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Validated hydrophilic interaction LC–MS/MS method for determination of N-methyl-2-pyrrolidinone residue: Applied to a depletion study of N-methyl-2-pyrrolidinone in swine liver following intramuscular administration of drug N-methyl-2-pyrrolidinone formulation

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ABSTRACT

A hydrophilic interaction high-performance liquid chromatography coupled with tandem mass spectrometry method for determination of N-methyl-2-pyrrolidinone in swine liver was developed and validated. After the fortification of N-methyl-d_3-2-pyrrolidinone-d_6 as the deuterium-labeled internal standard, N-methyl-2-pyrrolidinone in swine liver was extracted by acetonitrile and the supernatant was led through a C18 + WAX mixed-mode SPE cartridge for removal of the matrix interferences. The final eluate was acidified by formic acid and then injected onto a 3 μm 15 cm × 2.1 mm TX column for hydrophilic interaction chromatographic analysis. Mass spectrometry detection was carried on a PE Sciex API 4000 triple quadrupole mass spectrometer using positive turbo-ion spray ionization mode. The MRM transitions were 100 → 58 for N-methyl-2-pyrrolidinone and 109 → 62 for N-methyl-d_3-2-pyrrolidinone-d_6. Solvent calibration standards could be readily used for quantitative analysis of N-methyl-2-pyrrolidinone with excellent precision and accuracy, although there are endogenous levels of N-methyl-2-pyrrolidinone in many blank matrices. The true recovery was nearly 100% and the MRM signal of N-methyl-2-pyrrolidinone was suppressed about 30% because of the matrix effect. Nevertheless, N-methyl-d_3-2-pyrrolidinone-d_6 completely compensated the ion-suppression effect and the injection-to-injection variation. The detection limit was 5 ng g^{-1} swine liver. The validated method was applied to a depletion study of N-methyl-2-pyrrolidinone in swine liver following intramuscular administration of a drug N-methyl-2-pyrrolidinone formulation.

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1. Introduction

N-methyl-2-pyrrolidinone has a very wide spectrum of uses, including applications as a chemical intermediate, organic solvent, binder in many pharmaceutical tablets, food additive, excipient in animal drug formulations and personal care products. It is also an endogenous low molecular weight compound in mammals and ubiquitous in the human diet. N-methyl-2-pyrrolidinone has also been suggested as a skin penetration enhancer in transdermal therapy [1]. Based on these applications, there are many opportunities for human, animal and environmental exposures. Thus a robust analytical method has to be developed for the investigation of its toxicological kinetics and depletion.

Some publications have investigated the toxicokinetics and metabolism pathways of N-methyl-2-pyrrolidinone [2–7]. Carnerup et al. [2–4] developed and validated assay methods using ENV+ solid phase extraction and then liquid chromatography–tandem mass spectrometry. Cohen and Jönsson [5] upgraded the extraction method into the automated solid phase extraction. Kubota et al. [6] developed a SPE-GC/FID method for determination of N-methyl-2-pyrrolidone and its metabolites in urine. Mehdinia et al. [7] developed a solid-phase micro-extraction and gas chromatography-nitrogen–phosphorous method for determination of N-vinyl-2-pyrrolidone and N-methyl-2-pyrrolidone in drugs. However, the quantitative analysis of N-methyl-2-pyrrolidone in the matrices still remains as a technical challenge. First, the compound is extremely hydrophilic,

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having insufficient retention on various solid phase extraction phases (SPE) used in the literatures above [2–7]. Second, due to the same reason, in the reversed phase HPLC methodology including these in the previous literatures [2–5], N-methyl-2-pyrrolidone elutes nearly at the void volume and it is extremely difficult to isolate N-methyl-2-pyrrolidone from matrix interferences even under an extremely high selective MS/MS MRM detection. Also, the conventional reverse phase HPLC columns have short column life in nearly 100% aqueous mobile phase. Third, in the LC–MS or LC–MS/MS analysis, the ESI and/or APCI mass spectrometric detection suffers low sensitivity when nearly 100% aqueous mobile phase is used. Fourth, although GC and/or GC–MS technologies may not have the second problem mentioned above, conventional liquid–liquid and solid phase extraction technologies reported hitherto [2–7] cannot eliminate the matrix effect and the bio-specificity from different lots of matrices. The challenge is particularly obvious when tissue samples are tested. To the best of our knowledge, matrix ion-suppression effect has not been evaluated in the previous publication [2–7]. According to the current US-CVM preference, solution calibration standards should be used for regulatory surveillance method development. Therefore, the elimination or compensation of LC/MS/MS matrix effect is a big challenge. Fifth, there are endogenous levels of N-methyl-2-pyrrolidone in many blank tissue matrices. Thus it is also difficult to use matrix-matched calibration standards for quantitative analysis of N-methyl-2-pyrrolidinone at practically low concentration levels.

