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Quantification of Ricinine and Abrine in Human Plasma by HPLC-MS/MS: Biomarkers of Exposure to Ricin and Abrin

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Abstract

Ricin and abrin are toxic ribosome-inactivating proteins found in plants. Exposure to these toxins can be detected using the biomarkers ricinine and abrine, which are present in the same plant sources as the toxins. The concentration of the biomarkers in urine and blood will be dependent upon the purification of abrin or ricin, the route of exposure, and the length of time between exposure and sample collection. Here, we present the first diagnostic assay for the simultaneous quantification of both ricinine and abrine in blood matrices. Furthermore, this is the first-ever method that may detect abrine in blood products. Samples were processed by isotope-dilution, solid-phase extraction, protein precipitation, and quantification by HPLC-MS/MS. This analytical method detects abrine from 5.00 to 500 ng/mL and ricinine from 0.300 to 300 ng/mL with coefficients of determination of 0.996 ± 0.003 and 0.998 ± 0.002 (n=22), respectively. Quality control material accuracy was determined to have less than 10% relative error, and precision was within 19% relative standard deviation. The assay's time-to-first result is three hours including sample preparation. Furthermore, the method was applied for the quantification of ricinine in the blood of a patient who had intentionally ingested castor beans to demonstrate the test was fit-for-purpose. This assay was designed to support the diagnosis of ricin and abrin exposures in public health investigations.

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Conflict of Interest Disclosure

The authors declare no competing financial interest.

Keywords

abrine; isotope dilution; quantitative HPLC-MS/MS; ricinine

Introduction

Ricin is listed as a schedule 1 toxic chemical by the Organisation for the Prohibition of Chemical Weapons (1). It has been used in both assassination attempts and suicides (2–4). Although used less frequently than ricin, abrin poisonings have been reported in suicide attempts and accidental ingestions (5, 6). Both of these protein toxins can be extracted from plants. Specifically, ricin (LD_{50} i.v. mice 2 – 3 μg/kg) is derived from *Ricinus communis* and abrin (LD_{50} i.v. mice 0.4 – 0.6 μg/kg) from *Abrus precatorius*. *Ricinus communis* is commonly known as the castor bean plant, and its seeds are used to extract castor oil, which is used as a food preservative and an over-the-counter laxative among many other uses. *Abrus precatorius* is commonly known as the rosary pea plant due to the use of its seeds for jewelry such as ornamental beads for rosaries. The fact that these plants and their seeds are readily available to the public and contain highly toxic proteins make them a concern for both accidental or intentional poisonings (7).

Methodology for the detection of ricin and abrin in body fluids have been previously developed using immunoassays as well as HPLC-MS/MS (8, 9). However, the proteins are rapidly absorbed following exposure, making the detection window brief (2, 8, 9). For example, after treatment with ricin, only about 7% of the injected dose was detectable in blood after 24 hours (8). In fact, the detection of ricin in clinical exposure specimens has only been reported three times (10–12). As an alternative to the detection of the protein toxins, small molecule biomarkers can be used to detect exposure to ricin and abrin. These small molecules, ricinine and abrine (Figure 1), are found in the same plant sources as ricin and abrin, respectively. As compared to protein analyses, the sample preparation and analysis of small molecule biomarkers is faster and provides a higher-throughput detection of exposure.

Both ricinine and abrine (Figure 1) have been detected in urine following exposure to ricin and abrin, respectively (5, 13–17). Ricinine, the biomarker for ricin exposure, can be detected in blood products (16–18), but to-date no method has been developed for the detection of abrine in blood products. Abrine, or *N*-methyl-L-tryptophan, is a structural analogue of the amino acid tryptophan with one additional methyl group. Tryptophan is present in human plasma (19), and *N*-methyl-L-tryptophan may follow similar transport and metabolism mechanisms.

