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# Analysis of creatinine in mouse and rat serum by ion exchange high performance liquid chromatography for in vivo studies of renal function

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**Abstract**

An ion exchange high performance liquid chromatography method was developed for determining creatinine levels in both mouse and rat serum samples. Separation of creatinine from other serum components was achieved in 10 min using a 100 × 4.1-mm, 10 μm strong cation exchange column following acetonitrile precipitation of serum proteins. Incorporation of a guard cartridge placed in-line prior to the analytical column was employed to prevent interference from compounds used in renal disease animal trials. Creatinine levels in normal and diseased animals were accurately determined in the 0.01–10 mg/dL range, and average recovery of the method was approximately 85% for both mouse and rat serum. Addition of 0.5–1.0% acetic acid to the acetonitrile used for protein precipitation significantly improved creatinine recovery to above 97% in mouse serum. The method was used for routine preclinical diagnosis of rat and mouse model renal function, and for the evaluation of renal disease treatment efficacy.


*Keywords:* Creatinine; Renal disease; Acute renal failure; Cisplatin; Ion exchange; High performance liquid chromatography; Mouse serum; Cation exchange

## 1. Introduction

Renal diseases such as acute renal failure, chronic kidney disease, and end-stage renal disease can be detected, in part, by an increase in serum creatinine [1]. Creatinine is a normal metabolic breakdown product of creatine, and it is routinely excreted in the urine [2]. As kidney function becomes impaired, creatinine cannot be excreted, causing its level to rise in the blood. In patients with acute renal failure (sudden loss of kidney function), concentrations of serum creatinine increase to more than 1.5 mg/dL/day, with subsequent increases of 0.5 mg/dL/day. Normal levels of creatinine in human blood are approximately 0.6–1.2 mg/dL (adult males) and 0.5–1.1 mg/dL (adult females) [1].

The conventional method for determining creatinine levels in serum is spectrophotometric (Jaffé method) [3]. In alkaline solutions, creatinine forms a colored complex with picric acid, producing a reaction product that absorbs between 485 and 520 nm [4]. While this method provides accurate determination of creatinine in human serum, mouse serum contains components which interfere with creatinine measurement using the Jaffé method, and overestimations of up to five-fold have been reported [5].

As an alternative, high performance liquid chromatography (HPLC) has been investigated for measuring creatinine levels in animal models. However, there are several challenges associated with analyzing creatinine by HPLC. Creatinine has been shown to have three tautomers in $^{15}$N NMR [2], which can complicate selection of mobile phase composition and pH. Buffer type and concentration must also be considered carefully, as creatinine has a weak chromophore, and suffers from low sensitivity by ultraviolet (UV) detection. In addition, creatinine is very hydrophilic ($\log P = -0.63$), and therefore has poor retention in reversed phase (RP) HPLC. Further complications arise when analyzing creatinine in mouse and rat serum, as these matrices contain compounds with similar retention characteristics.

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Reproducible sample preparation and biological sample matrix complexity are also important considerations.

Despite the difficulty with analyzing creatinine by RP-HPLC, several reports have been published using this technique [6–10]. The mobile phases used in these studies are often phosphate-based, or consist of strong ion-pairing reagents such as sulfonic acid or triethylamine that may be detrimental to other detection methods (i.e., mass spectrometry). Additionally, none of these reports developed methodology specific to determining creatinine levels in rat and mouse serum. Liquid chromatography-mass spectrometry (LC–MS) methods developed for analysis of creatinine in serum using isotopically-labeled internal standards employed mobile phases comprised of 2–10 mM ammonium acetate [11–13]. This concentration of buffer in the mobile phase does not provide the optimal buffering capacity, thus impacting the reproducibility of creatinine analysis in a broad concentration range.

Ion exchange (IEX) HPLC has recently been shown to provide adequate retention for creatinine while still maintaining separation from interfering serum components in mouse serum. Yuen et al. and Dunn et al. developed IEX-based separation methods for creatinine analysis in mouse serum and plasma following protein precipitation with acidified acetonitrile [4,14]. Again, mobile phases contained low concentrations of sodium acetate, which is suitable only for selected levels of creatinine (range of 0.6–0.8 mg/dL). In addition, several sample preparation methods have been described in the literature for creatinine analysis in serum, and it is not clear what factors influence recovery from different biological matrices.