The present study develops and validates an innovative and robust LC–MS/MS method for quantification of N-methyl-2-pyrrolidinone in swine liver. The five key steps to the success include: (1) the use of C18 + WAX SPE cartridges for removal of the majority of the matrix interferences; (2) the use of a hydrophilic interaction LC–MS/MS method for retention and isolation of N-methyl-2-pyrrolidinone from the interference; (3) since the LC/MS/MS sensitivity is excellent, the extracted sample (1 g tissue) is diluted ten times for the analysis and the dilution greatly reduces the ion-suppression effect; (4) the use of solvent calibration standards for correction of the endogenous level of 2-pyrrolidine in the matrix blank; (5) the use of the deuterium-labeled internal standard makes for the compensation of the ion-suppression effect and the extraction variation. The validated method is applied to a depletion study of 2-pyrrolidinone in swine liver following intramuscular administration of drug N-methyl-2-pyrrolidinone formulation.

2. Experimental

2.1. Chemicals

N-methyl-2-pyrrolidinone (99.6% purity) and N-methyl-d_3-2-pyrrilidinone-d_6 (98% atom D purity) were purchased from Sigma–Aldrich (St. Louis, WI, USA). Acetonitrile and formic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). High-purity water was prepared with a Milli-Q water system (Millipore, Bedford, MA, USA). Innovation™ C18 + WAX mixed-mode SPE cartridges (2 g/10 mL) and Innovation™ TX 3 μm 15 cm × 2.1 mm LC–MS/MS column were from Chrom-Matrix Inc. (Richland, WA, USA).

2.2. Preparation of stock and spiking solutions

Stock solutions were prepared by addition of 15 mg N-methyl-2-pyrrolidinone or N-methyl-d_3-2-pyrrolidinone-d_6 into acetonitrile, made up a volume (correction for purity) that gave a concentration of 1.0 mg/mL. The N-methyl-2-pyrrolidinone stock solutions were made in duplicates. Stock solution stability was determined by freshly preparing two new stock solutions and comparing it to the old stock solutions. Both the N-methyl-2-pyrrolidinone stock solution and N-methyl-d_3-2-pyrrolidinone-d_6 stock solution were stored in a refrigerator (set point 4 °C) and were stable for at least 60 days.

One of the two N-methyl-2-pyrrolidinone stocks was used to make seven calibration standard spiking solutions by a serial dilution into acetonitrile. The spiking solutions contained seven standard levels at 0.50, 1.00, 2.00, 4.00, 7.50, 15.0 and 30.0 μg/mL. The other set of N-methyl-2-pyrrolidinone stock solutions were used to make three quality control standard spiking solutions at 1.50, 4.00 and 24.0 μg/mL.

2.3. Sample processing

Swine liver samples were chopped and ground to a powder with dry ice.

One (1.00 ± 0.05 g, accurately to ±0.1 mg) of the homogenized control liver sample was fortified with 10 μL of a N-methyl-2-pyrrolidinone spiking solution. After a 2-h room temperature storage, 10 μL of the deuterium-labeled internal standard spiking solution was added.

To prepare the matrix blank sample without N-methyl-2-pyrrolidinone fortification, 10 μL of acetonitrile was used instead of the N-methyl-2-pyrrolidinone standard spiking solutions for the fortification. To prepare the zero blank without N-methyl-2-pyrrolidinone fortification, 10 μL of the deuterium-labeled internal standard spiking solution was added to the matrix blank for the fortification.

For the assayed samples, 10 μL of the deuterium-labeled internal standard spiking solution was added for the fortification of each (1.00 ± 0.05 g, accurately to ±0.1 mg) of homogenized liver.

2.4. Extraction

To each sample mentioned above, 5.0 mL of acetonitrile was used for the extraction of N-methyl-2-pyrrolidinone from the liver tissue. 2 g of sodium chloride was added into each sample for prevention of water into acetonitrile supernatant.

The tubes were shaken for 2 h. Then the tubes were centrifuged at 3000 rpm for 5 min. The supernatant was led through a C18 + WAX mixed-mode SPE cartridge and then 3 mL acetonitrile was used for washing the SPE cartridge. The eluted solution was mixed with 2.0 mL acetonitrile containing 1% formic acid. The final volume of every eluted solution was adjusted as 10.0 mL by the addition of acetonitrile. Then 0.5 mL of the extracted sample was used for LC–MS/MS analysis.

