantitation. A number of different calibration regimes were also investigated. External calibration was shown to provide adequate calibration for most routine analysis with a relative associated expanded uncertainty ($k=2$) of 6% at the 95% confidence level. The use of a non-isotopically labelled internal standard was shown to improve the relative expanded uncertainty ($k=2$) to 4%. However, the difference in retention time between the internal standard and the creatinine was such that a matrix interferent produced an observed bias of over 16%. The use of an isotopically labelled internal standard was shown to reduce any bias to less than 0.2% with an expanded uncertainty ($k=2$) of less than 0.3%. The developed method was then used, in a blind trial organised jointly by IMEP and CCQM, to determine the amount of creatinine in human serum. The method performed well against the established reference method of ion-exchange chromatography followed by derivatisation gas chromatography (GC)–IDMS. The observed difference between the values determined by LC–IDMS and the key comparison reference value (average of all the submitted results) was less than 0.3%. The biggest advantage of the described method is in the speed of analysis. With a chromatographic run time of less than 10 min and sample preparation consisting of a simple protein precipitation, without the need for a derivatisation stage, the analysis is vastly simpler then the conventional GC–IDMS reference method.
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*Keywords:* Creatinine

## 1. Introduction

Creatinine (Fig. 1a) is one of the most important clinical biomarkers. Its measurement in body fluids is often used to indicate the degree of hydration of a sample (i.e., how dilute a urine sample is), enabling the comparison of many other analytes [1]. The measurement of creatinine is also used in the assessment of renal function. Creatinine is formed by the spontaneous nonenzymatic cyclization of creatine (Fig. 1b), a key component used in muscle contraction. Its routine chemical measurement is based on

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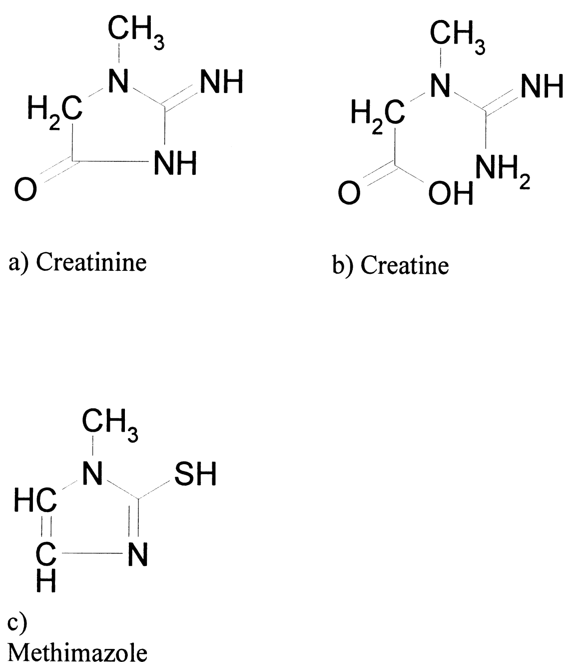


Fig. 1. Chemical structures of: (a) creatinine; (b) creatine; and (c) methimazole.

the Jaffé method [2], a colorimetric determination of the picrate derivative, or by several enzymatic assays. Whilst these methods have the required sample throughput, sensitivity and rigour for routine analysis, they should regularly be checked against a certified reference material for quality assurance and calibration purposes. A recent study [3] conducted by the Institute for Reference Materials and Measurements (IRMM) highlighted that out of more than 1000 laboratories the majority of the results submitted for the analysis of a creatinine sample were between 10 and 50% higher than the certified value. Therefore, methods of assigning reference values must be more precise than routine methods and should ideally be traceable to internationally agreed units of measurement.

Gas chromatography coupled to mass spectrometry (GC–MS) in conjunction with an isotope dilution procedure (GC–IDMS) has been the method of choice for certification purposes with relative standard deviations (RSDs) of $<0.3\%$ [4–6]. Due to the polarity of the molecule, creatinine requires derivatisation prior to analysis. However, creatine is derivatised into the same chemical species as creatinine, therefore separation of the two compounds is a prerequisite of GC analysis. This has been achieved by the use of a cation-exchange clean up, whereby the creatine is eluted off the column before the creatinine enabling fraction collection prior to derivatisation. An important assumption in using this method is that all the creatine has been removed from the creatinine fraction. This can be proven by the use of $^{14}C$ labelling experiments, however this has economic constraints. It is not unreasonable to assume that if the creatine/creatinine ratio is high in a sample then a small but significant bias may be observed using such a method.