Currently, exposures to ricin and abrin are determined using a method to quantify ricinine and abrine in urine (14, 20). We have now developed a method to quantify the same biomarkers in blood matrices to improve the capability to detect exposures to ricin and abrin regardless of whether urine or blood is collected from the patient. As in urine, the concentration of abrine and ricinine in plasma will vary as a result of the method of purification used to prepare the abrin and ricin, the route of exposure, and the length of time between exposure and sample collection. Although there is currently no literature threshold

for exposure, this method has been shown to be applicable up to at least 1000 ng/mL ricinine and abrine. This new assay is designed to support the detection of ricinine and abrine in blood matrices during public health investigations.

Materials and Methods

Safety Considerations

Universal safety precautions were followed for handling biological specimens such as blood products. Ricinine is toxic (mice i.p. LD_{50} = 0.34 g/kg) (21), and care should be taken to avoid inhalation, ingestion, skin, or eye exposure. Nitrile gloves should be worn during sample preparation.

Chemicals

HPLC grade acetonitrile, methanol, and water were purchased from Fisher Scientific (St. Louis, MO, USA). Formic acid (98%) was purchased from Sigma-Aldrich (Pittsburgh, PA, USA). Laboratory deionized water (18 MΩ-cm) was filtered in house using an Aqua Solutions Water Purification system (Jasper, GA, USA).

Stock solutions of ricinine (100 μg/mL in acetonitrile), abrine (100 μg/mL in water), $^{13}C_6$-ricinine (275 ng/mL in water), and $^{13}CD_3$-abrine (4125 ng/mL in water) were obtained from Cerilliant (Round Rock, TX, USA). Pooled plasma and individual plasma samples for unexposed matrix convenience set testing were purchased from Tennessee Blood Services (Memphis, TN, USA). These blood products used were acquired from a commercial source and did not meet the definition of human subjects as specified in 45-CFR 46.102 (f).

Preparation of calibrators and Quality Control materials

The internal standard (ISTD) mix was diluted to a final concentration of 11.0 ng/mL (0.0646 nM) $^{13}C_6$-ricinine and 165 ng/mL (0.742 nM) $^{13}CD_3$-abrine in water and stored in 500 μL aliquots at ≤ −20 °C. Calibrators and quality controls (QC) were prepared in filtered pooled plasma (0.22 μm polyethersulfone PES filter, EMD Millipore, Burlington, MA, USA), where calibrators ranged from 0.300 to 300 ng/mL (0.00183 to 1.83 nM) ricinine and 5.00 to 500 ng/mL (0.0229 to 2.29 nM) abrine (Table S1). QC concentrations were 200, 20.0, and 0.800 ng/mL (1.22, 0.122, and 0.00487 nM) ricinine and 350, 80.0, and 8.00 ng/mL (1.60, 0.367, and 0.0367 nM) abrine for QC-High (QH), QC-Mid (QM), and QC-Low (QL), respectively (Table S1). Calibrators and QCs were stored in 250 μL aliquots at ≤ −20 °C. The matrix blank (MB) consisted of pooled plasma.

Sample preparation

For all calibrators, QCs, MB, and samples, a 15 μL aliquot of ISTD mix was dispensed into each well of a deep 96-well plate. A 100 μL aliquot of each calibrator, QC, MB or sample was added to their respective wells. To each well, 85 μL of water was added. The plate was covered with adhesive foil and shaken at 1000 rpm for 1 minute. Solid-phase extraction (SPE) was then carried out on a vacuum manifold using a 96-well 60-mg Strata-X SPE plate (Phenomenex, Torrance, CA, USA). The sorbent was conditioned with 1000 μL of methanol and equilibrated with 1000 μL of water. The entire sample (~200 μL) was loaded onto the

sorbent bed. The sorbent was washed with 1000 μL of 5% methanol in water and then the analytes were eluted to a deep 96-well plate with 800 μL of acetonitrile. The eluent from each well was transferred to a 96-well protein precipitation plate (ThermoFisher Pierce, Waltham, MA, USA) and eluted to a deep 96-well plate. The samples were evaporated to dryness (~50 minutes) under nitrogen at 60 °C using a Porvair UltraVap96 (Porvair Sciences, Norfolk, UK). The samples were reconstituted in 100 μL of water, shaken at 1000 rpm for 0.5 minutes, and transferred to a 96-well PCR plate. The PCR plate was heat-sealed and then analyzed by HPLC-MS/MS.