2.5. Analysis

The instruments used were an Agilent 1100 HPLC system, a CTC high-throughput autosampler and a Sciex API 4000 triple quadrupole mass spectrometer with a turbo ion spray interface and the positive mode. The Analyst software (version 1.4.2) was used to operate the integrated system.

The current LC–MS/MS method employed a hydrophilic interaction chromatography with a TX LC–MS/MS column (3 μm 15 cm × 2.1 mm I.D.). Sample aliquots of 50 μL were injected into the system. The mobile phase A was acetonitrile containing 0.2% formic acid. The mobile phase B was water containing 0.2% formic acid. From 0 min to 0.2 min, isocratic run at 95% of mobile phase A. From 0.2 min to 2.8 min, a linear gradient from 95% of mobile phase A to 60% of mobile phase A. From 2.8 min to 3.0 min, back to 95% of mobile phase A. Isocratic run at 95% of mobile phase A until 4.0 min. The flow rate was 0.4 mL/min.

N-methyl-2-pyrrolidinone and N-methyl-d_3-2-pyrrolidinone-d_6 were determined by MRM scan mode. Dwell time was 100 ms for both of the compounds. The atmospheric pressure turbo ion spray interface was set to 550 °C and the ion spray voltage to 5000 V. For N-methyl-2-pyrrolidinone, analysis after collision induced fragmentation of the precursor ion at m/z 100.3 was performed at 58.2 (collision energy 30 V). The declustering potential was 50 V. For N-methyl-d_3-2-pyrrolidinone-d_6, analysis after collision induced fragmentation of the precursor ion at m/z 109.2 was performed at 62.2 (collision energy 13 V). The declustering potential was 66 V. The peak area ratios between the analyte and the internal standard were used for quantification. Chromatograms were automatically integrated using Analyst software to compute the $1/X$ weighted least-square linear regression.

2.6. Method validation

The validation was done according to US FDA CVM residue chemistry guidance. The calibration curves were prepared using 95% acetonitrile:4.8% water:0.2%formic acid.

Three days of solvent calibration curves, with six replicates of quality controls spiked onto the blank liver tissues at three concentration levels were performed to determine intra-assay and inter-assay variation and accuracy. After the LC–MS/MS analysis, the third batch was stored at room temperature for a re-injection to establish the extraction stability.

A blank matrix without endogenous level of N-methyl-2-pyrrolidinone was selected to evaluate absolute matrix effect and true recovery. The true recovery and absolute matrix effect were evaluated in both Low QC and High QC levels. Six extracted matrix blank samples were dried and then spiked with 1.0 mL of acetontrile:formic acid (99.8:0.2) containing Low-QC or High-QC concentration levels of N-methyl-2-pyrrolidinone. In addition, six replicate of 1.0 mL of acetontrile:formic acid (99.8:0.2) containing Low-QC or High-QC concentration levels of 2-pyrrolidinone were added at the end of the running sequence. The absolute matrix effect and the true recovery were calculated by the comparison of the integration area.

The fifth batch was used to evaluate the relative matrix effect from six unique matrix lots, at the LOW-QC level. Endogenous levels of N-methyl-2-pyrrolidinone in the six unique lots were also measured for correction.

Finally, the room-temperature matrix stability, one-cycle matrix freeze/thaw (F/T) stability and a six-month storage stability at −70 °C for quality control standards were evaluated.

MRM chromatograms were automatically integrated using Analyst software. The Analyst software was also used to perform the regression, calculate the regression constants, and calculate the concentration of the analyte in unknown samples and QCs using the peak area ratios of analyte vs internal standard. Individual precision and accuracy for the calibration standards and QC samples were also determined by Analyst.

2.7. Tissue sources, preparation and storage

Swines were treated with intramuscular administration of a proprietary drug N-methyl-2-pyrrolidinone formulation. Swine livers were transported with dry ice and stored at −70 °C.

