**Title page**

**Title: Serial ricinine levels in serum and urine after ricin intoxication**

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**Abstract**

Ricinine is an alkaloid present in the castor bean plant (*Ricinus communis*), and can be used as a biomarker for ricin poisoning. Serial ricinine levels are reported in serum and urine from a patient suffering from intentional ricin intoxication. The patient was brought to hospital 4 h after injection and oral intake of a castor bean extract, but died 38 h later despite intensive medical care. Ricinine was isolated from the samples by solid phase extraction and quantitatively determined by isotopic dilution liquid chromatography - mass spectrometry. The ricinine level in serum declined from 33 ng/mL to 23 ng/mL between 10 h and 29 h post exposure. Three urine samples collected from 12 h to 41 h after ricin intoxication showed ricinine concentrations in the range of 20-58 ng/mL. The creatinine corrected values (21-30 µg/g) indicated a concentration-time profile with a maximum ricinine level in urine between 12 and 29 h post exposure.


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## 1 Introduction

Ricin is a naturally occurring protein toxin and is among the deadliest poisons available. The fatal dose of ricin in humans is thought to be 5-10 µg/kg by injection (1) while ingestion has an $LD_{50}$ in the order of 1-20 mg/kg (2,3). The toxin is found in the castor bean plant (*Ricinus communis*) with the highest amounts (1-5% by weight) in the seeds (1). The castor bean plant is cultivated both as an ornamental plant and for its seeds, which contain up to 60 wt% castor oil (4). The castor oil is used in lubricants, polymers, coatings (5) and administered as a purgative in medicine (2) among other things. Also present in the castor seeds is an alkaloid called ricinine (Figure 1) with reported amounts of 0.3-0.8% by weight (6). The castor bean plant is the only known natural source of ricinine, and the alkaloid is co-extracted with ricin from the seeds of the plant. Hence the compound has been described as a biomarker for ricin exposure (6,7). In this work ricinine is utilised as a biomarker for conformation of assumed intentional ricin intoxication.

There are many suspected and some confirmed reports of ricin intoxication, and a summary of real cases in human and veterinary medicine was recently published by Worbs et al. (8). The identification of ricin in clinical samples has typically been performed by immunologically based methods (3,8,9). Animal experiments have shown that the toxin is rapidly absorbed in the body, however (10,11), and only three studies are found where ricin has been detected in urine or plasma from intoxicated patients (12-14). The difficulties associated with ricin determination in body fluids make the detection of ricinine a useful alternative for conformation of ricin intoxication. Ricinine is a small molecule (MW 164) easily extracted from clinical samples, and well suited for determination by liquid chromatography in combination


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with mass spectrometry (LC-MS). Darby et al. first proposed ricinine as a marker for complementary identification of ricin in unknown extracts (7). In 2005 Johnson et al. reported a method for trace level determination of ricinine in urine using solid phase extraction (SPE) in combination with LC-MS (6). The developed method was applied for determination of ricinine in urine from a person who had committed suicide by ricin intoxication. Since then, ricinine has been determined in urine and blood from a person who committed suicide by intentional ricin exposure (15), and in urine from patients with non-life-threatening symptoms (16,17). Moreover, two cases are described where ricin exposure to dogs has been confirmed by determination of ricinine (18,19).

We report serial ricinine levels in serum and urine from a hospitalised patient suffering from ricin intoxication, collected 10-35 h and 12-41 h after exposure, respectively. The analyte was isolated from the samples by SPE using a hydrophilic lipophilic balanced (HLB) column, and quantitatively determined by isotope dilution LC-MS. To our knowledge ricinine levels in human serum after ricin intoxication are reported for the first time.