HPLC-MS/MS

An HPLC gradient separation was carried out on an Agilent 1290 HPLC equipped with a 0.3 μm in-line filter. A Synergi 2.5 μm Polar-RP 100 × 2 mm column (Phenomenex, Torrance, CA, USA) was used for the separation, where mobile phase A (MPA) was 10% methanol in water with 5 mM formic acid and mobile phase B (MPB) was acetonitrile with 5 mM formic acid. The mobile phase composition was held at 7% MPB for 0.5 minutes and then ramped to 50% MPB over 1.5 minutes. The mobile phase was held at 50% MPB for 1.1 minutes and then returned to 7% MPB for 3.9 minutes for column equilibration. The total run-time for each injection was 7 minutes. A flow rate of 300 μL/minute was applied for the duration of the gradient elution. The injection volume was 10 μL. The needle was rinsed for 5 seconds using MPA. The autosampler was held at 8 °C and the column compartment was at 40 °C.

A triple quadrupole mass spectrometer (Sciex 4000 QTRAP, Framingham, MA, US) was used for analyte detection using positive mode ESI. The instrument was tuned and calibrated monthly using the Standards Chemical Kit (Chemical MS Chemical Kit 1, Low-High Conc PPGs, Sciex, Framingham, MA, US). The tuning and calibration was carried out using the automated instrument optimization option in Sciex Analyst v. 1.6. The following optimized instrument parameters were applied for the detection of the analytes and internal standards: collision gas, high; curtain gas, 10 psig; ion source gas 1, 75 psig; ion source gas 2, 10 psig; ion spray voltage, 5,500 V; temperature, 550 °C; entrance potential, 10 V; dwell time, 50 ms; a ‘unit’ resolution of 0.7 amu at full width half maximum; declustering potential (DP), collision cell exit potential (CXP), and collision energy (CE) were applied to each ion pair. Quantitation was determined by multiple-reaction monitoring, where ricinine quantification ion *m/z* 165.1 → 138.1, ISTD *m/z* 171.1 → 85.1, and confirmation ion *m/z* 165.1 → 82.1; abrine quantification ion *m/z* 219.1 → 188.1, ISTD *m/z* 223.1 → 188.1, and confirmation ion *m/z* 219.1 → 132.1. The DP and CXP for all ricinine transitions was 51 V and 8 V. The CE for the ricinine quantification ion was 25 eV, and 41 eV for the ISTD and confirmation ions. DP, CXP, and CE for abrine quantification and ISTD transitions were 46 V, 12 V, and 17 eV, and DP, CXP, and CE for the confirmation ion were 31 V, 16 V, and 29 eV.

Data acquisition and processing

Data acquisition and quantitative spectral analysis were carried out utilizing Sciex Analyst v. 1.6 build 3773. A linear regression was carried out for the peak area ratios of ricinine to $^{13}C_6$-ricinine and abrine to $^{13}CD_3$-abrine as a function of the theoretical concentration of the calibrator. An 1/x weighting was applied to the linear regression. Percent relative error was reported as $\%RE = [(C_e - C_t)/C_t] \times 100$, where C_e is the experimental concentration

determined from the calibration curve, and C_t is the theoretical concentration. Percent relative standard deviation was calculated as $\%RSD = (SD - C_{avg}) \times 100$, where C_{avg} is the average concentration calculated, and SD is the standard deviation. QC characterization was completed over 5 weeks, with three analysts participating and 22 curves analyzed with no more than two curves per day. The acceptable analyte QC ranges were determined using the Centers for Disease Control and Prevention's multi-rule quality control system (22).

The theoretical LODs for ricinine and abrine were determined by estimating the 95th percentile of the blank distribution (n=22) by the equation $LoB = \mu_B + 1.645\sigma_B$, where LoB is the limit of blank, μ_B is the mean calculated concentration for the matrix blank, and σ_B is the standard deviation of the calculated concentrations for the matrix blank (23, 24). The limit of detection was then calculated by $LoD = LoB + 1.645\sigma_s$, where LoD is the limit of detection, LoB is the limit of blank, and σ_s is the standard deviation of the calculated concentrations for the lowest calibrator (23, 24). The theoretical LOD in plasma for ricinine was determined to be 0.298 ng/mL and 2.11 ng/mL for abrine.