The current paper describes the development of a universal method for determining creatinine levels in both rat and mouse serum. Ion exchange HPLC was used for separation of creatinine from other serum components after protein precipitation with acetonitrile. A guard cartridge was placed in-line prior to the analytical column to eliminate effects from drug compounds dosed into serum samples. The method is sensitive and specific to creatinine detection, and covers a broader concentration range (0.01–10 mg/dL) than previously reported methods. This paper specifically addresses the previously described limitations of poor retention time reproducibility and accurate creatinine measurement at low levels in complex biological matrices. Recovery was consistently above 85% for serum sample sizes of 25 μL or less, and was significantly improved in mouse serum with the addition of 0.5–1.0% acetic acid to the acetonitrile used for protein precipitation. The method can routinely be employed during animal trials for discriminating changes in rat and mouse renal function.

## 2. Experimental

### 2.1. Chemicals and serum samples

Anhydrous creatinine and formic acid, ammonium salt, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water (18 MΩ-cm) was purified in-house using a NANOpure ultrapure water system (Barnstead, Dubuque, IA, USA). Formic acid, 98%, and HPLC grade acetonitrile (ACN) were purchased from EMD Chemicals (Gibbstown, NJ, USA). Glacial acetic acid (99.9%) was from J.T. Baker (Phillipsburg, NJ, USA). Trifluoroacetic acid (TFA), 99.5%, was purchased from Honeywell Burdick & Jackson (Muskegon, MI, USA), trifluoromethanesulfonic acid (TFMSA) hydrate, 99%, was from Alfa Aesar (Ward Hill, MA, USA), and anhydrous *N*-heptafluorobutyric acid (HFBA), 99.5%, was from Pierce (Rockford, IL, USA).

Rat serum was obtained from male, Sprague–Dawley (SD) rats, 350–400 g (Taconic Farms, Germantown, NY). Due to limited product availability, mouse serum for method development only was from female, BALB/c inbred mice, 20–24 g (Taconic Farms, Germantown, NY). Preclinical trials designed to evaluate renal disease induction and treatment used mouse serum from male, C57BL/6 mice, 25–30 g (Taconic Farms). Rats were induced with renal failure using cisplatin according to the published protocol [15]. Mice were injected intraperitoneally with 20 mg/kg cisplatin. Creatinine levels determined by the Jaffé method utilized the protocol and reagents supplied by Roche Diagnostics (Indianapolis, IN, USA). During animal trials, proprietary compounds were dosed into mouse and rat models to determine their effects on renal function. These compounds typically absorb in UV above 350 nm.

### 2.2. Sample preparation

A 1000 mg/dL (10 mg/mL) stock solution of creatinine was prepared weekly in 20 mM ammonium formate, pH 4.8. This stock solution was diluted daily to make ten standard solutions in the 0.01–10 mg/dL concentration range. A calibration curve was generated by injecting these 10 standards before and after each set of processed serum samples.

All serum samples were obtained fresh or frozen (−80 °C) within 48 h of collection. Samples were collected according to the published protocol [4]. Fresh serum was kept on ice until analysis, and frozen serum was thawed on ice for approximately 1–2 h prior to analysis. When appropriate, serum was spiked with standard solutions of creatinine prepared in 20 mM ammonium formate buffer, pH 4.8.

Prior to analysis by IEX-HPLC, samples were prepared by first adding 5 μL of 20 mM ammonium formate, pH 4.8, to 25 μL of serum in a clean 1.5 mL tube. The mixture was vortexed for 10 s and then centrifuged for 25 s at 13,000 rpm (Eppendorf centrifuge model 5417R, Westbury, NY). One milliliter of ACN containing 0–5% acetic acid was added, the mixture was vortexed again for 10–15 s, and centrifuged for 15 min at 13,000 rpm. Nine hundred ninety five microliter of the supernatant was transferred to another clean 1.5 mL tube, and the sample was dried completely in an SPD 1010 Speedvac system (Thermo Electron, Waltham, MA, USA) for 1.5–2 h. Samples were reconstituted in 100 μL of 20 mM ammonium formate, pH 4.8, and 20 μL was used for injection onto IEX-HPLC.