High-performance liquid chromatography (LC) and capillary electrophoresis (CE) are complementary separation techniques to GC. However, these separation techniques enable the separation of creatinine and creatine without derivatisation [7]. The last decade has seen many advances in the coupling of both LC and CE to MS, thus enabling the direct IDMS quantitation of creatinine. Previous studies on the use of LC–MS for the determination of creatinine in serum have reported a larger RSD ($RSD \geq 1\%$) than that observed when using GC [8,9]. Much of this imprecision can be attributed to the stability of the ionisation processes used. The use of electrospray ionisation coupled with orthogonal spraying, curtain gases and Z-spray inlets has vastly improved the precision obtainable with modern LC–MS instruments.

While previous studies on the use of LC–MS for the determination of creatinine in serum and blood have been reported [1,8,9], its use as an alternative to GC–MS for assigning certified reference values has been overlooked. This can be attributed to the belief that LC–MS is somewhat less precise than GC–MS. However, if the whole analysis protocol including extraction, is considered, the analytical results obtained by LC–MS are equivalent to those obtainable by GC–MS. This paper assesses several different extraction techniques for the isolation of creatinine from serum and details the results obtained from a recent inter-laboratory comparison for the determination of creatinine in human serum including GC and LC–IDMS data. Also discussed is an examination of different calibration regimes with an assessment made on the estimated uncertainty of such methods compared with the exact matching IDMS

approach. The method described is rapid, robust, requires minimal sample preparation and is suitable for the certification of creatinine reference materials.

## 2. Materials and methods

### 2.1. Sample preparation

Samples were received in two forms. The certified reference material (SRM 909b; NIST, Gaithersburg, MD, USA) was received lyophilised and was stored at 4 °C until required. Whole serum samples submitted as part of (IMEP-17/CCQM-K12) key comparison study were received frozen and stored at −70 °C until required.

### 2.2. Preparation of lyophilised serum

Lyophilised samples were reconstituted by adding 10 ml of deionised water (18 MΩ) 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 difference weighings. The serum was gently mixed at regular intervals for 2 h until fully reconstituted. The frozen samples were thawed and left to equilibrate to room temperature prior to analysis.

Two aliquots (2 g) of each serum sample were placed in separate amber vials (15 ml). Each aliquot was spiked with a known amount of isotopically labelled creatinine-d3, which was estimated to be equimolar in concentration to that of the natural creatinine in the serum sample. The spiked samples were then allowed to equilibrate at 6±2 °C overnight prior to extraction and analysis.

### 2.3. Extraction methodology

Different extraction and sample clean-up protocols were investigated for the analysis of creatinine in lyophilised human serum (NIST SRM 909b).

### 2.4. Ion-exchange chromatography

Three glass chromatography columns (20 cm× 1 cm I.D.) were filled to a height of 10 cm with Amberlite (120 mesh) ion-exchange resin (Aldrich, Dorset, UK. Part No. 21,653-4). The resin was conditioned by passing 1 *M* ammonium hydroxide (50 ml), deionised water (100 ml), 1 *M* hydrochloric acid (50 ml) and deionised water (100 ml), before applying the spiked serum (1 ml) to the top of the column. The column was then washed with deionised water (100 ml) in order to remove creatine. The creatinine was eluted using 1 *M* ammonium hydroxide (45 ml). The eluent was collected as two fractions. The first 30 ml was collected as fraction 1 and the final 15 ml was collected as fraction 2. The collected fractions were then individually freeze–dried before being reconstituted in 1 ml of water prior to analysis by LC–MS.

### 2.5. Solid-phase extraction

Solid-phase extraction (SPE) cartridges (Waters, Hertfordshire, UK, Oasis HLB 6 ml) were conditioned with methanol (5 ml) and deionised water (4 ml). A 1-ml volume of sample was then applied to the cartridge and allowed to pass through under gravity. The cartridge was then washed with deionised water (2 ml) and the creatinine eluted with methanol (2 ml). The extract was evaporated to dryness under nitrogen at room temperature before being reconstituted in deionised water (1 ml).

### 2.6. Plasma ultrafiltration

A 1-ml volume of spiked serum was added to an ultrafiltration tube (Sartorius 5000D, Goettingen, Germany). This was then centrifuged at 4000 rpm for 10 min. The ultrafiltrate was removed from the tube and analysed directly.

### 2.7. Protein precipitation

A 1-ml volume of spiked sample was added to the void created by gently vortex mixing ethanol (3 ml) in a 15-ml polypropylene sample tube. The solutions were allowed to stand for 5 min before being centrifuged at 4000 rpm. The supernatant liquid was transferred to an amber vial (4 ml) taking care not to remove any particulate matter. The extract was then evaporated at 40 °C to dryness under nitrogen before being reconstituted in deionised water (1 ml). Finally the reconstituted extract was passed through a What-

man 0.45 μm syringe filter before being presented to the LC–MS system for analysis. At this stage the high level samples were diluted by adding 1 ml of extract to 9 ml of water. This extraction procedure was performed in triplicate for each spiked sample aliquot.