Convenience Set and Application

A commercial convenience set of 100 individual human plasma samples presumed unexposed to castor seeds and rosary peas was analyzed in order to evaluate expected human concentrations. Additionally, ricinine concentrations in both plasma and urine were determined for a patient who intentionally ingested 6 castor beans. The plasma from the exposure case was prepared by isotope- dilution, SPE, and protein precipitation as described above. The urine was evaluated using the previously published method for ricinine and abrine quantification in urine (14, 20). Briefly, 30 μL of ISTD mix was added to each well. A 200 μL aliquot of calibrator, QC, MB (pooled urine), or sample was added to their respective wells. SPE was carried out according to the protocol above, but no protein precipitation was performed for the urine sample preparation. Calibrators and QCs for ricinine and abrine in urine were obtained from Cerilliant (Round Rock, TX, USA). Calibrators were diluted 1:25 with filtered pooled urine (0.22 μm PES filter) obtained from Tennessee Blood Services. The urinary creatinine concentration was also determined for the patient's urine sample in order to obtain the creatinine-corrected ricinine concentration. The exposure specimens did not have identifying key information and therefore did not meet the criteria for IRB approval of research under 45-CFR 46.111.

Results and Discussion

Separation and Detection

Ricinine and abrine are small molecules (Figure 1) that are chromatographically retained in reversed-phase separations. Using the linear gradient described earlier, ricinine eluted at 2.32 ± 0.02 minutes, and abrine eluted at 2.06 ± 0.04 minutes for 22 analyses (Figure 2). No interfering peaks were observed in the chromatogram for any of the six mass transitions monitored. The concentration at which the quantification and confirmation ions were detected at a signal-to-noise ratio greater than 3 was used as the lowest reportable limit (LRL) for the calibration curve. For ricinine, the LRL is 0.300 ng/mL, which corresponds to an on-column mass of 0.03 pg (18.3 amol) for a 10 μL injection volume. The average signal-

to-noise ratio for the ricinine LRL during characterization was 9.18. Abrine has been previously reported to be present in urine at low levels in the general population (20). As such, the LRL for abrine in urine was adjusted to 5.00 ng/mL to avoid non-critical exposure results (20). Similarly, the LRL for abrine in plasma is 5.00 ng/mL, which corresponds to an on-column mass of 0.5 pg (229 amol). The average signal-to-noise ratio for the abrine LRL during characterization was 178. Low levels of abrine are observed in pooled plasma, but the peak height for the LRL is over a factor of 10 greater than the peak height for the MB.

Linearity, Precision, and Accuracy

The peak area ratio of the quantification ion to the ISTD was linearly proportional to the expected concentration of ricinine in plasma over the range of 0.300 to 300. ng/mL. Over this linear range, the average (n=22) coefficient of determination (R^2) was 0.998 ± 0.002 with a line equation of $y = (0.773 \pm 0.055)x + (0.030 \pm 0.083)$. For abrine, the peak area ratio as a function of the expected concentration was linearly proportional from 5.00 to 500. ng/mL. Over this linear range, the average (n=22) R^2 was 0.996 ± 0.003 with a line equation of $y = (0.154 \pm 0.032)x + (0.084 \pm 0.16)$.

The accuracy and precision for the QC characterization (n=22) were calculated as a %RE and %RSD, respectively (Table 1). For ricinine calibrators and QCs, the %RE was ≤ 5.5% and the %RSD was ≤ 15%, except for the LRL and QL where the %RSD was ≤ 19%. For abrine calibrators and QCs, the %RE was ≤ 12% and the %RSD was ≤ 13, except for the LRL and QL where the %RSD was ≤ 19%. These values are in agreement with the FDA guidance for bioanalytical method validation which states that the accuracy and precision should be within 15% throughout the dynamic range, except near the LOD where the accuracy and precision may be up to 20% (25). The LRL and QL for both analytes is nearly three times the calculated theoretical LOD. Therefore, the method's accuracy and precision follow the guidelines in the FDA's guidance for bioanalytical method validation and demonstrate applicability for the analysis of clinical samples (25).