## 2 Case History

A 21 year old man was brought to hospital approximately 4 h after having deliberately intoxicated himself with a castor bean extract. One hour later he vomited, had diarrhoea and progressive generalised pain. The situation gradually deteriorated, and despite intensive medical care the man died of circulatory collapse and progressing multiple organ failure 42 h after intoxication. More details from the case history are given by Heyerdahl et al. (20). The patient informed that the castor bean extract was

both injected and ingested. A puncture mark was seen in his left arm, and he had an odour of acetone from his mouth. Acetone is often used to remove castor oil from the beans prior to ricin extraction, and residual solvent in the extract is therefore common (15). Unfortunately, no remain of the castor bean extract was available for analysis, and the amounts injected and ingested were not known. It was therefore not possible to estimate the exposed ricin dosage.

## 3 Experimental

### 3.1 Chemicals, reagents and equipment

Ricinine (1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-pyridinecarbonitrile, > 98%, CAS No. 524-40-3) was purchased from Latoxan, Valence, France. Isotopically labelled ricinine (ring-$^{13}C_5$; cyano-$^{13}C$, > 98%, 100 µg/mL in acetonitrile (ACN)) was provided by LGC Standards AB, Borås, Sweden. Anhydrous creatinine was from Sigma Aldrich, St. Louis, MO, USA. Methanol (HPLC grade) was from Rathburn, Walkerburn, Scotland, and ACN (99.9%) was delivered by Merck KGaA, Darmstadt, Germany. Ammonium formate (AF, 98%) was purchased from BDH Laboratory Supplies, Dorset, UK. Laboratory type I water (classified according to the American Society of Testing and Materials, D1193-91) was delivered in-house by Maxima ultra pure water system from ELGA Labwater, Marlow, UK.

A stock solution of ricinine was prepared by dissolving 0.241 mg in 0.970 mL methanol, weighed with an accuracy of ±0.001 mg on an MT5 Microbalance weight from Mettler Toledo (Greifensee, Switzerland). The stock solution was further diluted in type I water to a working solution of 2.49 µg/mL. An internal standard (IS) working solution was prepared by diluting the 100 µg/mL isotopically labelled

ricinine solution to a concentration of 5.0 µg/mL in ACN. A creatinine stock solution was prepared by dissolving 10.0 mg in 10 mL type I water.

Sample preparation by SPE was performed on a Visiprep DL vacuum manifold from Supelco Inc, Bellefonte, PA, USA. The Oasis HLB SPE cartridges (60 mg) were delivered by Waters AS, Milford, MA, USA. Filter unit for 0.45 µm filtration was from Millipore, Billerica, MA, USA. Sterile polyethylene sample tubes were purchased from Sarstedt AG & Co. (Nümbrecht, Germany), and centrifugation was performed on a Heraeus centrifuge (DJB Labcare Ltd, England).

### 3.2 Serum and urine samples

The serum and urine samples received for analysis were collected at the hospital over a time period of 10-41 h after ricin exposure. No autopsy was performed, and hence no post mortem samples were available for analysis. Serum samples were collected approximately 10, 22, 29, 31 and 35 h after intoxication. The urine samples were collected approximately 12 h and 29 h post exposure, in addition to a mean 29-41 h sample. The samples were transported to our institute in frozen condition and stored at -20 °C until analysis.

### 3.3 Blank samples

Blank samples of serum and urine for calibration and quality control were voluntarily provided by healthy persons. Blood samples were provided by three men in a total of 8 aliquots of 8 ml each. The samples were collected in Vacuette serum collection tubes with clot activator and separation gel from Greiner Bio-One, Austria. The tubes were inverted ten times, allowed 30 min clotting time, and then centrifuged for 10 min

at 1770 x g. The serum was combined, shaken for 40 min on a Multi Reax test tube shaker (Heidolph Instruments, Schwabach, Germany) and divided into 4 aliquots of 5 mL each. Urine samples were collected from five men and two women, combined to approximately 300 mL and shaken overnight on an HS 501 laboratory shaker from IKA (Staufen, Germany). The urine was then filtered (0.45 µm) and divided into 10 mL aliquots. Blank serum and urine samples were stored in sterile polyethylene tubes at -20°C.