### 2.3. Chromatographic conditions

All separations were performed on an Alliance® 2690 separations module equipped with a 2487 Dual λ Absorbance

detector (Waters Corporation, Milford, MA, USA) set to 227 nm. A Hamilton PRP-X200, 100 mm × 4.1 mm, 10 μm cation exchange column (Hamilton, Reno, NV) served as the analytical column. A Hamilton PRP-X200 guard cartridge was placed in-line prior to the analytical column to capture compounds dosed into mouse and rat animal models. The mobile phase consisted of 20 mM ammonium formate adjusted to pH 4.8 with formic acid. Isocratic separation was performed at 1.0 mL/min for 10 min. Separation temperature was 35 °C. Injection volume was 20 μL.

## 3. Results and discussion

### 3.1. HPLC method optimization

Initial experiments attempted to analyze creatinine by RP-HPLC, so that MS detection could be employed for molecular weight confirmation (MW = 113 Da). The use of ion-pairing reagents (i.e., formic acid) that are compatible with MS detection, however, did not provide any retention. Creatinine was only slightly more retained on commercial $C_{18}$ columns with TFA and TFMSA ($k' < 0.5$) as the ion-pairing agents. In addition, other components from mouse serum interfered with creatinine detection at these short retention times. Stronger ion pairing agents such as HFBA gave adequate retention but significant UV interference, which affected sensitivity of the method.

Reproduction of the method published by Yuen et al. [4] was successful using the PRP-X200 cation exchange column for a very narrow range of creatinine concentrations. Under the reported conditions (5 mM sodium acetate buffer, pH 5.1), there was a retention time decrease of 1.25 min from injection of the 0.01 mg/dL creatinine standard to the 0.1 mg/dL standard (data not shown). This was most likely due to the low buffering capacity of the mobile phase, and increasing the concentration of sodium acetate did not improve the irreproducibility of creatinine retention.

Therefore, ammonium formate was chosen as the mobile phase at a slightly elevated concentration (20 mM). This system gave reproducible retention times (RSD < 1.4%) and UV peak area values (RSD < 2.0%) for all creatinine standards analyzed in the range of 0.01–10 mg/dL. Day-to-day variablility of the slope from the calibration curve over one week was less than 0.4%. In addition, very little buffer interference was observed with UV detection. A representative calibration curve can be found in Fig. 1. The linear dynamic range of the IEX-HPLC method covers three orders of magnitude, which includes the concentrations expected in both rat and mouse serum. Standards were injected daily before and after analysis of all serum samples, giving two points per standard for calibration. Fig. 2 shows overlaid chromatograms of a creatinine standard, unspiked mouse serum, and mouse serum spiked with a known concentration of creatinine. The chromatograms of unspiked and spiked mouse serum samples are free from interferences in the region where creatinine elutes (~6 min) after sample processing. All other serum components that are reconstituted along with creatinine after protein precipitation elute between 1 and 3 min. It is important to note that the peak for the 1.0 mg/dL spiked serum sample in Fig. 2B is smaller than the peak for the 0.5 mg/dL creatinine standard in Fig. 2C. This is because the serum sample was spiked before HPLC analysis, and was therefore diluted during sample preparation. The final creatinine concentration in the 1.0 mg/dL spiked sample in Fig. 2B is 0.242 mg/dL.

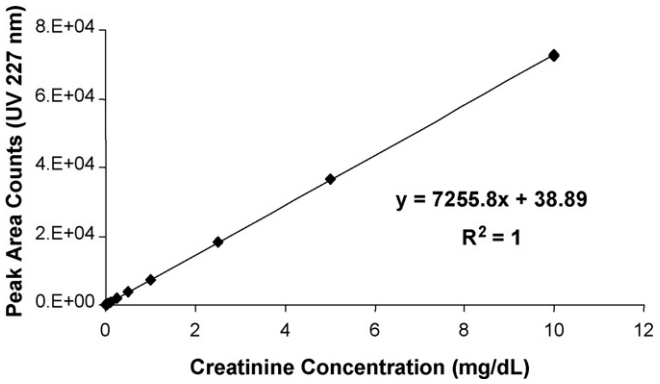


Fig. 1. Standard calibration curve for creatinine using IEX-HPLC. Ten standards were prepared in the 0.01–10 mg/dL range in 20 mM ammonium formate, pH 4.8. Two injections were performed for each standard. Hamilton PRP-X200 cation exchange column, 100 × 4.1-mm, 10 μm column. Mobile phase: 20 mM ammonium formate, pH 4.8. Isocratic separation for 10 min at 35 °C. 1.0 mL/min, UV 227 nm, 20 μL injection volume.