A dilution protocol was also evaluated for specimens above the highest reportable limit of the method (300 ng/mL ricinine, 500 ng/mL abrine). Pooled urine and plasma were enriched to a concentration of 1000 ng/mL ricinine and 1000 ng/mL abrine. These enriched matrices were diluted 1:10 with HPLC grade water (n=4). The accuracy and precision of the dilution protocol are provide in Table 2.

Recovery and Matrix Effects

The percent recovery for the sample preparation was determined by comparing the peak area for a 20 ng/mL abrine and ricinine in plasma sample (pre-spike) to the peak area of a blank plasma sample that was reconstituted in a 20 ng/mL solution of abrine and ricinine (post-spike). The percent recovery therefore is a recovery of the entire sample preparation, including SPE, protein precipitation, and sample evaporation. The recovery was calculated for 10 individual plasma samples, and the average recovery was 88 ± 6% for ricinine and 76 ± 4% for abrine.

The matrix effects for both analytes were evaluated by making an injection of prepared pooled plasma while infusing a neat solution of ricinine and abrine (26). The quantitative

mass transitions were plotted as a function of retention time and the results of this experiment are plotted in Figure S1. The extracted ion chromatogram for each analyte is overlaid in grey to show the expected retention time. Matrix effects are clearly observed at multiple points throughout the chromatogram for both analytes, but are not observed at a retention time overlapping with either analyte. An additional experiment was carried out with 10 individual plasma samples enriched to 20 ng/mL ricinine and abrine to ensure that matrix effects would not affect the accuracy and precision of the method. For ricinine, the average %RE was 9.9% and the %RSD was 11%. For abrine, the average %RE was −1.9% and the %RSD was 7.9%. These precision and accuracy values follow the guidelines in the FDA's guidance for bioanalytical method validation (25) and do not differ from the observed accuracy and precision measured for QCs prepared in pooled plasma.

Stability and Ruggedness

The stability of ricinine and abrine QCs was evaluated over a period of 30 days at three different temperatures as well as after three freeze-thaw (F/T) cycles. Three aliquots of each QC were stored at 4, 22, and 37 °C for 30 days and then quantified alongside a fresh calibration curve with QCs that had been stored at ≤ −20 °C. The stability of prepared plates was also evaluated. Three sets of QCs were prepared and quantified using a single calibration curve. The plate was left in the autosampler (8 °C) for 24 hours, and the wells were reinjected. Similarly, each QC was subjected to three F/T cycles and then quantified. The results of this stability study are provided in Table 3. Compared to the characterized QC concentrations for QCs stored at ≤ −20 °C (Table 1), these data indicate that the QCs in plasma remain stable for at least 30 days at up to 37 °C, 24 hours after preparation in the autosampler, and three F/T cycles.

QCs for both analytes under each stability condition tested remained within three standard deviations of the mean obtained during QC characterization. This stability indicated that the analytes are stable when stored in sealed cryovials for a month at up to 37 °C. However, for long-term storage, it is recommended that these materials are stored at ≤ −20 °C. It should be noted that in water, both labeled and unlabeled abrine degrades if left in open air. Therefore, the internal standard mix should remain sealed except when actively pipetting from the vial.

The ruggedness of the analytical method was explored during method validation (Tables S2–S3). Specifically, the QCs were quantified using an injection volume of 5 and 15 μL, a column compartment temperature of 20 and 60 °C, using a red PEEK tubing in place of the PEEKsil tubing, 0% methanol in mobile phase A, and 0% formic acid in mobile phase A and mobile phase B. Each changed parameter was treated as an individual unknown and compared to the characterized means were obtained from the n=22 characterization. Many of these changes resulted in a shift in the observed retention time of the analytes. However, the analytes were still quantified within three standard deviations of the mean concentration obtained during QC characterization. Three different column lots (5371–11, 5371–0015, 5371–0017) and four different SPE lots (S300–0204, S300–0217, S300–0224, S300–0226) were used during method characterization/validation and were not observed to affect the experimental QC concentration.

