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Reliable Determination of Food Additives in Baby Food Using UHPLC Combined with Tandem Mass Spectrometry

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Abstract

This application note describes a reliable and sensitive method for routine monitoring of four commonly used food additives including coumarin, vanillin, and methylated and ethylated vanillins in baby food. The LC/MS/MS method described delivered excellent linearity of quantitation over a concentration range of 50 to 5,000 µg/kg. The limit of detection (LOD) and the limit of quantitation (LOQ) for each analyte were 10 and 50 µg/kg, respectively. Spiking recovery in baby formula, baby cereal powder, and milk at 50, 100, and 500 µg/kg ranged from 90.7% to 98.5% with RSD below 10% (n = 6). These results suggest that the method is accurate, precise, reliable, and can be used for routine monitoring of these additives in the baby food matrices.

Introduction

Coumarin and vanillin, including alkylated vanillins, are two important classes of man-made food additives that are widely applied in the food industry to improve food taste characteristics. However, these food additives may induce potential heath effects, particularly for young babies. Hence, many countries strictly regulate these types of food additives.[1,2] In China, no food additive is allowed in baby food for babies under six months old according to GB 2760-2016.[3] Hence, a sensitive and reliable method is required to determine these food additives in baby food for routine monitoring in the market to ensure baby food safety. This study develops and evaluates an SPE extraction and cleanup followed with an LC/MS/MS analysis method in three types of baby food matrices: baby formula, baby cereal powder, and milk.

Experimental

Chemicals and Reagent

Standard compounds including coumarin, vanillin, methylated and ethylated vanillin were selected for monitoring, and their isotopically labeled analogs were selected for internal standard dilution calibration. LC/MS grade methanol and Milli-Q water were used to prepare mobile phase. Formic acid, ammonia, and ammonium acetate were HPLC grade, and all other reagents were analytical grade.
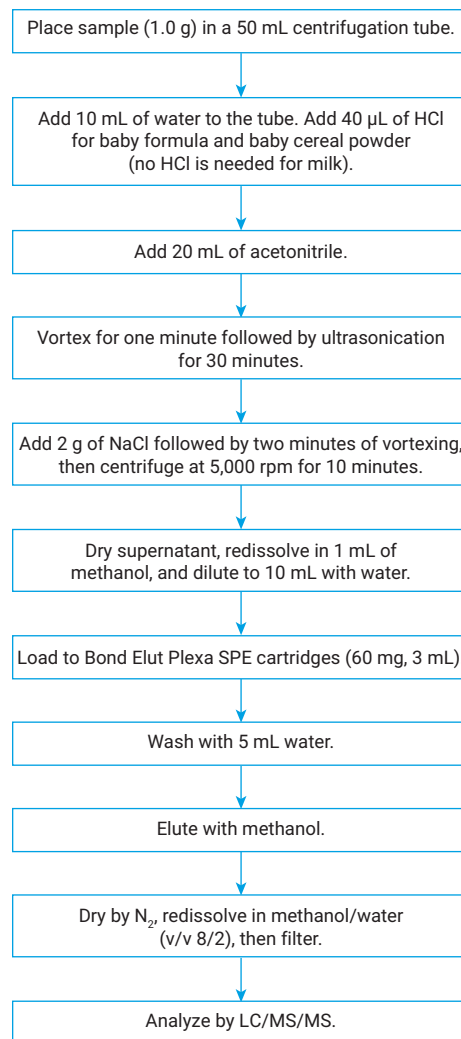
Instrument conditions