It is possible that compounds being dosed into rats and mice during animal trials can interfere with creatinine analysis, and thus compromise the accuracy of quantitation. Therefore, mixtures of creatinine and the proprietary drug candidates were injected directly onto the analytical column to observe their effects on creatinine analysis. Since most of the dosed compounds absorb above 350 nm in UV, they can be clearly detected in the chromatogram even if co-elution with creatinine occurs. It was discovered that most dosed compounds were strongly bound to the stationary phase of the cation exchange column under the method conditions. Such accumulation of the test compound(s)

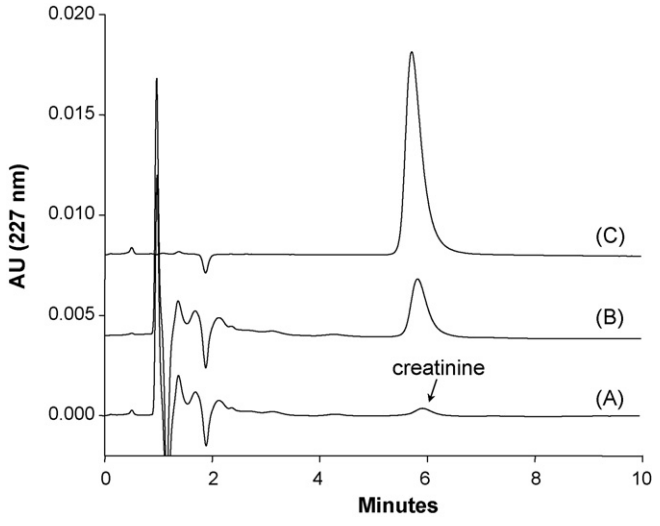


Fig. 2. Overlaid chromatograms of unspiked mouse serum (A), mouse serum spiked with 1 mg/dL creatinine (B), and 0.5 mg/mL creatinine standard (C). Serum was from female, BALB/c, 20–24 g mice. Samples were prepared according to Section 2. HPLC conditions as in Fig. 1.

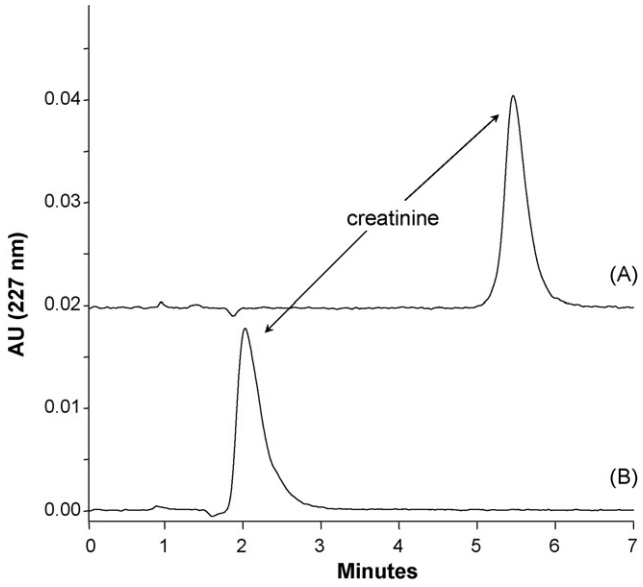


Fig. 3. Injection of a 1 mg/dL creatinine standard before (A) and after (B) the cation exchange column was washed with acetonitrile. HPLC conditions as in Fig. 1.

on the column will eventually result in a breakthrough of the adsorbed compounds, and subsequently interfere with creatinine analysis. High salt concentration washing steps were not sufficient for desorption of these compounds from the stationary phase. Only introduction of a strong organic modifier (ACN) was successful in eluting dosed compounds from the analytical column. However, this wash step not only increased the method run time, but also caused an irreversible shift in creatinine retention (Fig. 3). Attempts to regenerate the strong cation exchange column according to the manufacturer's protocol (repeated injections of 100 mM nitric acid) were unsuccessful.

In order to prevent eventual interferences from these compounds with analysis of creatinine, a disposable guard cartridge was placed in-line prior to the analytical column. The cartridge's ability to retain these drugs was tested by repeatedly injecting a 0.5 mg/mL solution (20 μL injection volume) of a proprietary compound onto the cartridge alone. Breakthrough of the drug was observed after 0.16 mg of total mass was injected onto the cartridge (Fig. 4). A rise in the baseline was observed after 0.15 mg of the drug was injected. Based on this information, and the estimated concentration of the compounds in serum samples (~0.026 mg/mL), the cartridge should be replaced every 300 injections.

