Application Note

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High-Throughput Determination of Multiple Toxic Alkaloids in Food by UHPLC/MS/MS

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Abstract

This Application Note presents a simple sample preparation procedure followed with an Agilent 1290 Infinity II LC combined with an Agilent 6470A triple quadrupole LC/MS for simultaneous, high-throughput determination of multiple alkaloids in food matrices. The matrix-matched calibration shows very good linear relationships for the 18 alkaloids in the concentration range of 0.5 to 50 µg/L in the diluted, extracted matrices of bread, milk, wine, and rice powder. This corresponds to 5.0 to 500 µg/kg in the matrices when considering the dilution factor of 10. The linear regression coefficients for all the analytes are higher than 0.99. The signal-to-noise ratios (S/Ns) for all analytes are higher than 10 at the lowest calibration level of 0.5 µg/L, corresponding to 5.0 µg/kg in the samples. The recoveries for the analytes in four different matrices at 5, 50, and 250 µg/kg levels ranged from 90 to 110%, and the corresponding relative standard deviations were within 2.3 to 7.9%. The results demonstrated that the developed method has the advantages of high sensitivity, accuracy, and precision. The simple sample preparation combined with rapid LC/MS/MS detection allows high-throughput screening of alkaloid residues in food matrices.

Introduction

Alkaloids are naturally occurring alkaline substances with nitrogen-containing heterocyclic structures. They often exhibit high bio-activity, and are commonly the primary components in traditional herb medicine. Alkaloids are found in some plants, and may cause adverse effects when ingested by accident.[1] According to Commission Regulation (EU) 2016/239, the maximum residue levels (MRLs) of tropane alkaloids including atropine and scopolamine in certain cereal-based foods for infants and young children are as low as 1 µg/kg.[2] Colchicine is in the prohibited list in Regulation (EU) No. 37/2010.[3] China allows atropine to be used in all food matrices of animal origin without MRL, and no other regulations restrict the amount of alkaloids in food matrices in China.[4] However, the accidental mixing of alkaloid-containing weeds into crops or the accidental ingestion of alkaloid-containing weeds by farm animals may increase the risk of exposure to the public. It is essential to develop sensitive and reliable methods for routine monitoring of alkaloids in food matrices. In reality, alkaloids vary greatly in physicochemical properties, and they are also involved in many complicated matrices. It is challenging to develop a reliable method for routine, simultaneous monitoring of multiclass alkaloids. Available reports are mainly focused on the detection of alkaloids with similar chemical properties.[5,6] This Application Note presents a simple sample preparation procedure followed by rapid LC/MS/MS analysis for simultaneous, high-throughput determination of five classes of alkaloids in four types of food matrices, including milk, wine, bread, and rice powder.

Experimental

Standards and sample preparation procedure

All five classes 18 alkaloid compounds were purchased from Anpu Tech. (Shanghai, China), which were prepared at 1.0 g/L in methanol for stock at –20 °C. Calibration standard solutions were then diluted from the stock solutions using the corresponding matrix extract.

Solid samples (2.0 g) were added to 50 mL centrifugation tubes, and 5 mL of water was transferred to the tube for hydration. Acetonitrile (10 mL) was then added to the tube, followed by 5 mL of Na_2EDTA-McIlvaine buffer (pH 3). The tube was vortexed thoroughly for two minutes, then centrifuged at 7,500 rpm for five minutes. The supernatant was collected and filtered through a 2.0 µm membrane and analyzed by LC/MS. Liquid samples such as milk and wine followed the same extraction procedure except without the initial hydration step.

LC system

Agilent 1290 Infinity II LC with the following module configuration:

- Agilent 1290 Infinity II binary pump with degasser
- Agilent 1290 Infinity II autosampler with needle seat backflush function
- Agilent 1290 Infinity II multiple column compartments

LC conditions

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18 (3.0 × 50 mm, 1.8 µm)
Mobile Phases	A: 0.1% formic acid in water; B: methanol
Flow Rate	0.50 mL/min
Column Temperature	40 °C
Injection Volume	2.0 µL
Gradient	Time (min) %A %B 0 90 10 1 80 20 7 20 80 9 90 10 10 90 10

MS conditions

Parameter	Value
MS	Agilent 6470A triple quadrupole LC/MS
Ion Source	Agilent Jet Stream ESI
Ionization Mode	Positive
Capillary Voltage	3,500 V
Nozzle Voltage	500 V
Nebulizer Gas Pressure	N_2 (35 psi)
Drying Gas (N_2) Temperature	300 °C
Drying Gas Flow Rate	8 L/min
Sheath Gas (N_2) Temperature	400 °C
Sheath Gas Flow Rate	12 L/min
Acquisition Mode	MRM

Results and discussion

Alkaloids can easily be protonated under acidic conditions. Therefore, MRM in positive ESI ionization mode was selected. Different mobile phase combinations were examined, including water/methanol, water/acetonitrile, 0.1% formic acid/methanol, and 0.1% formic acid/acetonitrile. Most alkaloids exhibited a better response in methanol than in acetonitrile. Compared to the narrower and more symmetrical peaks with higher intensity in the acidic water, tailing peaks with low intensity were observed if water without acid was used. Hence, acidified water/methanol was selected as the binary mobile phase in the final conditions. MRM parameters were then optimized under the selected mobile phase. Table 1 lists the resultant parameters.