3.4 Sample preparation

The polymeric Oasis HLB SPE cartridge was chosen for use since it has proved successful for isolation of ricinine from blood and urine (15), and from feed extracts (21). All urine samples were filtered (0.45 µm) prior to sample preparation. Aliquots of 1 mL serum or urine were added 20 µL of the IS working solution and shaken for 1 min. The SPE cartridge was conditioned with 2 mL methanol followed by 2 mL type I water. Then, the 1 mL sample was loaded and slowly passed through. The cartridge was rinsed with 3 mL type I water and dried under vacuum for 5 min. Retained ricinine was eluted with 3 mL methanol and collected in 15 mL polyethylene tubes. The eluate was evaporated to dryness under a mild flow of nitrogen at 40 °C, re-dissolved in 500 µL type I water with 5% ACN, and transferred to autosampler vials.

For determination of the urine creatinine levels, the samples were diluted 1:1000 prior to analysis. Hence no sample cleanup was necessary. Urine samples of 100 µL were first diluted to 10 mL in type I water, and further 1:10 with the LC start gradient ($H_2O$/ACN 95/5 with 10 mM AF). The diluted samples were transferred to autosampler vials for LC-MS determination (n=1).

3.5 Liquid chromatography - mass spectrometry

Liquid chromatography was performed on an Ultimate 3000 RSCL (Dionex Corporation, Idstein Germany). Chromatographic separation was achieved on an Acclaim $C_{18}$ column (150 mm x 1.0 mm i.d., 3 µm particle size) from Dionex Benelux, Amsterdam, the Netherlands. Mobile phases were (A) type I water, (B) ACN and (C) 200 mM AF. Sample amounts of 1 µL were injected, and gradient elution was performed at a flow rate of 40 µL/min. The gradient was 5% B 0-2 min, 5-50 % B 2-8 min, 50-90% B 8-10 min. Channel C was 5% during the run, ensuring a constant buffer concentration of 10 mM AF.

The LC was coupled to a MicroTOF-Q II mass spectrometer from Bruker Daltonics, Bremen, Germany. Electrospray ionisation (ESI) was operated in positive ionisation mode with a capillary voltage of 4500 V and end plate offset of -500 V. The collision cell energy was 8.0 eV and collision RF peak-to-peak voltage was 150. Nitrogen for nebulising gas (0.8 bar) and drying gas (6.0 L/min, 190 °C) was provided by a high purity generator from Domnick Hunter, Durham, UK. Compressed $N_2$ (purity 6.0) was used as collision gas. Mass spectra were acquired in the *m/z* range of 50-500, and quantitative calculations were performed with peak areas of the extracted quasi molecular ions $[M+H]^+$ ± 5 mDa.

3.6 Calibration and quality control

Recoveries from the SPE procedure were investigated by adding ricinine to blank serum and urine samples at two different extraction steps. Ricinine was added from the working solution to a concentration of 50 ng/ml, and 1 ml aliquots (n=3) were


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treated as described in Section 3.4 and analysed by LC-MS. The obtained peak areas ($PA_{prior}$) were compared with those where ricinine was added at the same amount to the re-dissolved SPE eluates of blank serum and urine ($PA_{after}$), (n=3): Recovery (%) = 100% x $PA_{prior}/PA_{after}$.

Calibration samples were prepared by adding appropriate amounts of the ricinine working solution into blank serum and urine. Ricinine was added to serum at 15, 36 and 60 ng/mL (n=3), and to urine at 14, 36 and 60 ng/mL (n=3). After addition of IS (98 ng/ml), the calibration samples were subjected to the described SPE procedure and analysed by LC-MS. For determination of creatinine in urine, calibration samples were prepared at 0.50, 1.0 and 2.0 µg/mL (n=2) by diluting appropriate amounts of the creatinine stock solution in type I water containing 5% ACN and 10 mM AF. The samples were transferred to autosampler vials for LC-MS determination, and external calibration was performed.

## 4 Results and discussion

The main purpose of the present work was to confirm intentional ricin intoxication of a hospitalised patient by identification of ricinine as a biomarker. Several serum and urine samples were collected over a time period of 10-41 h post exposure, and concentrations of ricinine in these samples are reported.