LC Conditions	
Agilent 1290 Infinity II UHPLC System	• 1290 Infinity II binary pump with degasser • 1290 Infinity II autosampler with needle seat backflush function • 1290 Infinity II multiple column compartment
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 100 mm, 1.8 µm
Mobile Phases	A) 0.1% Formic acid in water B) Acetonitrile
Flow Rate	0.15 mL/min
Column Temperature	30 °C
Injection Volume	10.0 µL
Post Time	3 minutes
Gradient	Time (min) %A %B 0 55 45 5 40 60 5.01 10 90 8 10 90
MS Conditions	
MS	Agilent 6470 QQQ
Ion Source	Agilent Jet Stream ESI
Ionization Mode	Positive
Capillary Voltage	4,000 V
Nozzle Voltage	600 V
Nebulizer Gas (N_2) Pressure	50 psi
Drying Gas (N_2) Temperature	300 °C
Drying Gas Flow Rate	8 L/min
Sheath Gas (N_2) Temperature	350 °C
Sheath Gas Flow Rate	10 L/min
Acquisition Mode	MRM

Method

Baby food samples including baby formula, baby cereal powder, and milk were collected from local vendors. The sample was prepared following the flowchart below.

Sample preparation procedure

Place sample (1.0 g) in a 50 mL centrifugation tube.
↓
Add 10 mL of water to the tube. Add 40 µL of HCl for baby formula and baby cereal powder (no HCl is needed for milk).
↓
Add 20 mL of acetonitrile.
↓
Vortex for one minute followed by ultrasonication for 30 minutes.
↓
Add 2 g of NaCl followed by two minutes of vortexing, then centrifuge at 5,000 rpm for 10 minutes.
↓
Dry supernatant, redissolve in 1 mL of methanol, and dilute to 10 mL with water.
↓
Load to Bond Elut Plexa SPE cartridges (60 mg, 3 mL).
↓
Wash with 5 mL water.
↓
Elute with methanol.
↓
Dry by N_2, redissolve in methanol/water (v/v 8/2), then filter.
↓
Analyze by LC/MS/MS.

Results and discussion

The target analytes can easily be protonated under acidic conditions; hence, MRM under positive ESI ionization mode was selected. The corresponding MRM acquisition parameters for the analytes and their isotopically labeled internal standards (ILIS) were then optimized to achieve high sensitivity and selectivity for each analyte. Table 1 lists the resulting acquisition parameters. By using 0.1% formic acid/acetonitrile as the binary mobile phase and a C18 column under the optimized gradient elution, the four analytes can be eluted out of the column within seven minutes. Methylated and ethylated vanillin are one pair of isomers, and cannot be separated by baseline. Nonetheless, each isomer has specific fragment ions, which allows selective detection and accurate quantitation of individual ones. Figure 1 shows the typical MRM chromatograms for the analytes and the corresponding ILISs.

Table 1. Optimized MRM parameters for sensitive and selective detection of the analytes (* quantitation ion).