Calculation of creatinine levels in serum was relatively straightforward. Once serum samples were processed and analyzed by IEX-HPLC, chromatograms were integrated and peak areas were obtained for creatinine in each sample. Eq. (1) was then used to calculate the concentration of creatinine present in the serum:

$$x = 4.14\left(\frac{y-b}{m}\right), \quad (1)$$

where $x$ is the concentration of creatinine in serum, $y$ is the peak area obtained from integration of the creatinine peak in IEX-HPLC, and $b$ and $m$ are the $y$-intercept and slope, respectively, obtained from the calibration curve (as in Fig. 1). The constant 4.14 is the dilution factor calculated from the sample preparation procedure, and is based on a starting serum volume of 25 μL. The dilution factor is 5.15 for 20 μL of starting serum volume and 6.83 for 15 μL of serum, assuming all other volumes in the sample preparation procedure remain constant.

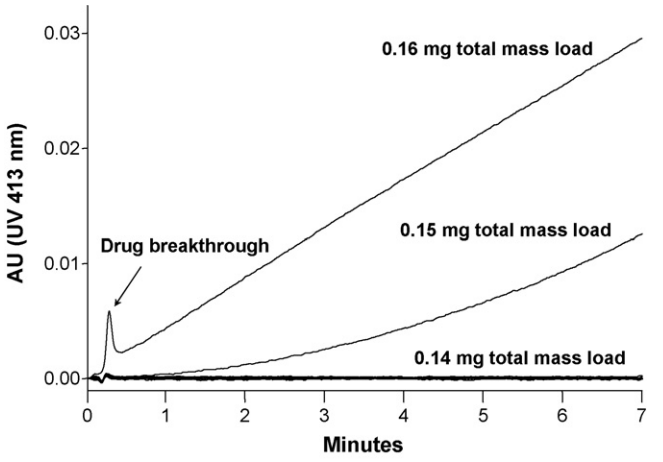


Fig. 4. Evaluation of the guard cartridge mass load capacity for one proprietary compound used for renal disease animal trials. The compound was prepared at a concentration of 0.5 mg/mL, and 20 μL was used for injection onto the cation exchange guard cartridge alone. HPLC conditions as in Fig. 1, except UV detection was 413 nm.

### 3.2. Optimization of sample preparation

One of the preliminary goals of this study was to determine the correlation of the IEX-HPLC method to the Jaffé method for analysis of creatinine in rat serum. This served as a validation of the IEX-HPLC method, due to the fact that rat serum generally does not contain components that interfere with accurate creatinine level determination using the Jaffé method [16–18]. Fig. 5 shows the correlation between the two methods for the same set of rat serum samples. For IEX-HPLC, rat serum samples were prepared and analyzed in triplicate, and proteins

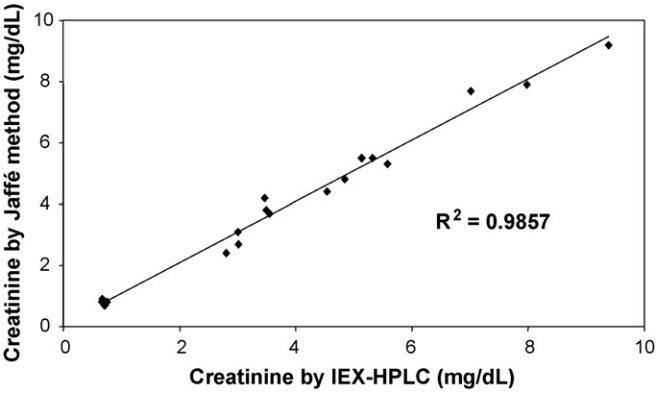


Fig. 5. Correlation between Jaffé method ($y$-axis) and IEX-HPLC ($x$-axis) for determination of creatinine levels in rat serum. For IEX-HPLC, samples were prepared and analyzed in triplicate (25 μL starting serum volume). Serum was from male, Sprague–Dawley, 350–400 g rats.