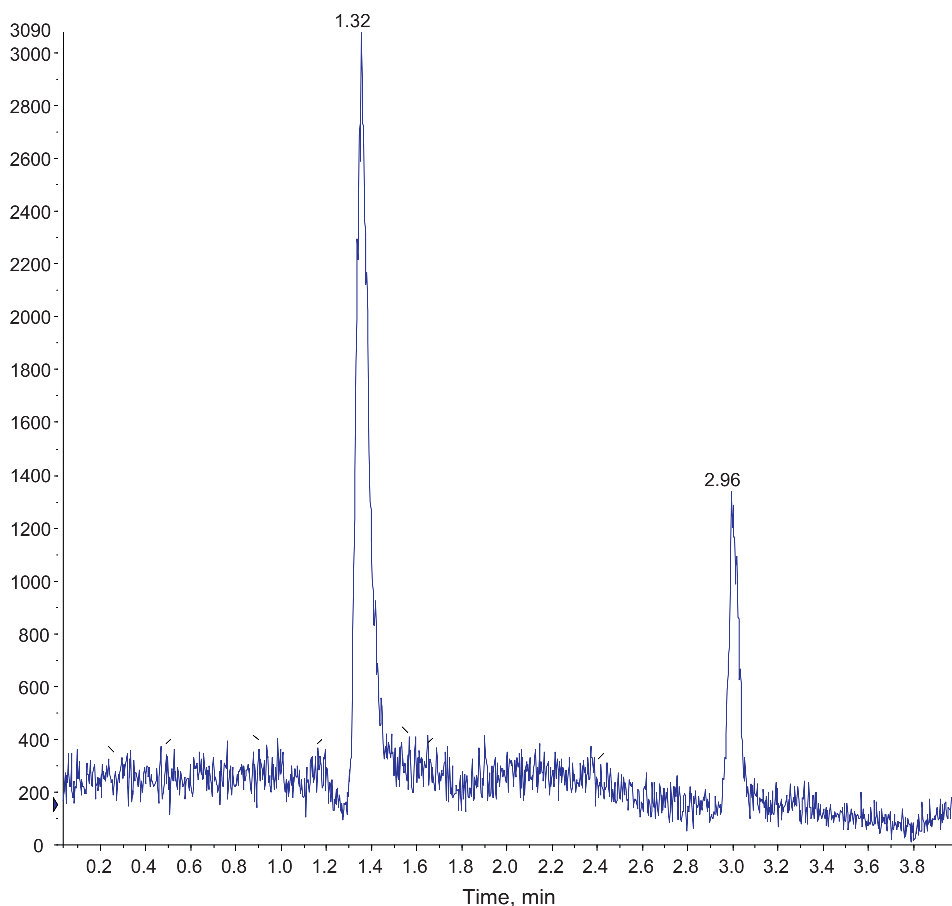

Fig. 1. N-methyl-2-pyrrolidinone LC–MS/MS MRM chromatogram (5 ng g^{-1} swine tissue extract: LLOQ sample).