| Analyte	Retention Time (min)	Precursor Ion (m/z)	Frag. Voltage (V)	Product Ion (m/z)	CE (V)
D3-Vanillin	4.40	156.2	106	93	17
				65*	29
Vanillin	4.40	153	111	93.2	13
				65.2*	29
D5-Ethylvanillin	5.35	172	101	112	13
				93.1*	21
Ethylvanillin	5.35	167.1	111	110.9	13
				92.9*	17
D3-Methylvanillin	5.56	170.2	106	142.1*	13
				127	21
Methylvanillin	5.56	167.1	123	139*	13
				124.1	21
D4-Coumarin	6.08	151.1	122	107.1	21
				95.1*	29
Coumarin	6.08	147	117	103	17
				91.1*	30
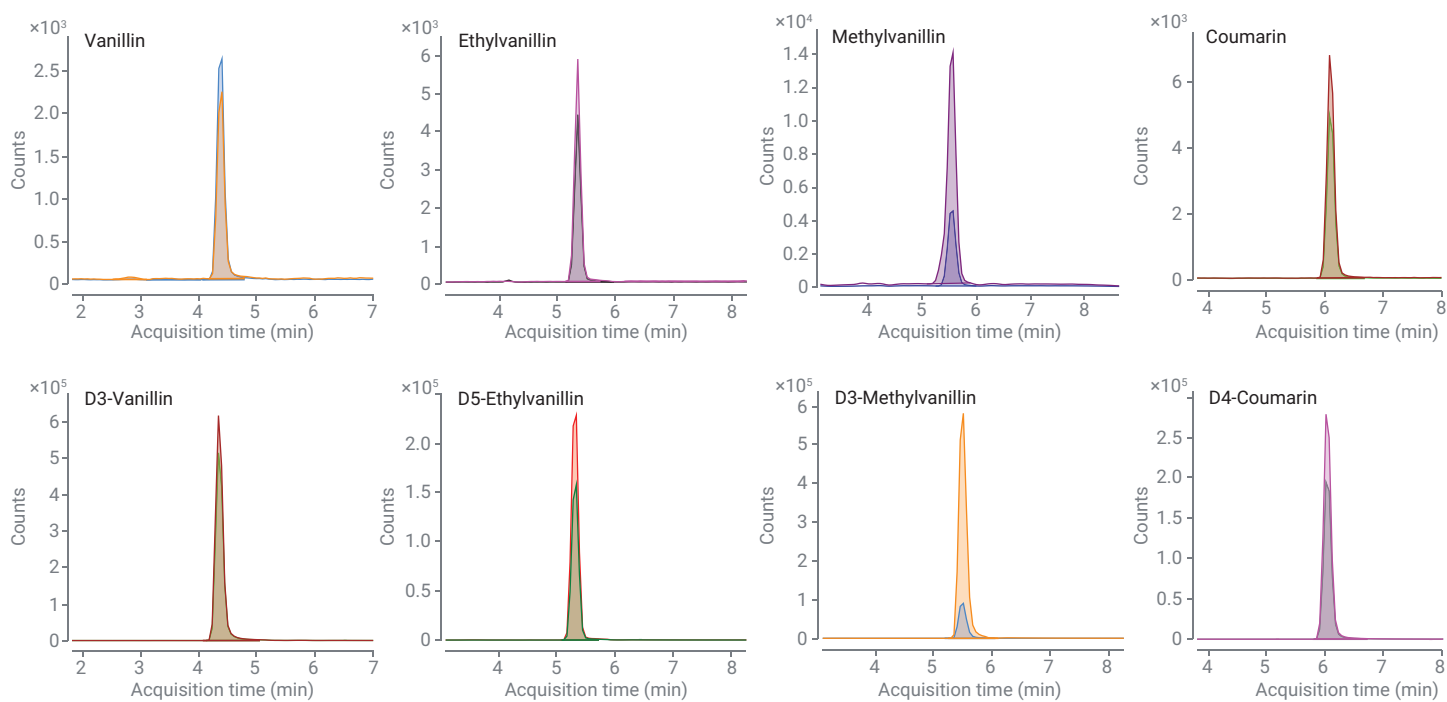
Figure 1. Extracted MRM chromatograms for the four food additives (top) and their corresponding internal isotopic analogs (IS, bottom).

Sample preparation optimization and method performance

Three types of acidified solvents were investigated for sample extraction. As shown in Figure 2, acidified acetonitrile showed the highest recovery for each analyte among the three selected solvents including acetonitrile, ether, and ethanol under acidic conditions, and was selected as the extraction solvent. The amount of acid was also examined for each food matrix, using baby cereal powder as an example. Figure 3 shows that the addition of 40 µL of HCl exhibited the highest recovery for each analyte. Other parameters for extraction and cleanup were also carefully optimized to maximize recovery. The optimized sample extraction and cleanup procedure is shown in the Method section.