For those compounds not separated by chromatography, the characteristic MRMs enabled accurate compound quantitation. Figure 1 shows the typical MRM overlays of 18 alkaloids from different classes.

Table 1. Optimized MRM parameters for sensitive detection.

Analyte	Retention Time (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor Voltage (V)	Collision Energy (V)
Physostigmine	2.250	275.8	219.2, 162.2*	110	7, 22*
Scopolamine	1.898	303.8	137.9, 156.1*	120	23, 15*
Colchicine	4.771	399.9	358.1, 309.9*	135	22, 32*
Strychnine	2.368	334.9	156.1, 184.1*	135	55, 44*
Cortisone	5.307	360.9	162.9, 121.2*	135	25, 36*
Pseudoephedrine	1.719	166	148.1, 133.0*	80	10, 20*
Camptothecin	5.262	348.8	305.0, 249.0*	135	25, 33*
1-(1-Naphthyl)thiourea	3.912	202.8	185.8, 144.1*	135	20, 20*
Atropine	2.554	290	124.1, 93.0*	135	26, 35*
Mesasonitine	4.906	632.1	571.8, 105.0*	135	35, 60*
Hypaaconitine	5.059	616.2	556.6, 105.3*	135	50, 50*
10,11-Dimethoxystrychnine	2.594	394.9	243.6, 323.9*	135	46, 46*
Nuciferin	4.122	295.8	265.1, 249.9*	135	15, 17*
Podophyllotoxin	5.532	414.8	246.6, 397.1*	135	10, 5*
10-Hydroxycamptothecin	4.773	364.8	320.5, 235.7*	135	30, 33*
Aconitine	5.119	646.2	586.6, 105.3*	135	45, 50*
Gelsemine	2.171	322.8	70.1, 235.8*	135	32, 37*
Solanine	4.965	868.5	98.3, 398.1*	135	92, 92*

* Quantitative ion

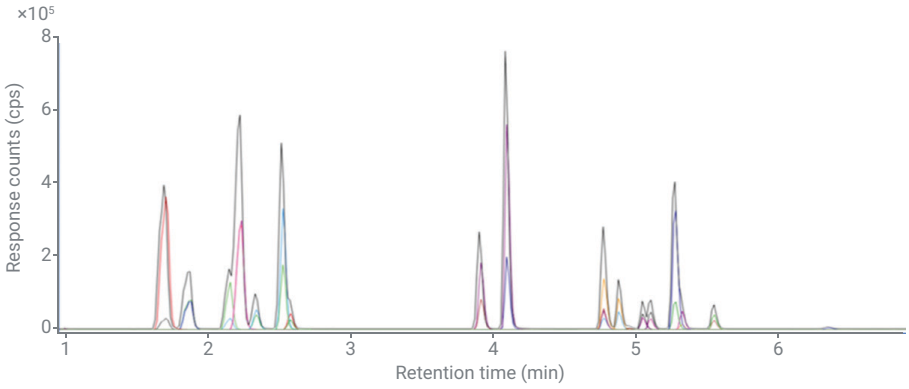

Figure 1. MRM overlay of 18 alkaloids from different classes at a level of 50 µg/L for each.

Sample preparation optimization and method performance

Methanol and acetonitrile were investigated as extraction solvents. It was found that acetonitrile can provide better recovery. The volume of extraction solvent required was also examined. As shown in Figure 2, with the extraction volume increasing from 5 to 10 mL, the recoveries for all alkaloids increased, with the majority of values higher than 60%. When the volume increased from 10 to 15 mL, recoveries for two compounds decreased, though others continued to increase. Considering the sensitivity in the real samples with extraction-induced dilution, 10 mL of extraction solvent was selected for target compounds extraction.

Alkaloids are easily dissolved in water under acidic conditions, and present as cations. The Na_2EDTA-McIlvaine buffer was added to the extraction solvent to achieve acidic pH. As shown in Figure 3, higher recovery values can be achieved for most compounds at pH 3 and 4 than at pH 5. Therefore, pH 3 was selected for the buffer during extraction.

Matrix effect was evaluated using the linear response slopes obtained in the matrix and in the pure solvent. Matrices affected cortisone and podophyllotoxin more severely, particularly bread (Figure 4).