### 4.1 Quality control

The method employed in this work was established ad-hoc by modifications of previously validated methods (6,15), with the purpose of qualitative and quantitative determination of ricinine in the received samples. Method validation was therefore


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limited to recovery from sample preparation and linearity within the relevant concentration range (semi-quantitatively determined in advance). Ricinine was identified by accurate mass measurement of $[M+H]^+$ ± 5 mDa, and by retention time matching with isotopically labelled ricinine. Hence, the use of MS/MS was not considered necessary for unambiguous identification of the compound. The extracted ion chromatograms (EICs) of ricinine and isotopically labelled ricinine from the analysis of a serum extract are shown in Figure 1, together with the structural formula of ricinine. Virtually no baseline noise was observed when the ions were extracted at the accuracy of ± 5 mDa, and the compound showed a distinct $[M+H]^+$ ion with no fragmentation at the present settings.

Figure 1 in approximately here

Ricinine was not found in the serum and urine provided by volunteers. Hence the compound was added at 50 ng/mL for determination of recoveries from the SPE procedure. High recoveries were obtained both from serum (94 ± 4% ) and urine (91±2%), proving the effectiveness of the polymeric HLB column. For quantitative determination of ricinine and creatinine, the concentrations of the calibration samples were accommodated to the levels of the analytes in the received samples. Hence, three point calibration curves were considered sufficient. Linear curve fit with no weighting gave $R^2$= 0.995 with a relative standard deviation (RSD) of the slope of 4.0% for ricinine in serum; $R^2$= 0.987, RSD = 2.4% for ricinine in urine; and $R^2$= 0.999, RSD = 1.3% for creatinine in urine. The limits of detection (LODs) for ricinine in serum and urine were estimated to 3 and 4 ng/mL, respectively, from linear regression of the peak heights of the calibration samples. If needed, the LODs could have been lowered

somewhat by adjusting the MS settings and by optimising the sample and reconstitution volumes in sample preparation.

### 4.2 Background levels of ricinine in general population

A prerequisite for the use of ricinine as a biomarker for ricin poisoning is that no background is present in the general population, or that the measured ricinine concentration is well above any expected background level. In a study by Johnson et al. in 2009, background levels of ricinine (< 4 ng/mL) were found in the urine from two out of 113 individuals (22). These low levels were probably caused by exposure of one or more castor oil products, for instance a laxative. Caution should therefore be taken before concluding with ricin poisoning from determination of ricinine at ppb- to sub ppb-levels in urine. No thorough studies have been found on background levels of ricinine in blood.

### 4.3 Ricinine levels in serum

Ricinine was determined in five serum samples collected from 10 h to 35 h after ricin intoxication. Figure 2 shows the concentrations (average values ± SD) plotted as a function of the elapsed time after exposure. Larger variations were measured in some of the parallel extracts compared to those of the calibration samples, even though IS correction was employed. This may be due to a more complex serum matrix from the patient, resulting in partial protein binding of the IS. At 30 h post exposure the patient was subjected to dialysis treatment. Since ricinine has a low molecular weight and is slightly soluble in water (7), it is reasonable to assume that it might be removed to some extent during dialysis (23). Hence, natural elimination of ricinine from serum is probably illustrated only by the three samples 10-29 h post exposure (33-23 ng/ml).

Most extraneous compounds in blood are eliminated by first-order kinetics, which gives an exponential decrease in concentration (24). Assuming this is the case for ricinine as well, the mathematical model of the data 10-29 h post exposure is $y = 39e^{-0.018x}$ ($R^2 = 0.98$), giving a half-life of ricinine in serum of 39 h. However, the data set within this time range is not strong enough to conclude whether the elimination actually follows an exponential curve, or if it may be linear. In any case, the elimination of ricinine was probably diminished due to ricin poisoning, which among other factors leads to renal dysfunction (1).