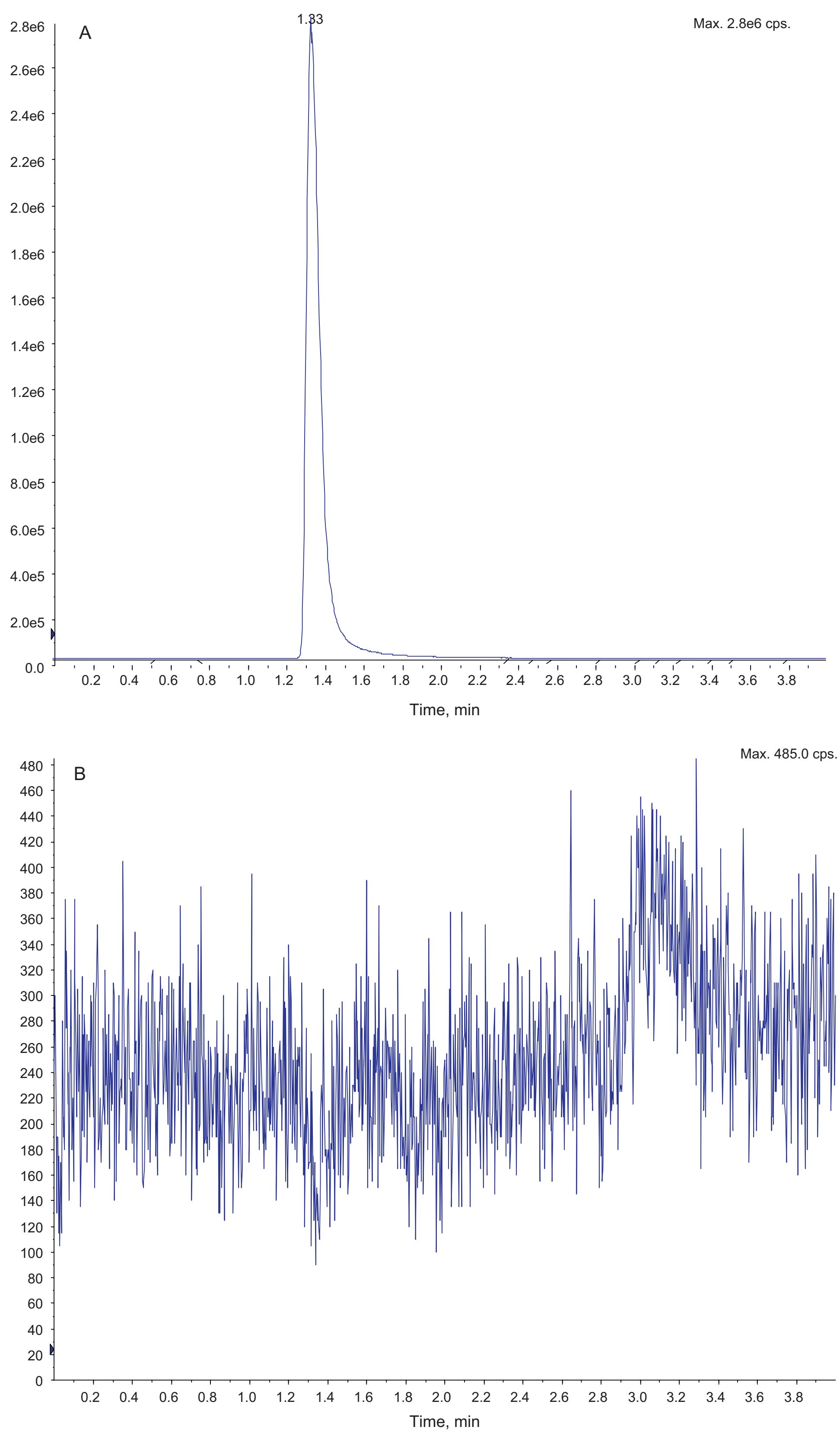

Fig. 2. (A) LC–MS/MS MRM chromatogram of 1000 ng/mL N-methyl-2-pyrrolidine solution. (B) LC–MS/MS chromatogram of the blank injection following the previous 1000 ng/mL N-methyl-2-pyrrolidinone injection.

Table 1
Effect of sample cleanup on the absolute matrix effect (%) and true recovery of N-methyl-2-pyrrolidinone.

2-Pyrrolidinone	Ion-suppression/ enhancement (%)	CV % ($n=6$)	True recovery (%)	CV % ($n=6$)
Low QC	69.5	8.4	98.5	6.4
High QC	71.2	6.7	99.2	4.3

3. Results and discussion

3.1. HPLC method development

The first technical challenge is how to develop a robust HPLC method.

In the previous studies, reversed phase HPLC methods had been used [2–5]. Unfortunately, N-methyl-2-pyrrolidinone is extremely hydrophilic, thus having no sufficient retention on all kinds of reversed phase HPLC columns, and having insufficient separation from the matrix interferences. In addition, the uses of nearly 100% aqueous mobile phase not only significantly shorten the column life, but also reduce the sensitivity of the mass spectrometric detection.

In the present study, a novel method using a 3 μm 15 cm × 2.1 mm TX LC–MS/MS column is developed. The method uses 95% acetonitrile:4.8% water:0.2% formic acid as the starting mobile phase. When the flow rate is 0.4 mL/min, the retention times for both N-methyl-2-pyrrolidinone and its isotope labeled internal standard are about 1.33 min.

Although the retention time is still short, the retention times of N-methyl-2-pyrrolidinone and its deuterium-labeled internal standard are 1.7 times than that at solvent front, at where the majority of interferences emerge. The LC–MS/MS MRM chromatogram of N-methyl-2-pyrrolidinone at 5 ng g^{-1} liver tissue is shown in Fig. 1. The corresponding LC/MS/MS MRM chromatogram of its deuterium-labeled internal standard is not shown because it has a very identical character. The LC/MS/MS method is rugged and over two thousands of injections have been done during the study with the use of a TX column, without peak distortion and shift.