Clinical Relevance

Ricinine was not observed in a convenience set of 100 individual plasma specimens presumed unexposed to castor seeds. Although not observed in the convenience set analyzed in this study, baseline exogenous levels of ricinine may be observed in the plasma of individuals that have not been exposed to ricin. This is due to the presence of ricinine in pharmaceutical and cosmetic products containing castor oil (27). In a previous study, a larger convenience set of 989 individual urine specimens was analyzed, and ricinine was present in 1.2% of the specimens (28). The LRL for ricinine in urine and serum was determined to be fit-for-purpose at 0.300 ng/mL based on observations in unexposed convenience sets, animal studies, and previously reported exposures (13–17, 28). Example chromatograms of ricinine in a representative convenience set sample as compared to the LRL are plotted in Figure 3A.

Baseline-level contributions to abrine were observed in 85% of the 100 individual plasma specimens presumed unexposed to rosary peas. These results were consistent with values previously reported in urine, where abrine was observed in 87% of 113 specimens (20). The LRL for abrine at 5.00 ng/mL in both urine and serum was determined to be fit-for-purpose based on these individual unexposed specimens, animal studies, and previously reported exposures (5, 20). Example chromatograms of abrine in a representative convenience set sample as compared to the LRL are plotted in Figure 3B. Based on animal studies, exposures near the LD_{50} of abrin would result in urinary concentrations at least 40 times greater than the lowest reportable limit (20). In an exposure where urine was collected within 48 hours of the ingestion of rosary peas, over 700 ng/mL of abrine was observed (5). Abrine has not been previously identified in blood products following exposure but may follow similar transport and metabolism mechanisms as the amino acid tryptophan which is present in plasma (19).

The concentrations of ricinine and abrine in blood or urine matrices will vary based on route of exposure and the length of time between exposure and sample collection. These biomarkers should be evaluated within the context of the specific case, other exposures, and the clinical symptoms of the patient. The detection of both ricinine and abrine by this method supports public health investigations by providing a blood biomarker of exposure for ricin and abrin, respectively.

Application of the Method

Although the abrine biomarker has not been observed in a case study with a plasma specimen to-date, the identification of the abrine biomarker is expected with this newly developed diagnostic assay in the event of an abrin exposure. The ricinine biomarker, however, was quantified from a case study with a plasma specimen obtained from a patient who intentionally ingested six castor beans. A urine sample obtained from the same patient was also analyzed using a previously published method (14, 20). The patient experienced symptoms of significant GI discomfort and moderate diarrhea. It is unknown when the plasma and urine samples were collected following the ingestion of the castor beans. The concentration of ricinine was 63.7 ng/mL in plasma, 1470 ng/mL in urine, and the creatinine-corrected concentration was 583 ng/mg-cr. Since the urine sample was above the highest reportable limit for the method (300 ng/mL), a 1:10 dilution (urine:water) was

prepared for quantitative analysis. The urinary ricinine concentration is consistent with a previous case reporting 1400 ng/mL ricinine (591 ng/mg-cr) at 14 hours post-ingestion of six castor beans (13).

Conclusion

For the first time, a quantitative HPLC-MS/MS method was developed for the simultaneous monitoring of ricinine and abrine in human blood matrices. Furthermore, this is the first-ever method that may detect abrine in blood products. These biomarkers of ricin and abrin can be quantified from 0.300 to 300 ng/mL and 5.00 to 500 ng/mL, respectively. A 1:10 dilution (at least) with water allows the quantification of samples above the assay's highest reportable limits. QC material accuracy was within 10% RE, and precision was within 19% RSD for both analytes. Including sample preparation and instrument analysis of calibrators and QC materials, this assay provides a time-to-first result of approximately three hours. Moreover, the method presented herein utilizes the same HPLC-MS/MS platform as the previously published method for the quantification of ricinine and abrine in urine. Therefore, both matrices can be analyzed on the same instrument. The newly developed assay was demonstrated using not only 100 plasma samples with no expected ricin or abrin exposure but also with a case specimen from a patient who ingested six castor beans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclaimer

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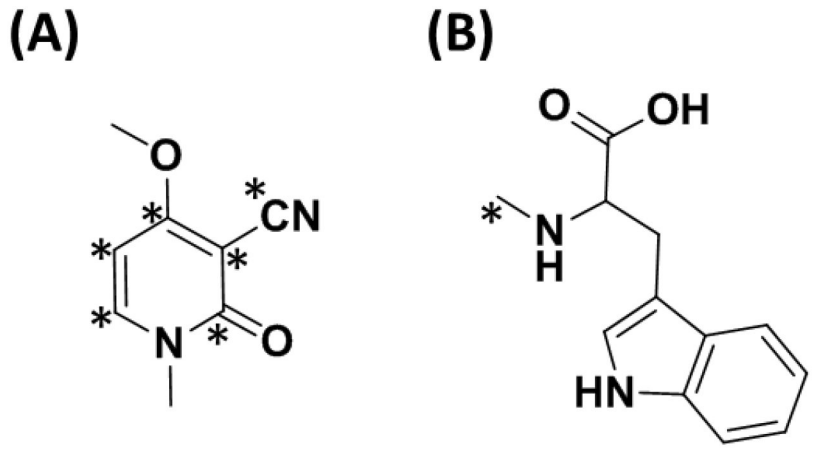

Figure 1.

Structures of (A) ricinine and (B) abrine. Asterisks denote isotope-label sites for internal standards $^{13}C_6$-ricinine and $^{13}CD_3$-abrine.

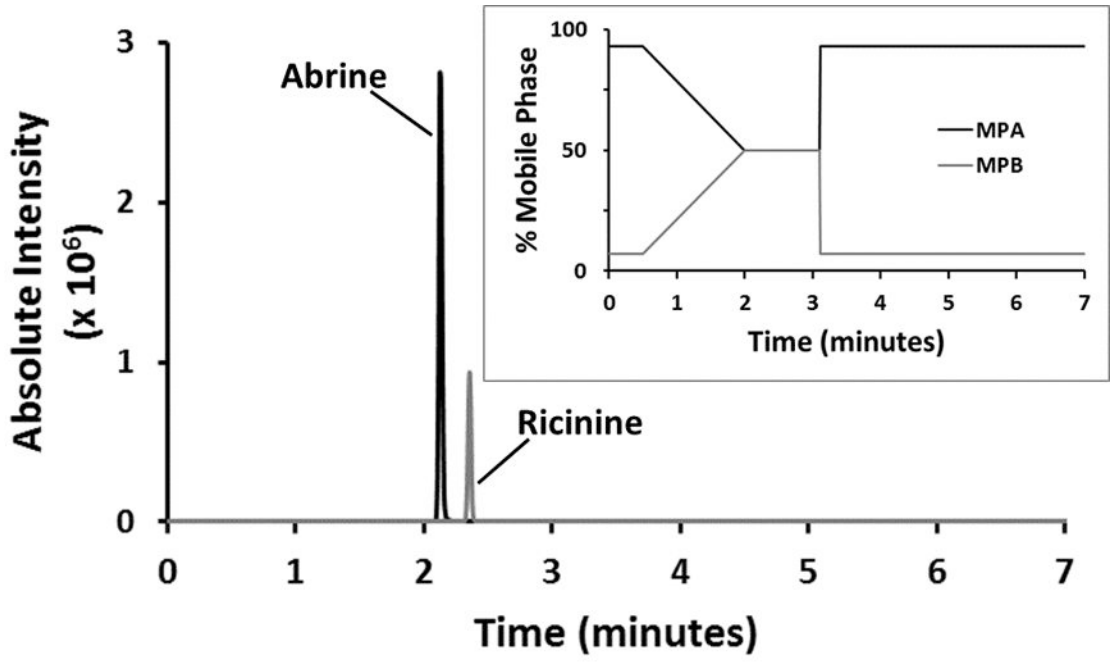

Figure 2.

HPLC separation of ricinine and abrine in plasma where the chromatogram of QH (200 ng/mL ricinine and 350 ng/mL abrine) is plotted; the gradient mobile phase composition is plotted in the insert.