### 2.8. Instrumental analysis

1×5 μl aliquots of the sample extracts were analysed by LC (Waters, 2690 Separations Module, Hertfordshire, UK) coupled with an electrospray ionisation MS system (Micromass, Quattro Ultima, Manchester, UK). The chromatography was performed on a $C_{18}$ column [Luna $C_{18}$ (II), 3 μm 150×2.1 mm, Phenomenex, Cheshire, UK] and the mobile phase consisted of ammonium acetate (10 m*M*) at a flow-rate of 0.2 ml $min^{-1}$. This combination resulted in the total separation of creatinine and creatine (Fig. 2).

The electrospray probe was operated at 2.2 kV and the cone voltage was ~20 V. The source block and desolvation temperatures were 100 and 380 °C, respectively. Analyte ionisation was by protonation resulting in $[M+H]^+$ ions at *m/z* 114 and 117 for the natural and deuterated compounds, respectively.

### 2.9. Calibration methods

The data obtained from the analysis of the creatinine extracts following protein precipitation as described above were processed using a number of different calibration options. Details of theses different approaches and their results are described in detail below.

### 2.10. Standard preparation

Standards were freshly prepared, by mass, in water (18 MΩ) using natural creatinine (99.8±0.2%, NIST SRM 914) from NIST, deuterated isotopically labelled creatinine-d3 (methyl-d3) >98 atom%, Isotec (OH, USA), and methimazole (98%) from Sigma (Dorset, UK).

### 2.11. External calibration (without an internal standard)

The LC–MS system was externally calibrated with five gravimetrically prepared calibration solutions containing creatinine in the range 5 to 50 μg $g^{-1}$. Injections of the sample extracts were bracketed by standard solutions. This was repeated three times, in series, for each of the three replicate protein precipi-

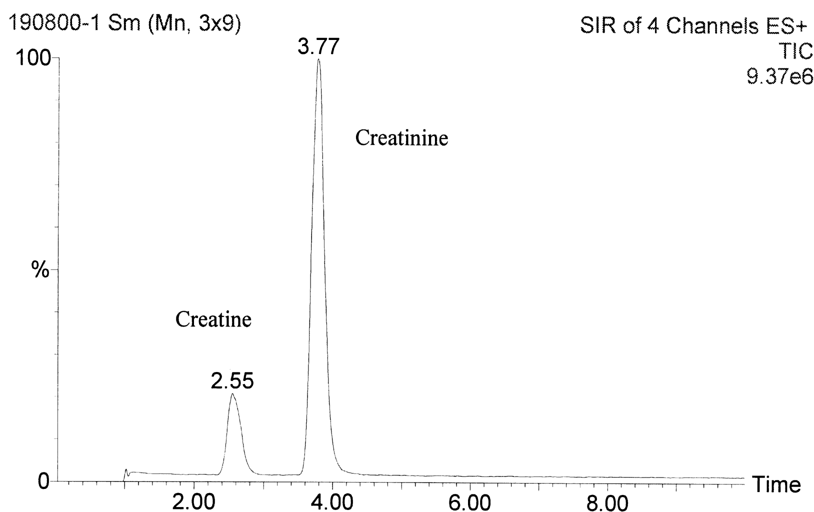


Fig. 2. Chromatogram of a mixed standard of creatine and creatinine using selected ion recording (SIR) for the $[M+H]^+$ ions of creatinine (*m/z* 114) and creatine (*m/z* 132).

tation extractions. This resulted in 10 repeat injections for each point on the standard curve and three repeat injections of each sample extract. A calibration graph was constructed using the mean value of the repeat injections and the sample concentration was calculated from the slope and intercept of a least squares fitted line through the calibration points. The combined standard uncertainties were calculated according to the recommendations for least squares calibrations given by ISO and EURACHEM [10,11].

### 2.12. Internal calibration (with an internal standard)

A set of five quantitative standards was prepared as above. However, creatinine-d3 and methimazole (Fig. 1c) were added to all five standards and the blank at a concentration of 18 μg $g^{-1}$. All sample aliquots were also prepared with 18 μg $g^{-1}$ of creatinine-d3 and methimazole added. The standards and samples were run in the same format as that used for the external calibration. A calibration graph was constructed using the mean creatinine–internal standard peak area ratio. Creatinine concentration was calculated from these graphs in the same manner as that described for the external standards.

### 2.13. Exact matching double IDMS (the ideal form of internal standardisation)

A solution standard was prepared such that it contained nominally the same concentration of creatinine as the sample. Both samples and standards were prepared so that they also contained equal concentrations of creatinine-d3. Ideally this results in both sample and standard solutions producing identical signals when measured quantitatively on the mass spectrometer. The solutions were prepared to yield an ideal measured ratio close to unity for the natural and isotopic analogue. Five repeat injections were performed for each sample extract, resulting in a total of 15 repeats for each spiked sample aliquot. Each sample injection was bracketed by an injection of the spiked standard of equal concentration and isotope ratio to that of the sample. The peak areas of the resulting selected ion chromatograms were used to calculate the measured ratios of the two isotopic forms (i.e., deuterated and natural).