The novel LC–MS/MS method offers another four significant advantages. First, N-methyl-2-pyrrolidinone and N-methyl-d_3-2-pyrrolidinone-d_6 cannot be dried by rotor evaporator or Zymark Turvo LV evaporator. In the present hydrophilic interaction LC–MS/MS method, acetonitrile is a weak solvent and thus the extracted supernatants can be directly used for the LC–MS/MS analysis. Second, in comparison with nearly 100% aqueous mobile phase in the previous methods organic ratio [2–5], both the compounds have much better mass spectrometric sensitivity when acetonitrile containing 0.2% formic acid is used as the mobile phase. With the use of the present method, the LLOQ for N-methyl-2-pyrrolidinone can reach 0.2 ng/mL or even less if necessary. Third, the present method has zero carryover. Fig. 2A is the MRM chromatograms of 1000 ng/mL N-methyl-2-pyrrolidinone solution and the next injection for its carryover evaluation. No detectable N-methyl-2-pyrrolidinone was observed in the MRM chromatogram of the next blank injection (Fig. 2B). Fourth, since the LC/MS/MS sensitivity is excellent, the extracted sample (1 g tissue) is diluted ten times for the analysis and the dilution greatly reduces the ion-suppression effect.

3.2. Absolute matrix effect

N-methyl-2-pyrrolidinone is extracted from liver by acetonitrile. A few incurred samples are used to establish the extraction. A 2-h extraction is sufficient when the values from the 1-h extraction reach 95% of the maximum.

The acetonitrile extracts are too dirty. The direct injection of the extracts onto the LC/MS/MS system results in a severe contamination of mass source and the precipitated loss of the MRM signals of N-methyl-2-pyrrolidinone very fast.

In order to solve the problem, the supernatant above is led through a C18 + WAX mixed-mode SPE cartridge. Weak anionic exchange sorbent is one of the most powerful sorbent for removal of impurities in vegetables and minimization of matrix-enhancement effect in the GC–MS analysis of pesticide multi-residues [8,9]. All negative and zwitterionic components including phospholipids, fatty acids, carbohydrates, proteins, peptides and amino acids are readily and promptly adsorbed on WAX surfaces. On the other hand, neutral and basic components including N-methyl-2-pyrrolidinone have no retention on the SPE cartridge. In addition, it is well known that C18 is also useful for removal of fatty substances. The novel matrix cleanup strategy proves very effective for the present study. Phospholipids and fatty substances are considered as two of the major causes for the matrix effect [9,10]. Since both of them are removed by WAX and C18, a significant relief on ion-suppression is expected. As a matter of fact, the supernatants become much cleaner and over a few hundreds of samples can be continuously injected for the quantitative LC/MS/MS analysis without severe contamination of the system.

The absolute matrix effect is measured according to Matuszewski et al.'s method [11]. The value is characterized as ion-suppression or enhancement. When the value is below 100%, there is an ion-suppression. When the value is above 100%, there is an enhancement [11]. At both Low-QC and High QC levels, the integration areas of the six post-spiked QC samples are divided by the integration areas of the six solution QC samples. The data are shown in Table 1.

The absolute matrix effect is 69.5% for Low QC and 71.2% for High QC. It means that the MRM signals are suppressed by about 30%!

Further attempt for the elimination of the matrix effect remains unsuccessful. N-methyl-2-pyrrolidinone has no retention on strong cationic exchange SPE cartridge. On the hand, polymer-based reverse phase SPE cartridges such as HLB and ENV+ solid phase extraction cartridges have little role for minimization of the matrix effect.

Table 2
Relative matrix effect from six unique low QC standards on quantitative assessments.

N-methyl-2-pyrrolidinone	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Mean endogenous level found (ng g^{-1})	0	11.2	23.5	34.5	41.4	47.3
%CV	0	5.7	6.1	7.2	7.7	5.1
Total amount after spiked (ng g^{-1})	14.7	26.8	37.8	49.8	56.8	61.9
%CV	4.3	7.3	6.1	3.8	11.2	8.8
Low QC amount after correction (ng g^{-1})	14.7	15.6	14.3	15.3	15.4	14.6
Difference (%)	−0.3	0.6	−0.7	0.3	0.4	−0.4
n	6	6	6	6	6	6

Table 3
Summary of precision and accuracy of N-methyl-2-pyrrolidinone QC samples from three core runs.

N-methyl-2-pyrrolidinone	Theoretical concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy derivation (%)	Intra-assay CV (%) ($n=6$)	Inter-assay CV (%) ($n=18$)
LLOQ	5.00	5.03	106%	11.5	
LOW QC	15.0	14.7	98.0%	9.4	6.7
MID QC	40.0	41.2	103%	6.9	4.4
HIGH QC	240	232	96.7%	7.8	5.6

However, the isotope labeled internal standard completely compensates the absolute matrix effect. In addition, the isotope labeled internal standard perfectly tracks the extraction process and completely compensates the injection-to-injection variation.