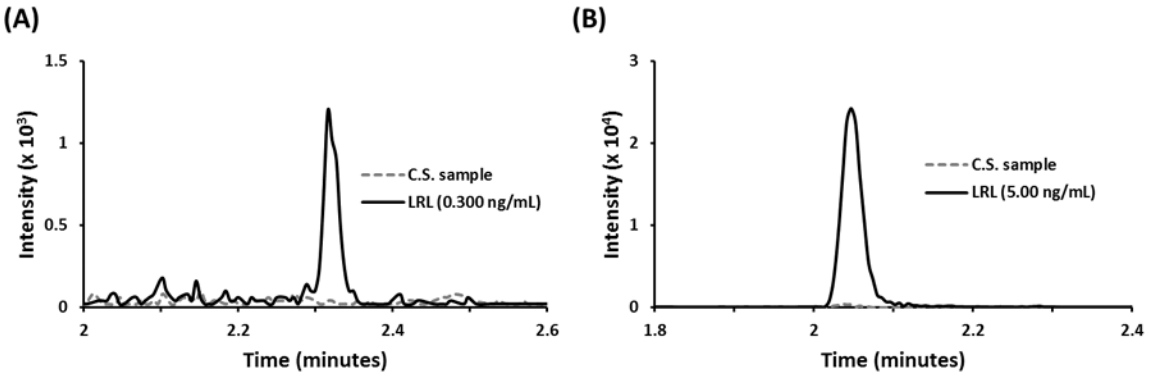

Figure 3.

Representative chromatograms of an individual plasma convenience set sample (C.S. sample, grey dashed line) as compared to the LRL (black line) for (A) ricinine and (B) abrine.

Table 1.

Accuracy (%RE) and precision (%RSD) for ricinine and abrine QC pools.*

Analyte	QC	Conc (ng/mL)	%RE	%RSD
	QH	199 ± 19	-0.55	9
Ricinine	QM	20.8 ± 2.3	3.8	11
	QL	0.831 ± 0.155	3.9	19
	QH	347 ± 47	-0.88	13
Abrine	QM	88.2 ± 8.6	10.	10.
	QL	7.72 ± 1.50	-3.5	19

* n=22, except abrine QM, n=21

Table 2.

Accuracy and precision of ricinine and abrine above the highest reportable limit.*

Analyte	Matrix	Conc (ng/mL)	%RE	%RSD
Ricinine	Urine	894 ± 39	-11	4.4
	Plasma	911 ± 56	-8.9	6.2
Abrine	Urine	938 ± 38	-6.2	4.1
	Plasma	1045 ± 44	4.5	4.2

*urine and plasma diluted 1:10 with HPLC grade water (n=4)

Table 3.

Stability of ricinine and abrine QCs in plasma.*

Analyte	QC Level	30 Day Stability			Post-Prep Stability** (8 °C)	3 F/T Stability
		4 °C	22 °C	37 °C		
Ricinine	QH	184 ± 40	220 ± 3	200. ± 34	207 ± 29	183 ± 33
	QM	21.8 ± 2.6	19.7 ± 3.2	22.2 ± 5.5	19.5 ± 0.9	16.9 ± 5.6
	QL	1.01 ± 0.11	0.928 ± 0.080	0.980 ± 0.099	0.852 ± 0.086	0.810 ± 0.104
Abrine	QH	399 ± 30	455 ± 51	390. ± 95	362 ± 31	324 ± 45
	QM	96.2 ± 9.1	87.4 ± 15.1	85.9 ± 17.2	86.5 ± 4.1	67.3 ± 14.6
	QL	7.42 ± 3.13	7.09 ± 1.55	5.12 ± 1.17	7.86 ± 0.12	7.75 ± 0.88

* n=3 for each QC at each condition, control conditions (≤ −20 °C, n=22) provided in Table 1

** QCs reinjected after 24 hours in autosampler

Table 4.

Concentrations of ricinine in the urine and plasma in an individual, following the ingestion of castor beans.

Matrix	Ricinine (ng/mL)	Creatinine (mg/dL)	Creatinine-Corrected ng/mg-cr
Plasma	63.7	NA	NA
Urine	1470	252	583