Results were calculated using the double IDMS equation [12] (Eq. (1)). 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 the weighings and the concentration of the natural standard solution (Eq. (2))

$$W_x = W_z \cdot \frac{m_z}{m_{yc}} \cdot \frac{m_y}{m_x} \cdot \frac{R'_B}{R'_{BC}} \tag{1}$$

where: $W_x$ = the concentration of creatinine in sample (μg $g^{-1}$); $W_z$ = the concentration of natural creatinine solution used to prepare the calibration blend (μg $g^{-1}$); $m_z$ = mass of the natural creatinine standard added to the calibration blend; $m_x$ = mass of the sample used; $m_{yc}$ = mass of the labelled creatinine standard added to the calibration blend; $m_y$ = mass of the labelled creatinine standard added to the sample blend; $R'_B$ = measured ratio (peak area $m/z$ 114/peak area $m/z$ 117) of the sample blend; and $R'_{BC}$ = average measured ratio (peak area $m/z$ 114:peak area $m/z$ 117) of the calibration blend injected before and after the sample.

$$u = w_x\sqrt{\left(\frac{u_{Cz}}{c_z}\right)^2 + \left(\frac{u_{pm}}{p_m}\right)^2 + \left(\frac{um_x}{m_x}\right)^2 + \left(\frac{um_y}{m_y}\right)^2 + \left(\frac{um_z}{m_z}\right)^2 + \left(\frac{um_{yc}}{m_{yc}}\right)^2} \tag{2}$$

where $p_m$ is the precision of the method:

$$u_{pm} = \left(\frac{SD}{\sqrt{n}}\right)$$

where SD = standard deviation of all results and $n$ = number of analytes.

## 3. Results and discussion

The chromatography procedure developed resulted in the elution of creatinine, with its complete separation from creatine, in less than 4 min (Fig. 2). This separation was still deemed necessary, even when using MS as the detection method, as loss of water from creatine resulted in an isobaric interference

with creatinine. Even though creatinine had totally eluted by 4 min the analysis time for each injection was extended to 10 min due to a peak eluting at 7.5 min. This interferant caused suppression of the analyte signal and therefore had to be allowed to elute before proceeding with the next injection.

### 3.1. Extraction method

For the assessment of the extraction processes the double IDMS calibration protocol was used. The addition of the isotopic analogue prior to sample preparation/extraction provided the ideal mechanism for compensating for incomplete recovery. Therefore, analyte recovery had little bearing on the analytical error observed. However, poor recovery of the creatinine and its labelled analogue resulted in smaller analyte peak areas and a poorer signal-to-noise ratio. This had a detrimental effect on the measurement precision resulting in larger uncertainties.

### 3.2. Ion exchange

Isolation of creatinine from serum was readily achieved using ion exchange. Under acidic conditions creatinine was preferentially retained on the column while creatine and other potential interferences were removed. Elution of creatinine required a relatively large volume of elution buffer (45 ml) which was removed by freeze–drying. This whole process was laborious and time consuming and took over 12 h to complete. The creatinine was observed in both fractions of the ammonium hydroxide solution, with the second fraction yielding a peak area approximately 60% of the first fraction. The mean concentration of creatinine in serum determined by each fraction was slightly lower than the certified value ($6.217 \pm 0.061$ μg $g^{-1}$) however the measurement uncertainty encompassed the reference value. The results of the amount of creatinine determined in serum with their associated uncertainties for the different extraction methods are shown in Fig. 3.

### 3.3. Solid-phase extraction

The SPE method investigated consisted of a generic SPE protocol. The peak area observed for the resulting eluted analyte was 10% of that observed for the first ion-exchange fraction collected. The mean overall determined concentration for the SPE extraction was greater than the CRM reference value