Figure 2 in approximately here

One study is found where ricinine has been measured in human blood after ricin intoxication. Coopman et al. reported a concentration of 2.3 ng/mL in the blood from a man who died approximately 35 h after having injected himself with a ricin solution, with an estimated dosage of 0.01 mg/kg (15). Any estimation of ricin exposure in the present case by comparison of the measured ricinine levels between these two cases would be speculative, since the ricin/ricinine level in different extracts may vary with extraction technique and amounts of the compounds in various seeds. We do note, however, that our measured ricinine level in serum 35 h post exposure was approximately seven times higher compared to the findings by Coopman et al.

### 4.4 Ricinine levels in urine

Three urine samples collected from 12 to 41 h after ricin exposure were investigated for the levels of ricinine. The concentrations of creatinine were measured as well, to correct for the dilution effect altering with urinary output (25). Table 1 shows the

measured concentrations of ricinine together with the creatinine corrected levels. All samples contained ricinine at concentrations well above earlier reported background levels (22). The creatinine corrected concentrations 12 h and 29 h after intoxication were at the same level, with a subsequent decrease in the mean 29-41 h sample. This implies a concentration-time profile with a maximum level between 12 and 29 h post exposure. In general, the creatinine corrected ricinine concentration was relatively stable during the measured time period after intoxication, which is consistent with earlier reported serial ricinine levels in urine (16).

Table 1 in approximately here

Two incidents are reported where ricinine was measured in post mortem urine samples after intravenous injection of castor bean extracts. A ricinine concentration of 5.7 ng/mL was measured in the case with an estimated ricin dosage of 0.01 mg/kg (15). In the other study ricinine was found at 4.2 ng/mL in urine (6), but the exposed dosage and time of death after exposure were not known. The higher ricinine levels in our findings indicate a higher exposure level of ricin, but it may also be a result of the additional oral intake of the extract. It has been shown that elevated levels of ricinine should be expected in urine when castor beans are ingested (16). In a case where six castor beans were chewed and swallowed, serial ricinine levels were measured with a maximum as high as 674 µg/g-creatinine 23 h post ingestion (16). Moreover, various procedures for extracting ricin from the castor beans may also give different levels of co-extracted ricinine.

## 5 Conclusions

Ricin intoxication was confirmed by determination of ricinine as a biomarker in serum and urine from a hospitalised patient. The biomarker was effectively isolated from the samples by SPE using a polymeric HLB cartridge (> 90% recoveries) and quantitatively determined by isotope dilution LC-MS. Elimination of ricinine from serum was slow (33-23 ng/ml 10-29 h post exposure) until start dialysis treatment at 30 h. To our knowledge this is the first study of serial ricinine levels in serum after ricin intoxication. The urine ricinine concentration was relatively stable (21-30 µg/g creatinine) between 12 h and 41 h post exposure. The slow elimination of ricinine suggests that the biomarker can be measured in both serum and urine for several days after ricin intoxication, in cases of survival.

## References


[1] Bradberry, S.M., Dickers, K.J., Rice, P., Griffiths, G.D., Vale, J.A. (2003) Ricin poisoning. *Toxicological Reviews*, **22**, 65-70.

[2] Audi, J., Belson, M., Patel, M., Schier, J., Osterloh, J. (2005) Ricin poisoning. *JAMA: The Journal of the American Medical Association*, **294**, 2342-2351.

[3] Musshoff, F., Burkhard, M. (2009) Ricin poisoning and forensic toxicology. *Drug Testing and Analysis*, **1**, 363-364.

[4] Barnes, D.J., Baldwin, B.S., Braasch, D.A. (2009) Ricin accumulation and degradation during castor seed development and late germination. *Industial Crops and Products*, **30**, 254-258.

[5] Mutlu, H., Meier, M.A.R. (2010) Castor oil as a renewable resource for the chemical industry. *European Journal of Lipid Science and Technology*, **112**, 10-30.

[6] Johnson, R.C., Lemire, S., Woolfitt, A.R., Ospina, M., Preston, K.P., Olson, C.T., Barr, J.R. (2005) Quantification of ricinine in rat and human urine: A biomarker for ricin exposure. *Journal of Analytical Toxicology*, **29**, 149-155.