Table 1
Recovery for rat and mouse serum spiked with various concentrations of creatinine (25 μL starting serum volume)

| Serum sample description | SD rats | | | BALB/c mice | | |
|---|---|---|---|---|---|---|
| | Creatinine concentration (mg/dL)[a] | Experimental concentration increase (mg/dL)[b] | Recovery % | Creatinine concentration (mg/dL)[a] | Experimental concentration increase (mg/dL)[b] | Recovery % |
| Unspiked | 0.266 ± 0.008 | – | – | 0.169 ± 0.006 | – | – |
| 0.25 mg/dL spike | 0.494 ± 0.003 | 0.228 | 91.2 | 0.39 ± 0.01 | 0.22 | 88.0 |
| 0.75 mg/dL spike | 0.94 ± 0.02 | 0.68 | 90.7 | 0.900 ± 0.008 | 0.731 | 97.5 |
| 1.0 mg/dL spike | 1.19 ± 0.03 | 0.92 | 92.0 | 1.102 ± 0.005 | 0.933 | 93.3 |
| 1.5 mg/dL spike | 1.68 ± 0.03 | 1.42 | 94.7 | 1.58 ± 0.01 | 1.41 | 94.0 |
| 2.5 mg/dL spike | 2.71 ± 0.05 | 2.45 | 98.0 | 2.45 ± 0.02 | 2.28 | 91.2 |
| 4.0 mg/dL spike | 4.01 ± 0.05 | 3.75 | 93.8 | 3.87 ± 0.05 | 3.70 | 92.5 |

[a] All values in the table are based on the average of triplicate analyses.
[b] Calculated as the average concentration of the spiked serum minus the average concentration of the unspiked serum.

were precipitated with acetonitrile only. A good correlation was obtained between these two methods, confirming the utility of the IEX-HPLC method for accurately determining creatinine in rat serum.

In order to determine the recovery of the current method, several concentrations of creatinine standard were spiked into healthy rat and mouse serum (25 μL starting serum volume). Protein precipitation was performed using acetonitrile alone, and the supernatants were dried, reconstituted, and analyzed by IEX-HPLC. Concentrations of the unspiked and spiked rat and mouse serum samples were then determined using Eq. (1), and recovery was calculated as the difference between the amounts of creatinine in each of the spiked serum samples and the amount present in unspiked serum. Results from this experiment can be found in Table 1. All concentrations were determined with low standard deviation (RSD < 4%). Recovery was consistently above 85% for both rat and mouse serum. A linear relationship was observed between recovered and expected creatinine levels ($R^2 = 0.9989$ for rats; $R^2 = 0.9997$ for mice).

In some cases, additional creatinine measurements are required in the middle of animal trials. Hence, sample volume is limited because the animals cannot be sacrificed. This is especially true when animal trials are performed with mouse models, as each mouse affords only a small amount (~75 μL) of serum. Therefore, a spike and recovery experiment was performed with mouse serum using a reduced serum starting volume of 15 μL (Table 2). Even with a 40% reduction in starting mouse serum volume, recovery was still above 85% for all spiking concentrations, and a linear relationship was obtained for recovered and expected creatinine levels ($R^2 = 0.9996$).

It was reported that the use of acidified solvents allows for more complete protein precipitation from biological matrices [14,19]. The effect of using acidified acetonitrile for protein precipitation was investigated to determine the impact on creatinine recovery from serum. Spike and recovery experiments were again conducted using rat and mouse serum, and proteins were precipitated with acetonitrile containing 0.0, 0.5, 1.0, and 5.0% acetic acid. While the presence of acetic acid in acetonitrile during precipitation only slightly impacted creatinine recovery in rat serum, the effect was much more pronounced in mouse serum (Fig. 6). In SD rats, the best results were obtained with 0.5% acetic acid in acetonitrile for protein precipitation. For BALB/c mice, the presence of acid in acetonitrile improved recovery by about 10% over non-acidified acetonitrile. When rat and mouse serum samples were treated with 5% acetic acid during the precipitation step, a large and bulky pellet was observed. Particulates were present even after centrifugation, and supernatant transfer was not reproducible. Therefore, data for creatinine recovery from rat and mouse serum using 5% acetic acid in the precipitation step are not shown.