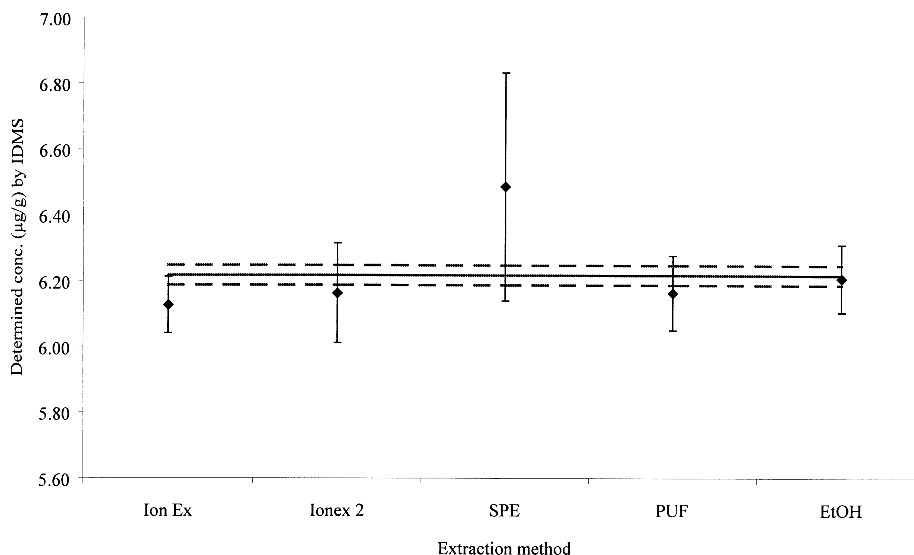


Fig. 3. Comparison of different sample preparation methods for the quantitation of creatinine in serum (SRM 909b). Solid line, certified value; dashed line, ±expanded uncertainty.

however the low peak area counts resulted in poor peak area reproducibility. Therefore the associated uncertainty for this measurement was large in comparison to the other extraction methods investigated. While not being as time consuming as the ion-exchange cleanup, due to the absence of the freeze–drying step, the SPE method was still rather laborious.

### *3.4. Plasma ultrafiltration*

Plasma ultrafiltration was by far the quickest and easiest extraction process investigated. It consisted of simply centrifuging the spiked serum sample in an ultrafiltration tube. The filtrate was then collected and injected straight onto the LC column. The mean determined concentration of creatinine in the serum was slightly low, however the associated uncertainty encompassed the certified value. The observed peak area for the ultrafiltration method was twice that observed for the ion-exchange method and was consistent with the peak area observed for a gravimetric standard prepared in water at 6.2 $\mu g\ g^{-1}$ of creatinine. The ultrafiltration method did have some associated drawbacks. After 20 or so injections of the sample the analyte peak shape started to deteriorate and the column back pressure increased. This was simply remedied by replacing the guard column. However, this along with the price of the ultrafiltration tubes was considered a costly method of extraction.

### *3.5. Protein precipitation*

The final extraction method investigated was a simple protein precipitation followed by concentration by evaporation and filtration. The observed peak areas using this method were comparable to those obtained for a standard solution of a similar creatinine concentration. The determined concentration of creatinine in the serum agreed well with the certified value and the uncertainty was comparable with the more thorough ion-exchange cleanup. The peak shape remained unaffected even after over 150 repeat injections. This method of sample cleanup was simple and cost effective and was without any adverse effects to the analytical performance (Fig. 4).

Based on the above the protein ''crash'' method of sample extraction was deemed appropriate for the LC–MS analysis of creatinine in serum. Thus, this procedure was chosen for the international IMEP-17/CCQM-K12 certification exercise.

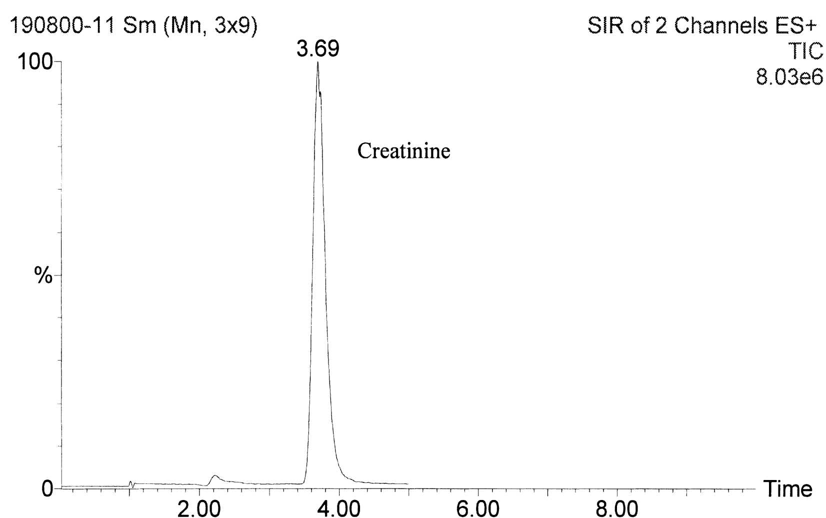


Fig. 4. Chromatogram of ethanol protein precipitated serum sample using selected ion recording (SIR) for the $[M+H]^+$ ions of creatinine ($m/z$ 114) and creatinine-d3 ($m/z$ 117).

### 3.6. Method of calibration

For the comparison of different calibration protocols the IMEP-17/CCQM-K12 serum sample was prepared for analysis using the ethanol protein precipitation method.