[7] Darby, S.M., Miller, M.L., Allen, R.O. (2001) Forensic determination of ricin and the alkaloid marker ricinine from castor bean extracts. *Journal of Forensic Sciences*, **46**, 1033-1042.

[8] Worbs, S., Köhler, K., Pauly, D., Avondet, M.-A., Schaer, M., Dorner, M.B., Dorner, B.G. (2011) Ricinus communis intoxication in human and veterinary medicine - A summary of real cases. *Toxins*, **3**, 1332-1372.

[9] Ler, S.G., Lee, F.K., Gopalakrishnakone, P. (2006) Trends in detection of warfare agents - Detection methods for ricin, staphylococcal enterotoxin B and T-2 toxin. *Journal of Chromatography A*, **1133**, 1-12.

[10] Cook, D.L., David, J., Griffiths, G.D. (2006) Retrospective identification of ricin in animal tissues following administration by pulmonary and oral routes. *Toxicology*, **223**, 61-70.

[11] He, X.H., McMahon, S., Henderson, T.D., Griffey, S.M., Cheng, L.W. (2010) Ricin toxicokinetics and its sensitive detection in mouse sera or feces using immuno-PCR. *PLoS ONE*, **5**, e12858. doi:10.1371/journal.pone.0012858

[12] De Paepe, P., Gijsenbergh, F., Martens, F., Piette, M., Buylaert, W. (2005) Two fatal intoxications following ricin injection. *British Journal of Clinical Pharmacology*, **59**, 125-126.

[13] Kopferschmitt, J., Flesch, F., Lugnier, A., Sauder, P., Jaeger, A., Mantz, J.M. (1983) Acute voluntary intoxication by ricin. *Human Toxicology*, **2**, 239-242.

[14] Lim, H., Kim, H.J., Cho, Y.S. (2009) A case of ricin poisoning following ingestion of Korean castor bean. *Emergency Medicine Journal*, **26**, 301-302.

[15] Coopman, V., De Leeuw, M., Cordonnier, J., Jacobs, W. (2009) Suicidal death after injection of a castor bean extract (Ricinus communis L.). *Forensic Science International*, **189**, 13-20.

[16] Hamelin, E.I., Johnson, R.C., Osterloh, J.D., Howard, D.J., Thomas, J.D. (2012) Evaluation of ricinine, a ricin biomarker, from a non-lethal castor bean ingestion. *Journal of Analytical Toxicology*, **36**, 660-662.

[17] Smith, S.W., Graber, N.M., Johnson, R.C., Barr, J.R., Hoffman, R.S., Nelson, L.S. (2009) Multisystem organ failure after large volume injection of castor oil. *Annals of Plastic Surgery*, **62**, 12-14.

[18] Mouser, P., Filigenzi, M.S., Puschner, B., Johnson, V., Miller, M.A., Hooser, S.B. (2007) Fatal ricin toxicosis in a puppy confirmed by liquid chromatography/mass spectrometry when using ricinine as a marker. *Journal of Veterinary Diagnostic Investigation*, **19**, 216-220.

[19] Roels, S., Coopman, V., Vanhaelen, P., Cordonnier, J. (2010) Lethal ricin intoxication in two adult dogs: toxicologic and histopathologic findings. *Journal of Veterinary Diagnostic Investigation*, **22**, 466-468.

[20] Heyerdahl, F., Haavind, A., Jacobsen, D. (2012) A Fatal Case of Suicidal Injection and ingestion of self-extracted ricin. *Clinical Toxicology*, **50**, 290.

[21] Wang, Z.Y., Li, D.F., Zhou, Z.Q., Li, B.Y., Yang, W.J. (2009) A simple method for screening and quantification of ricinine in feed with HPLC and LC-MS. *Journal of Chromatographic Science*, **47**, 585-588.

[22] Johnson, R.C., Zhou, Y., Jain, R., Lemire, S., Fox, S., Sabourin, P., Barr, J.R. (2009) Quantification of L-abrine in human and rat urine: A biomarker for the toxin abrin. *Journal of Analytical Toxicology*, **33**, 77-84.