We evaluated the method performance using the optimized sample preparation method. Using the ILIS dilution calibration, it was found that all analytes showed excellent linearity in the range of 50 to 5,000 µg/kg, with R^2 higher than 0.9995 (Table 2). The LOD and LOQ for each compound in each matrix were 10 and 50 µg/kg, respectively.
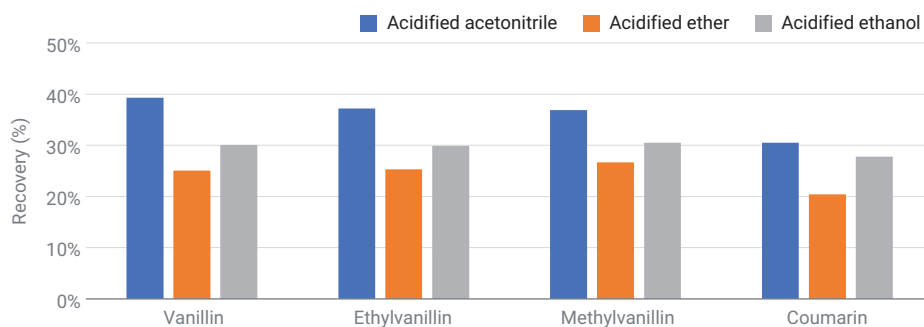

Figure 2. Effect of different solvents on extraction efficiency.
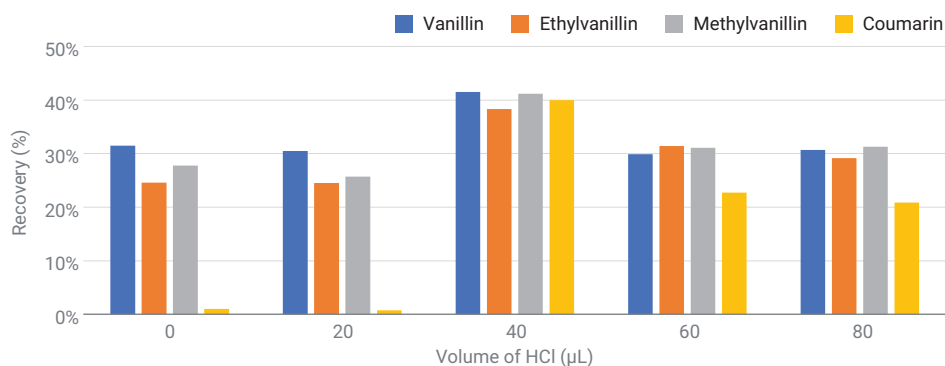

Figure 3. Effect of acid volume on extraction efficiency.

Table 2. Linearity, LOD, and LOQ of the method.

Compound	Calibration Equation	R^2	Linear Range (µg/L)	LOD (µg/kg)	LOQ (µg/kg)
Vanillin	y = 0.009664x − 2.789625E-004	0.99978	50 to 5,000	10	50
Ethylvanillin	y = 0.046100x − 5.051333E-004	0.99962	50 to 5,000	10	50
Methylvanillin	y = 0.037052x − 4.161242E-004	0.99966	50 to 5,000	10	50
Coumarin	y = 0.050603x − 8.280931E-005	0.99962	50 to 5,000	10	50

The method recovery and precision were evaluated by analyzing samples spiked at 1×, 2×, and 5× LOQ levels in three matrices with six replicates each. Figure 4 shows that the recoveries at all levels in all matrices ranged from 81.7% to 102%, with %RSD below 10%, suggesting that this method is reliable.

Conclusion

This study demonstrates a reliable UHPLC-MS/MS method for the determination of four common additives in baby formula, baby cereal powder, and milk. The method has excellent linearity, accuracy, and precision, and should prove useful in the routine monitoring of these additives in baby food.