### 3.7. External standard

Five external standards and a blank were injected 10 times throughout the analysis sequence. Samples were analysed in triplicate. The calibration graph was constructed using the mean values for the 10 standard injections. The $R^2$ value (measure of linear correlation) for a line of best fit was 0.998 with the lowest standard having an RSD of 6%. The observed sample RSD was 8%. The associated uncertainty was calculated as described by the GUM [8], whereby the largest contribution to the uncertainty was the position of the line drawn through the calibration points. The determined concentration by external standard calibration differed from the key comparison reference value (KCRV) by 6%. This difference was encompassed by the expanded uncertainty of the analysis and would be suitable for most routine analytical analyses (Fig. 5).

### 3.8. Deuterated isotopically labelled internal standard

Most laboratories, in an effort to reduce errors and uncertainty, incorporate the use of internal standards. The ideal internal standard for MS is an isotopically labelled analogue of the analyte of interest. This, if added to the sample and fully equilibrated, forms the basis of isotope dilution mass spectrometry. The amount of analyte in the sample is determined by the natural:labelled ratio. A set of standards equivalent to those used for the external calibration were prepared such that each standard contained the same concentration of labelled creatinine. The samples were prepared to contain the same concentration of creatinine-d3 as the standards. The standards and samples were run in the same sequence as those used for external calibration. A calibration curve was prepared by plotting the measured creatinine:creatinine-d3 ratio versus natural creatinine standard concentration. The $R^2$ value for a line of best fit was 1.00 with the lowest standard

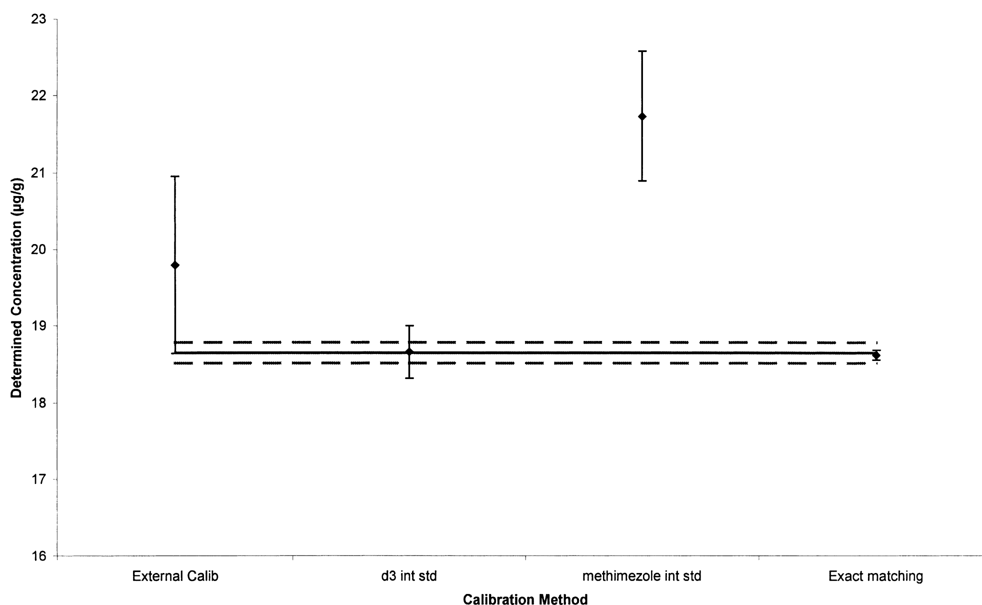


Fig. 5. Comparison of different calibration methods for the quantitation of creatinine in serum. Solid line KCRV for CCQM-K12 with its associated expanded uncertainty (dashed lines).

having an RSD of 0.5%. The observed sample RSD was 1%. The amount of creatinine in the sample was determined from the slope and intercept of this plot. The determined concentration differed from the KCRV by less than 0.1% with an associated uncertainty of less than 2%. The agreement between the determined value and the KCRV is a testament to the success of internal standardisation with isotopically labelled materials. The use of isotopic ratio measurements resulted in improved repeatability and uncertainty making accurate and precise quantitation possible by LC–MS.

### *3.9. Non isotopically labelled internal standard*

Isotopically labelled analogues are considered the best internal standards. However, these are generally expensive and are not always available for every analyte of interest. Therefore other forms of internal standardisation must be considered. For MS a compound of similar mass and structure would be considered the best alternative. In an effort to investigate this methimazole was placed in the above standards and samples to replace the labelled creatinine The presence of methimazole in the samples and standards resulted in a peak at 8.5 min in the chromatogram at a $m/z$ (+1) greater than creatinine. A calibration curve was constructed by plotting the creatinine:methimazole ratio versus creatinine concentration and a line of best fit was drawn through the calibration points. The resulting $R^2$ value was 0.998 with repeat injections of the lowest standard having an RSD of 3%. The observed sample RSD was 5%. The amount of creatinine in the sample was determined from the slope and intercept of this plot. The determined concentration differed from the KCRV by over 16% with an associated uncertainty of less than 4%. Therefore the determined value and its uncertainty did not agree with the KCRV. The lack of agreement between the determined creatinine concentration and the KCRV was of concern for an internal standard method of calibration. As expected the use of any internal standard did improve the repeatability of measurements. The difference between the determined creatinine concentration and the KCRV can be explained by the difference in elution times of the creatine and methimazole. The methimazole eluted at 8.5 min, which coincided with a large matrix peak from the serum samples (Fig. 6). This resulted in