Finally, sensitivity of the method to small changes in creatinine concentration was determined for both rat and mouse serum. Healthy samples from both types of animals were spiked with 0.05, 0.10, 0.15, 0.20, and 0.25 mg/dL of creatinine. Based on the results shown in Fig. 6, all serum samples were precipitated with acidified acetonitrile (0.5% for SD rats; 1.0%

Table 2
Recovery for BALB/c mouse serum spiked with various concentrations of creatinine (15 μL starting serum volume)

| Serum sample description | Creatinine concentration (mg/dL)[a] | Experimental concentration increase (mg/dL)[b] | Recovery % |
|---|---|---|---|
| Unspiked | 0.143 ± 0.008 | – | – |
| 0.25 mg/dL spike | 0.36 ± 0.01 | 0.22 | 88.0 |
| 0.75 mg/dL spike | 0.85 ± 0.01 | 0.71 | 94.7 |
| 1.0 mg/dL spike | 1.011 ± 0.006 | 0.868 | 86.8 |
| 1.5 mg/dL spike | 1.488 ± 0.006 | 1.345 | 89.7 |
| 2.5 mg/dL spike | 2.37 ± 0.02 | 2.23 | 89.2 |
| 4.0 mg/dL spike | 3.78 ± 0.03 | 3.64 | 91.0 |

[a] All values in the table are based on the average of triplicate analyses.
[b] Calculated as the average concentration of the spiked serum minus the average concentration of the unspiked serum.

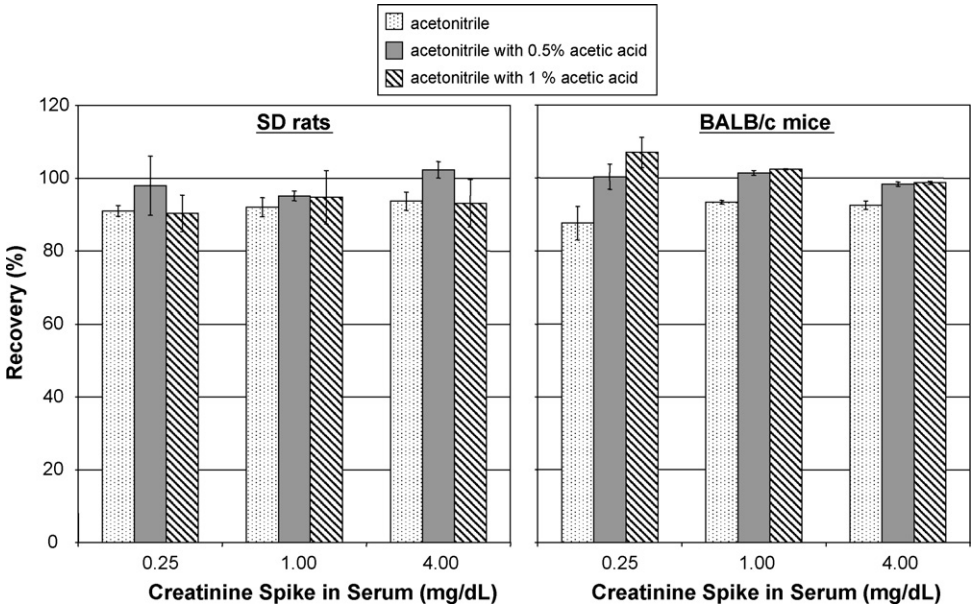


Fig. 6. Recovery comparison between three concentrations of acetic acid added to the acetonitrile used to precipitate proteins in serum from SD rats and BALB/c mice. Three representative creatinine spiking levels are shown for each animal type. All samples were processed in triplicate according to Section 2.

for BALB/c mice) in order to maximize creatinine recovery. The results in Table 3 demonstrate that even minute changes in creatinine concentration can be differentiated in both rat and mouse models. The recovered versus expected concentrations of creatinine exhibited a linear relationship with good correlation ($R^2 = 0.9918$ in rats; $R^2 = 0.9994$ in mice). The slightly lower recovery seen in BALB/c mice over SD rats can be attributed to the fact that mouse serum is a more challenging and heterogeneous matrix.