[23] Cantilena Jr., L.R., (2001) Clinical toxicology. In Klaassen, C.D. (ed.), Casarett and Doull's toxicology: The basic science of poisons, 6th edition, Chapter 32. McGraw-Hill, New York, NY, pp. 1109-1122.

[24] Medinsky, M.A., Valentine, J.L., (2001) Toxicokinetics. In Klaassen, C.D. (ed.), Casarett and Doull's toxicology: The basic science of poisons, 6th edition, Chapter 7. McGraw-Hill, New York, NY, pp. 225-237.

[25] Smith-Palmer, T., (2005) Clinical analysis. In Worsfold, P., Townshend, A. and Poole, C. (eds.), Encyclopedia of Analytical Science, 2th edition. Elsevier, Oxford, pp. 166-174.

Table 1 Levels of ricinine in urine from a ricin intoxicated patient, measured as absolute concentrations and creatinine corrected levels.

| Hours post exposure | Ricinine<br>ng/mL ± SD (n=3) | Ricinine/creatinine<br>µg/g ± SD (n=3) |
|---|---|---|
| 12 | 57.6 ± 0.6 | 28.1 ± 2.0 |
| 29 | 20.0 ± 1.4 | 29.8 ± 0.3 |
| 29-41 | 39.9 ± 4.0 | 21.3 ± 2.1 |


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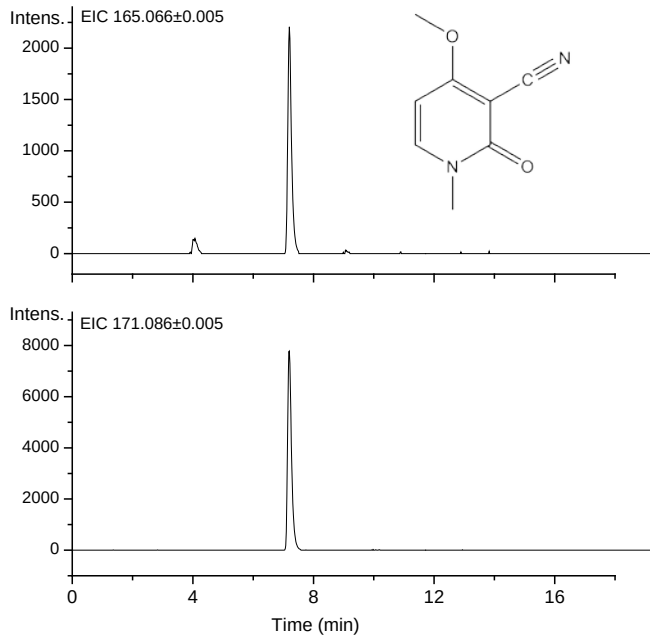


Figure 1 EICs ($[M+H]^+$ ± 5 mDa) from LC-ESI-MS determination of ricinine (upper chromatogram, calculated to 27 ng/mL) and IS (lower, 98 ng/mL) in a serum sample extract. The structural formula of ricinine is also shown.

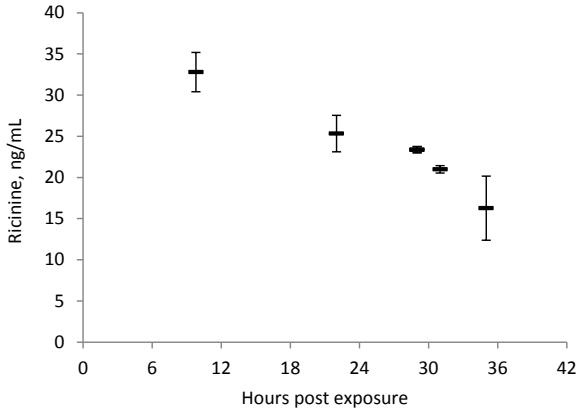


Figure 2 Concentration of ricinine in serum as a function of the elapsed time after ricin intoxication, plotted as mean values ± SD (n=2).