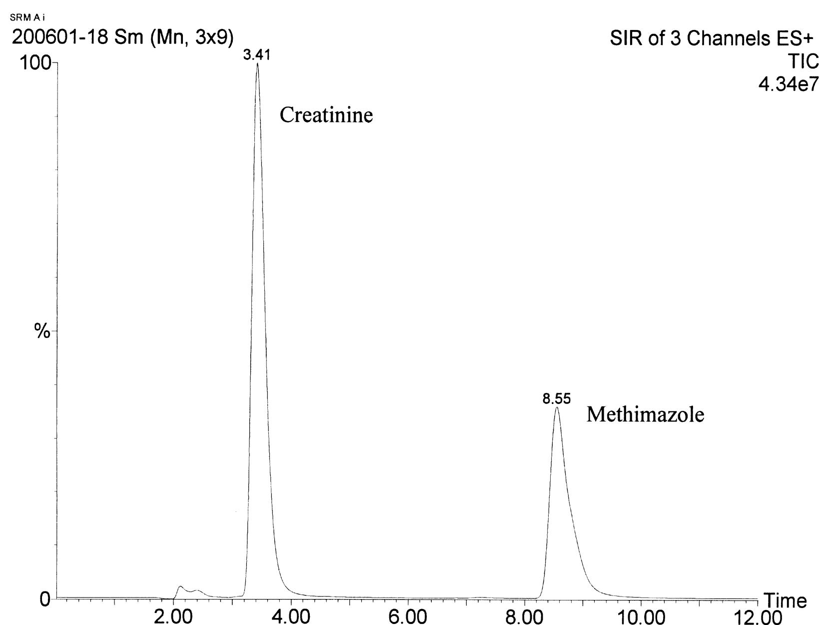


Fig. 6. Chromatogram of a mixed standard of creatinine and methimazole using selected ion recording (SIR) for the $[M+H]^+$ ions of creatinine ($m/z$ 114) and methimazole ($m/z$ 115).

suppression of the internal standard signal yielding an erroneously high creatinine level, when using non matrix matched calibration standards.

### 3.10. Exact matching optimisation

An exact matching form of IDMS was first developed by Henrion [13] and further described in a recent IDMS guide [14]. The exact matching technique is an iterative process and for samples of unknown creatinine concentrations it may be necessary to make a preliminary measurement in order to determine the approximate creatinine concentration. Using this information samples and standards can be prepared to contain equimolar concentrations of analyte and labelled analogue. Ideally this results in the ratio measurements for both sample and standard being indistinguishable on a mass spectrometer. As the standard has been gravimetrically prepared the sample concentration can be easily calculated from its measured ratio. A large component of the overall measurement uncertainty for exact matching IDMS comes from the precision of replicate ratio measurements. In an effort to improve the measurement precision the quadrupole ion dwell time was reduced from 500 to 10 ms. The effect on measurement precision is shown in Fig. 7. While the ratio measurement precision increased with reduced dwell time the measured ratio increased. It is difficult to explain this phenomenon however it highlights the necessity for both sample and standard blends to be measured under exactly the same MS conditions.

Using the exact matching method of analysis the determined value of creatinine in serum differed from the KCRV by less than 0.2% with an expanded uncertainty ($k=2$) of less than 0.3%. The reduced error and uncertainty make this type of quantitative analysis ideal for certifying high quality reference materials.

### 3.11. Analysis of IMEP-17 serum

The IRMM launched IMEP-17, trace and minor constituents in human serum under the auspices of its international measurement evaluation programme (IMEP). This international intercomparison exercise involved the analysis of two serum samples (levels I and II) in order to determine their creatinine content. The same sample was also distributed as part of an international comparison between the National Mea-