3.3. True recovery

At both Low-QC and High QC levels, the integration areas of the six QC samples are divided by the integration areas of the six post-spiked QC samples to generate the data for true recovery. The data are shown in Table 1. The true recovery is about 100%.

Table 4
Stability of N-methyl-2-pyrrolidinone in swine liver.

Analyte (QC)	Concentration (ng/mL)	Accuracy derivation (%)	CV (%) ($n=6$)
One freeze–thaw cycle			
Low	15.0	92.4	7.8
High	240	94.1	6.6
72-h room temperature extraction stability			
Low	15.0	91.2	8.9
High	240	92.3	7.2
24-h room-temperature stability in matrix			
Low	15.0	94.6	6.1
High	240	92.8	7.4
Six-month freezer storage stability in matrix (−70 °C)			
Low	15.0	88.5	13.2
High	240	90.4	9.8

3.4. Endogenous level of N-methyl-2-pyrrolidine

In the six unique lots of matrix blanks, five of them have endogenous level of 2-pyrrolidine at 10–50 ng g^{-1} (Table 2). Thus it is difficult to use matrix-matched calibration standards for the validation. Also, according to US FDA CVM guidance, solvent calibration standards should be used.

Since the deuterium-labeled internal standard, N-methyl-d_3-2-pyrrolidinone-d_6, fully compensates the absolute matrix effect, solvent calibration standards can readily be used. The use of the solvent calibration standards readily determines endogenous level of 2-pyrrolidine. In the bio-specific experiment, six blank matrices are spiked with the labeled internal standards to determine endogenous level of N-methyl-2-pyrrolidine, followed the spiking Low QC samples. After deduction of endogenous level of 2-pyrrolidine, the spiking Low QC values in six unique lot show no bio-specificity (Table 2).

3.5. Precision and accuracy

Over the range of 5–300 ng g^{-1} tissue for N-methyl-2-pyrrolidinone, the calibration curve is better fitted with linear model using $1/x$. The least-squares linear regression constants (r^2) are always equal to higher than 0.996. Concentrations of quality control standards are calculated from each curve. The accuracy is obtained by comparing the averaged calculated concentrations to their nominal values (% of nominal) and the precision by the percent coefficient of variation (%CV). The results from a three-day precision and accuracy batches are listed in Table 3. The current US FDA regulatory requirements expect that the lower limit of quantitation (LLOQ) standard back-calculated concentrations must be within ±20% of theoretical nominal concentrations and all other standards' back-calculated concentrations must be within ±15% of their nominal theoretical concentrations. The results in Table 3 fully meet with the acceptance criteria.

Table 5
Data from a depletion study.

	Swine liver 1	Swine liver 2	Swine liver 3	Swine liver 4
Day 0				
Weight (g)	1448	1812	1915	2020
N-methyl-2-pyrrolidine (ng g^{-1})	24.3	11.5	34.5	50.2
	Swine liver 5	Swine liver 6	Swine liver 7	Swine liver 8
Day 1				
Weight (g)	1557	1734	2020	2114
N-methyl-2-pyrrolidine (ng g^{-1})	205	233	254	275
	Swine liver 9	Swine liver 10	Swine liver 11	Swine liver 12
Day 4				
Weight (g)	1532	1779	1950	2105
N-methyl-2-pyrrolidine (ng g^{-1})	142	147	168	157
	Swine liver 13	Swine liver 14	Swine liver 15	Swine liver 16
Day 7				
Weight (g)	1669	1825	2075	2155
N-methyl-2-pyrrolidine (ng g^{-1})	67.5	88.4	92.5	104