### 3.3. Application to animal trials

The IEX-HPLC method was used to determine creatinine levels in samples obtained during animal studies. In general, mouse models were used to study renal disease induction and treatment. Fig. 7 shows the results of one of the animal trials, in which seven C57BL/6 mice induced with renal failure were compared with an averaged baseline creatinine level. The baseline creatinine concentration of $0.24 \pm 0.01$ mg/dL was determined by analyzing serum from seven pre-bled healthy animals (in triplicate), and averaging all measurements to obtain one value (RSD = 3.6%). The animals were then induced with renal failure by intraperitoneal injection with cisplatin (20 mg/kg), and sacrificed 4 days later. Creatinine values obtained from disease-induced mice were all determined within 4% RSD. It is important to note that some variation in disease progression is observed on day 4 after animal sacrifice. However, this variation is expected when comparing individual mice. Typically, serum samples from animal trials are not homogenous, due to the presence of leftover clotting factors and other artifacts from whole blood processing. Results from the study in Fig. 7 demonstrate the reproducibility of sample preparation prior to IEX-HPLC analysis of creatinine, even for challenging sample matrices such as mouse serum. It also illustrates the ability of the method to discriminate between different stages of renal disease and evaluate efficacy of treatment.

Table 3
Recovery for serum containing 0.05 mg/dL incremental spikes of creatinine

| Serum sample description | SD rats | | | BALB/c mice | | |
|---|---|---|---|---|---|---|
| | Creatinine concentration (mg/dL)[a] | Experimental concentration increase (mg/dL)[b] | Recovery % | Creatinine concentration (mg/dL)[a] | Experimental concentration increase (mg/dL)[b] | Recovery % |
| Unspiked | 0.30 ± 0.02 | – | – | 0.215 ± 0.004 | – | – |
| 0.05 mg/dL spike | 0.35 ± 0.01 | 0.05 | 100.0 | 0.253 ± 0.006 | 0.038 | 76.0 |
| 0.10 mg/dL spike | 0.382 ± 0.001 | 0.082 | 82.0 | 0.301 ± 0.004 | 0.086 | 86.0 |
| 0.15 mg/dL spike | 0.432 ± 0.006 | 0.132 | 88.0 | 0.341 ± 0.003 | 0.126 | 84.0 |
| 0.20 mg/dL spike | 0.463 ± 0.006 | 0.163 | 81.5 | 0.384 ± 0.003 | 0.169 | 84.5 |
| 0.25 mg/dL spike | 0.522 ± 0.007 | 0.222 | 88.8 | 0.428 ± 0.004 | 0.213 | 85.2 |

[a] All values in the table are based on the average of triplicate analyses.
[b] Calculated as the average concentration of the spiked serum minus the average concentration of the unspiked serum.

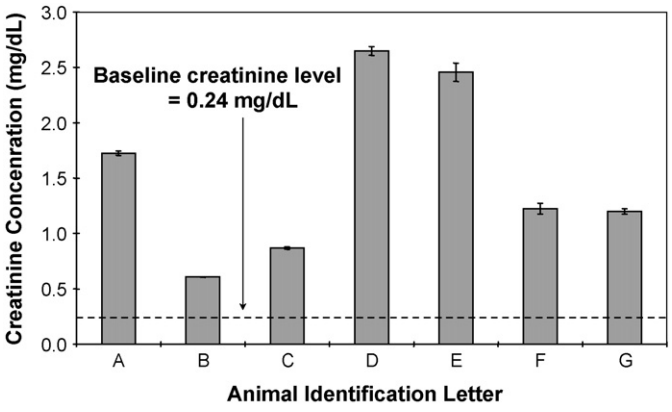


Fig. 7. Creatinine levels of serum obtained from seven C57BL/6 mice induced with renal failure during an animal trial. The baseline creatinine level was determined by averaging the creatinine levels of the same seven mice before disease induction with cisplatin. All measurements are the average of triplicate analyses.

## 4. Conclusions

An IEX-HPLC method has been developed for determining creatinine levels in both rat and mouse serum following protein precipitation with acetonitrile. The method is sensitive and specific to creatinine, and chromatographic separation is sufficient for eliminating interferences from other serum components within 10 min. Additional interferences from compounds dosed into serum were eliminated by placing a guard cartridge in-line prior to the analytical column. Average recovery of the method was consistently above 85% for low starting volumes of serum. With the addition of 0.5–1.0% of acetic acid to the acetonitrile used for serum protein precipitation, creatinine recovery from both mouse and rat serum was greater than 97%. Excellent correlation was observed between the Jaffé and IEX-HPLC methods for the same set of rat serum samples. Creatinine levels were reproducibly and accurately determined in the 0.01–10 mg/dL range, demonstrating the ability of the method to routinely diagnose renal function in animal models.

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