References

1. US Food and Drug Administration. 21 CFR 189.130 - Coumarin. **2002**.

2. The European Parliament and the Council. Regulation (EC) No. 1334/2008.

3. China GB 2760-2016 on National Standard on Food Safety-the Standard for Usage of Food Additives.
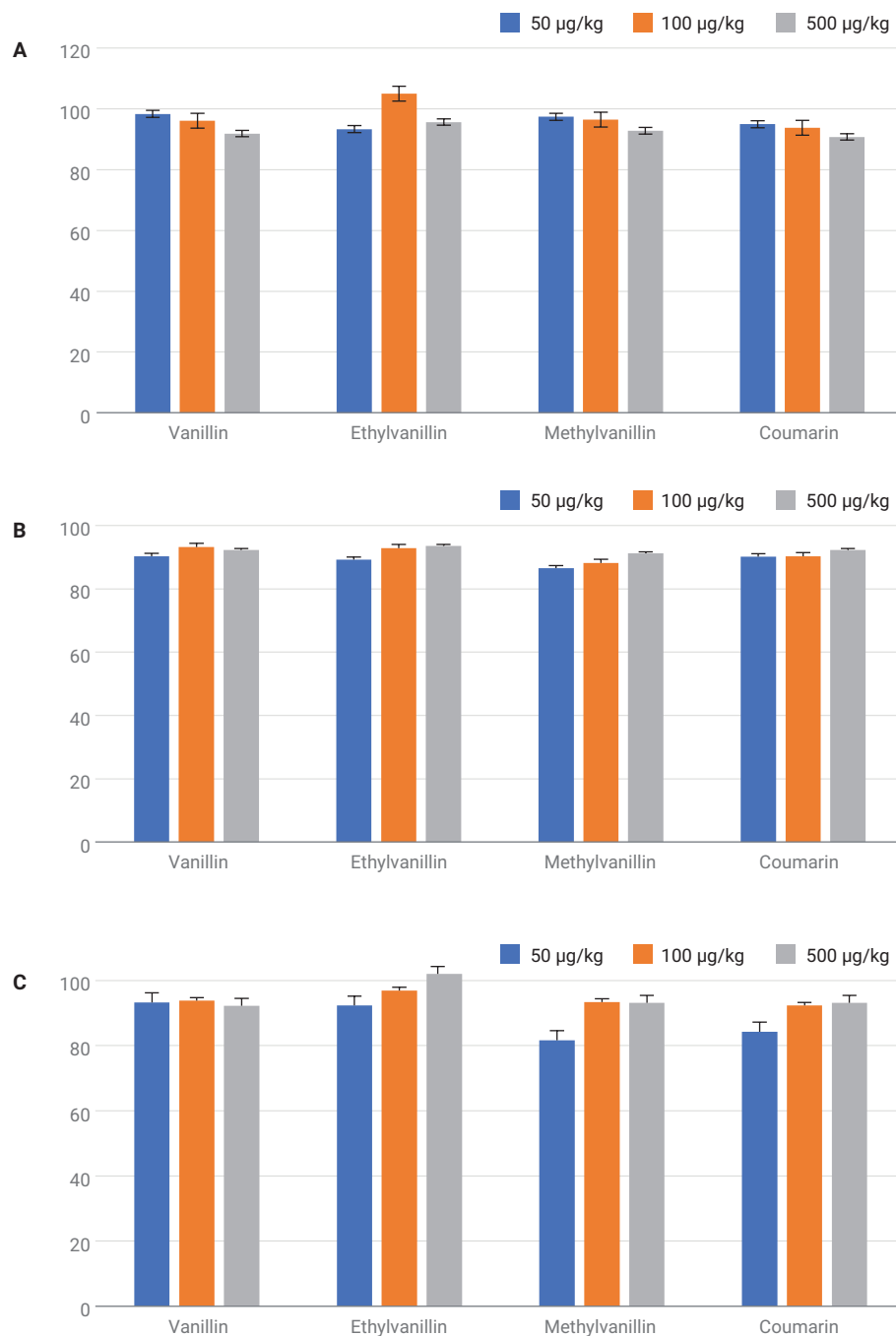
Figure 4. Recoveries and precisions for the additives spiked into three matrices at three levels (n = 6). A) Baby formula; B) Baby cereal powder; C) Milk.

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