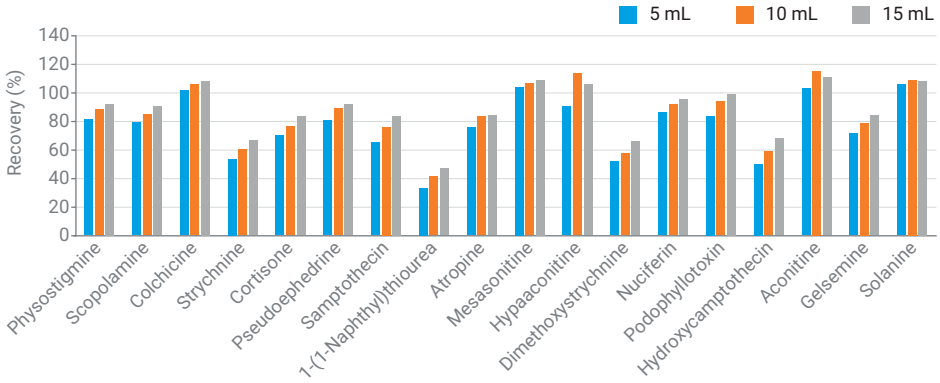

Figure 2. Effect of extraction solvent volume on recovery.

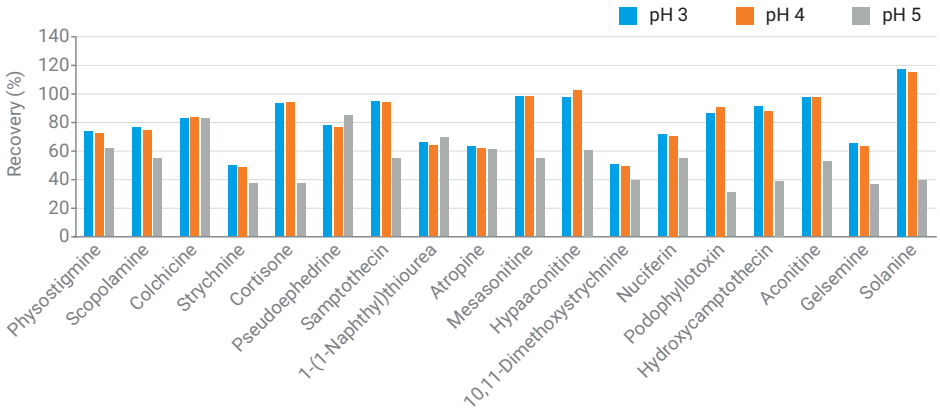

Figure 3. The effect of pH for the extraction solvent on the recovery of each analyte.

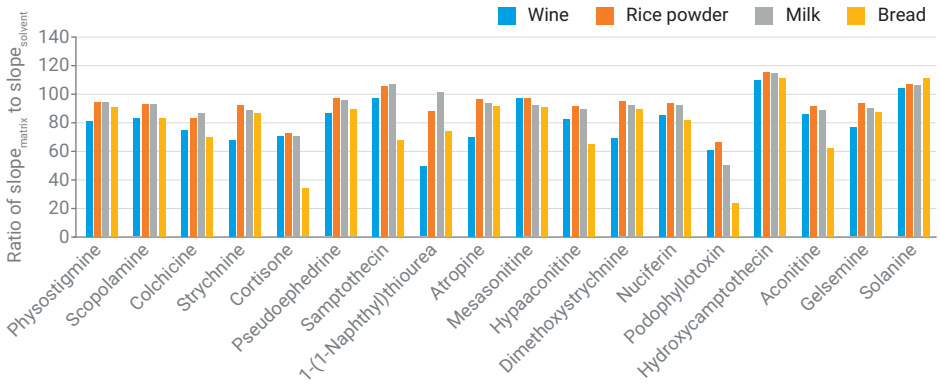

Figure 4. Matrix effect of alkaloid detection. **Note:** relative slope was used to evaluate the matrix effects.

A matrix-matched calibration curve was applied for quantitation. The result demonstrated that the alkaloid compounds exhibited excellent linearity within 0.5 to 50 µg/L, with regression coefficients higher than 0.995. The S/Ns are higher than 10 for 18 alkaloids at the lowest calibration level of 0.5 µg/L, corresponding to 5.0 µg/kg in the samples when considering the 10-fold dilution factor of the method. Therefore, the uniform limit of quantitation (LOQ) for the 18 alkaloids was set at 5.0 µg/kg.

The method recovery and precision were evaluated by analyzing the spiking samples at three levels (5, 50, and 250 µg/kg) in four matrices with six replicates of each matrix. The corresponding concentrations of the injection solution were 0.5, 5.0, and 25 µg/L. Table 2 shows that the recoveries at each level in all matrices range from 82 to 119%, with %RSD within 2.3 to 7.9%, suggesting that this method is reliable.