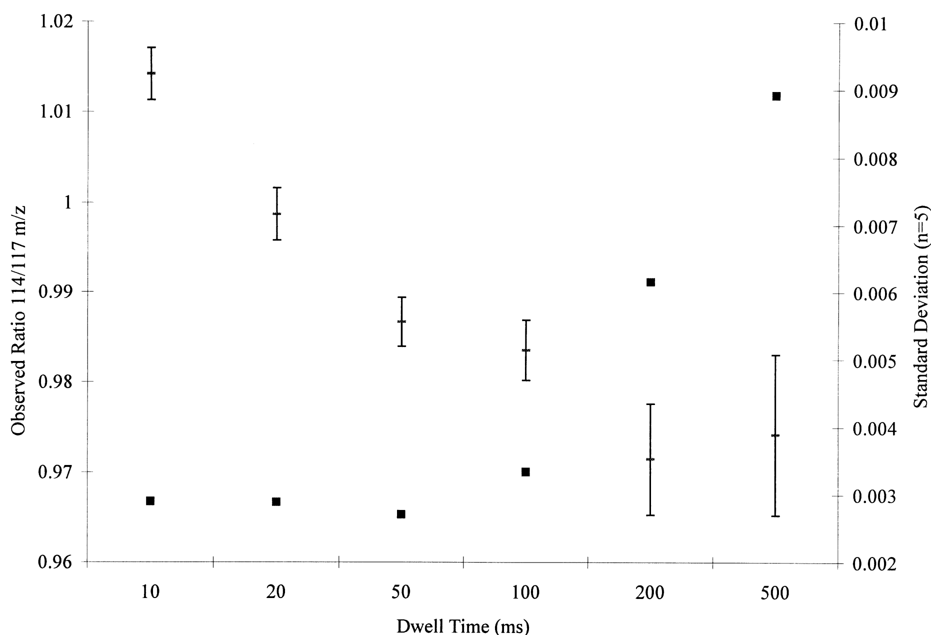


Fig. 7. Effect of quadrupole analyser dwell time on observed ratio (—) and measurement precision (■).

surement Institutes (NMIs) by the organic working group of the Comité Consultatif pour la Quantité de Matière (CCQM).

### *3.12. Sample preparation and extraction*

The concentration of creatinine in the two IMEP/CCQM samples and the NIST SRM were determined using the protein precipitation method of extraction and quantified using the exact matching double isotope dilution approach to calibration.

### *3.13. Determined concentration of creatinine*

The LC–MS determined concentrations of creatinine in the serum samples are shown in Table 1. The values obtained for the NIST CRM agree well with the certified value, which was obtained using the conventional ion-exchange/GC–MS approach. The values obtained for the IMEP-17/CCQM-K12 serum were submitted as part of a blind trial. The values obtained were in excellent agreement with the three other laboratories used to establish the reference value. Two of these laboratories used the conventional GC–MS approach. What was more interesting was that the calculated uncertainty for the ion-exchange GC–MS method and the relatively quick protein crash LC–MS method were almost equivalent. This was a significant finding as conventionally LC–MS has been considered poorer at quantitative analysis when compared with GC–MS methods, due to the inferior ion signal stability observed in LC–MS. However, a large proportion of the uncertainty for the GC–MS can be attributed to sample preparation steps (i.e., the separation of creatine and derivatisation) which are removed when using LC–MS methods.

## 4. Conclusions

A high-accuracy LC–MS method has been developed that is much quicker than the conventional GC–MS methods used for the high accuracy determination of creatinine in serum. A simple protein crash clean up has been shown to provide a suitable sample for LC–MS analysis. This compares favourably with the more involved selective clean ups necessary for GC–MS, which also required a derivatisation step. A blind trial, international interlaboratory comparison has shown that the LC–MS data is comparable in accuracy and uncertainty to that provided by the laboratories using the traditional GC–MS methods with an observed bias of less than 0.2% and an expanded uncertainty ($k=2$) of less than 0.3%.

The choice of calibration method and internal standard can dramatically affect the accuracy and precision of the final result. External calibration was shown to have sufficient accuracy and precision for most everyday applications. The inclusion of an internal standard was shown to improve the precision of the analysis. However, in non-matrix matched standards an internal standard whose mass and structure were similar to that of the analyte, was shown to reduce the accuracy of the method. Therefore, where non-isotopically labelled analogues are to be used as internal standards matrix matched standards may still be required.

## Acknowledgements

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Table 1
Summary of results for the analysis of creatinine by IDMS in the international blind trials

| Sample | Calculated concentration ($\mu g\ g^{-1}$) | Uncertainty ($\mu g\ g^{-1}$) ($k=2$) | Reference value ($\mu g\ g^{-1}$)[†] |
|---|---|---|---|
| SRM I (NIST) | 6.181 | 0.023 | 6.217±0.061 |
| SRM II (NIST) | 50.994 | 0.182 | 50.984±0.58 |
| IMEP-17 I | 8.193 | 0.016 | 8.217±0.066* |
| IMEP-17 II | 18.614 | 0.031 | 18.645±0.135* |

* Key comparison reference value as determined by CCQM-K12.
[†] Expanded uncertainty expresses at the 95% confidence level.

ment System Valid Analytical Measurement (VAM) programme.

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