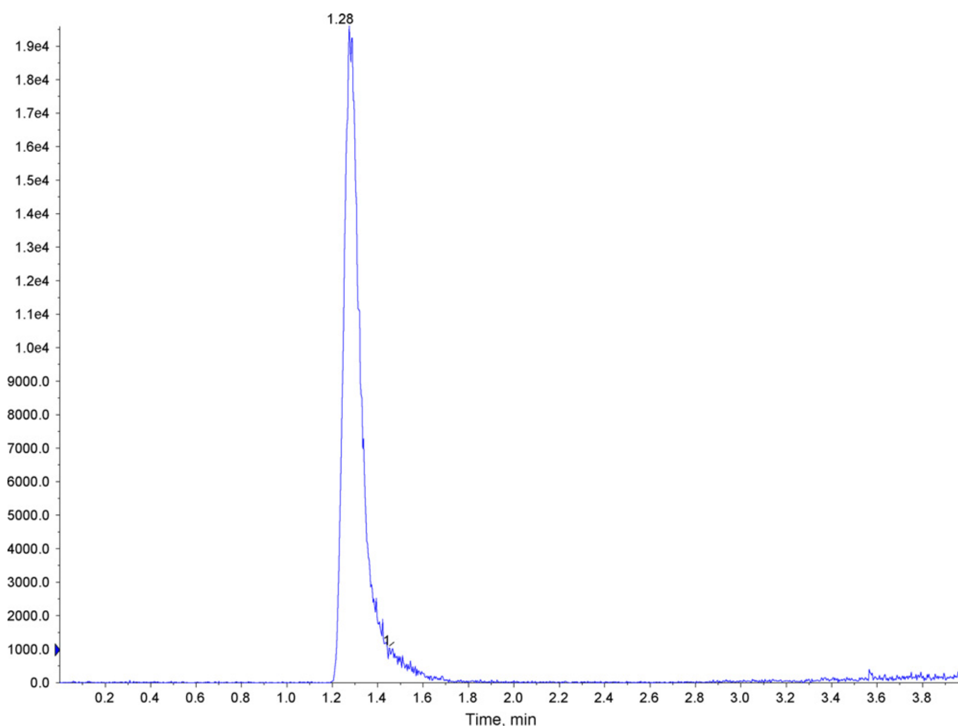

Fig. 3. N-methyl-2-pyrrolidinone LC–MS/MS MRM chromatogram of an incurred swine liver (N-methyl-2-pyrrolidinone concentration: 24.3 ng g^{-1}).

3.6. Freeze-thawed stability, 24-h room temperature stability, extraction stability and six-month storage stability

The one-cycle 24-h freeze-thawed stability, the 24-h room stability, the 72-h extraction stability and the six-month storage stability at −70 °C have been successfully established. The data are shown in Table 4.

3.7. A depletion study of N-methyl-2-pyrrolidinone in swine liver

Sixteen real samples are assayed and the results are shown in Table 5.

A representative LC/MS/MS MRM chromatogram for an incurred swine liver extract is shown in Fig. 3.

The data show that N-methyl-2-pyrrolidinone in the investigated drug formulation has a C_{max} at 275 ng g^{-1} after administration.

4. Conclusion

A hydrophilic interaction LC/MS/MS method is successfully developed and validated for the determination of N-methyl-2-pyrrolidinone residue in swine liver. The five key steps to the success include: (1) the use of C18 + WAX SPE cartridges for removal of the majority of the matrix interferences; (2) the use of a hydrophilic interaction LC–MS/MS method for retention and isolation of N-methyl-2-pyrrolidinone from the interference; (3) since the LC/MS/MS sensitivity is excellent, the extracted sample (1 g tissue) is diluted ten times for the analysis and the dilution greatly reduces the ion-suppression effect; (4) the use of solvent calibration standards for correction of the endogenous level of 2-pyrrolidine in the matrix blank; (5) the use of the deuterium-labeled internal standard for the compensation of the ion-suppression effect and the extraction variation.

References

[1] B.W. Barry, J. Control. Release 6 (1987) 85.
[2] M.A. Carnerup, B. Åkesson, B.A.G. Jönsson, J. Chromatogr. B 761 (2001) 107.
[3] M.A. Carnerup, A.M. Saillenfait, B.A.G. Jönsson, Food Chem. Toxicol. 43 (2005) 1441.
[4] M.A. Carnerup, M. Spanne, B.A.G. Jönsson, Toxicol. Lett. 162 (2006) 139.
[5] A.S. Cohen, B.A.G. Jönsson, Chromatographia 65 (2007) 407.
[6] R. Kubota, Y. Endo, A. Takeuchi, Y. Inoue, H. Ogata, M. Ogawa, T. Nakagawa, N. Onda, G. Endo, J. Chromatogr. B 854 (2007) 204.
[7] A. Mehdinia, H. Ghassempour, R. Rafati, Heydari, Anal. Chim. Acta 587 (2007) 82.
[8] F.J. Schenck, S.J. Lehotay, J. Chromatogr. A 868 (2000) 51.
[9] Y. He, Y.H. Liu, Chromatographia 65 (2007) 581.
[10] P. Bennett, K.C. Van Horne, 2003 AAPS Annual Meeting and Exposition, Salt Lake City, Utah, October 2003.
[11] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.