Table 2. The average recoveries and relative standard deviations for alkaloids spiked at three levels in the tested food matrices (n = 6).

Analyte	Spiking Level (µg/kg)	Wine		Milk		Rice Powder		Bread	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Physostigmine	0.5	99	4.5	112	2.3	113	2.7	101	5.7
	5	112	3.7	116	4.3	101	3.5	112	6.2
	50	100	4.8	105	4.8	116	3.7	109	4.5
Scopolamine	0.5	99	3.8	111	6.8	110	3.7	92	2.5
	5	117	3.1	119	4.4	90	2.8	114	3.6
	50	97	3.8	104	4	119	3.1	110	4
Colchicine	0.5	85	4.5	112	3.5	83	2.6	88	2.8
	5	110	4.8	117	3.4	107	3.3	112	3.8
	50	101	2.5	103	3	119	3.9	107	3.3
Strychnine	0.5	86	3.2	90	4	103	2.5	111	4.3
	5	86	3.4	118	4.9	96	4.8	116	4.8
	50	103	4.2	107	2.4	116	4.6	107	3.3
Cortisone	0.5	92	3.4	117	4.5	103	3.5	106	4.8
	5	102	4.4	96	4	100	2.6	105	2.4
	50	94	6.3	99	3.8	119	2.4	110	4.8
Pseudoephedrine	0.5	91	3.2	110	2.4	113	6.8	96	2.5
	5	101	2.4	118	2.9	95	2.5	112	4.9
	50	99	3.8	106	7.1	118	5.6	109	7.3
Camptothecin	0.5	109	5.1	104	3	96	3.2	103	4.9
	5	112	5	118	2.8	111	7.6	109	3.8
	50	97	5	103	2.9	117	6.4	106	5.2
1-(1-Naphthyl)thiourea	0.5	100	2.5	108	7.4	110	3.5	88	4.3
	5	115	4.3	112	2.6	114	5.6	112	4.9
	50	98	3.9	104	6	110	5.6	105	2.7
Atropine	0.5	82	2.8	111	2.6	110	7.3	92	4.9
	5	106	5.6	115	3.3	103	6.2	115	4.9
	50	102	3.4	107	4.5	101	7.1	109	3.4
Mesasonitine	0.5	91	6.6	110	3	109	3	102	4.5
	5	106	3.5	112	4.4	98	4.3	101	2.7
	50	93	2.9	104	4.3	117	4.7	105	2.3
Hypaaconitine	0.5	88	3.8	112	2.7	107	3.6	95	2.8
	5	118	2.9	99	4.8	92	2.5	108	2.4
	50	102	2.7	100	4.2	112	7.1	103	4.7

Analyte	Spiking Level (µg/kg)	Wine		Milk		Rice Powder		Bread	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
10,11-Dimethoxystrychnine	0.5	86	3.7	105	3.6	109	7	106	2.9
	5	105	5.1	101	4.2	103	5.2	116	3.5
	50	103	4.7	108	5.5	119	3.7	108	3.3
Nuciferin	0.5	91	2.4	117	3.6	112	2.9	97	4.7
	5	117	6.3	115	5.3	98	2.5	114	4.3
	50	96	5.6	104	4.4	117	3.5	109	3.2
Podophyllotoxin	0.5	83	6.5	108	6.5	110	7.9	85	2.4
	5	106	3.3	109	2.4	99	4.4	111	3.1
	50	101	6.4	94	6.5	107	2.7	109	3.6
10-Hydroxycamptothecin	0.5	86	4.4	93	2.8	83	6.6	106	3.5
	5	113	6.3	110	2.4	96	3.5	103	3.9
	50	90	2.7	102	5.7	114	2.7	105	3.1
Aconitine	0.5	89	5.1	105	5.3	107	6.3	90	3.4
	5	116	2.9	111	2.9	93	5.9	111	2.7
	50	100	3.6	103	2.6	119	4.6	105	3.5
Gelsemine	0.5	91	4.1	107	2.9	113	5.5	92	2.8
	5	115	2.4	119	4.3	107	6.7	115	2.8
	50	100	2.6	105	3.2	119	3.7	107	2.6
Solanine	0.5	97	3.2	101	4.2	89	6.1	115	2.7
	5	106	3	104	2.7	93	2.9	92	6.7
	50	89	2.8	102	3	104	2.3	106	5.5

Conclusion

This Application Note demonstrates a reliable UHPLC/MS/MS method for simultaneous determination of 18 alkaloids in wine, milk, bread, and rice powder. The method has excellent linearity, high sensitivity, good accuracy, and precision. It is easy to perform, and can reliably be applied in the routine monitoring of alkaloids in food.

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