Method of Test for Phthalate Plasticizers in Foods (Draft)

1. Scope

This method is applicable for the determination of phthalates in foods.

2. Method

After extraction, phthalates are determined by liquid chromatograph/tandem mass spectrometer (LC/MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: positive ion electrospray ionization, ESI^+.

2.1.1.2. Column: ACQUITY BEH C18, 1.7 μm, 2.1 mm i.d. × 100 mm, or equivalent product.

2.1.2. Sonicator.

2.1.3. Centrifuge: rotation speed ≧ 3500 ppm.

2.1.4. Vortex mixer.

2.2. Chemicals

Methanol, HPLC grade;

Ammonium acetate, AR grade;

Mili-Q water, resistivity ≧ 18 MΩ・cm (@25℃);

Benzyl butyl phthalate (BBP), reference standard;

Dibutyl phthalate (DBP), reference standard;

Di(2-ethylhexyl)phthalate (DEHP), reference standard;

Di-*n*-octyl phthalate (DNOP), reference standard;

Diisononyl phthalate (DINP), reference standard;

Diisodecyl phthalate (DIDP), reference standard.

2.3. Apparatus

2.3.1. Glass burette: 1mL and 5 mL.

2.3.2. Glass volumetric flask: 10 mL and 50 mL.

2.3.3. Glass centrifuge tube: 10 mL.

Note: all apparatus used for testing should be made by glass not plastics, and need be rinsed by methanol and dried before use.

2.4. Reagents

2.4.1. 5 mM Ammonium acetate solution

Dissolve 0.39 g of ammonium acetate in Mili-Q water and dilute to 1000 mL.

2.4.2. Mobile phase solution

Methanol: 5 mM ammonium acetate solution (9:1, v/v).

2.5. Standard solution preparation

Accurately weigh 50 mg of BBP, DBP, DEHP, DNOP, DINP and DIDP reference standard to each 50-mL volumetric flask, dissolve and dilute to volume with methanol as standard stock solution. Transfer 0.25 mL of each standard stock solution into a 50-mL volumetric flask and dilute to volume with methanol as mixed stock solution. When to use, dilute mixed stock solution with methanol to 0.02 ~ 0.5 μg/mL as working standard solution.

2.6. Sample solution preparation

Transfer about 1 g of the sample accurately weighed to a 50-mL volumetric flask and add 45 mL of methanol, sonicate for 30 min., then cool to room temperature, dilute with methanol to volume and mix, stand still for several minutes, transfer about 5 mL of the upper solution to a centrifuge tube, and centrifuge at 3500 rpm for 10 min. Use the supernatant as the sample solution.

2.7. Identification and quantification

Accurately inject 10 μL of the sample solution and standard solution into LC/MS/MS respectively according to below LC/MS/MS operating conditions. Identify and quantify each phthalate against the retention time and relative intensity of multiple reaction monitoring (MRM) transitions. Calculate the content of each phthalate in the sample by the following formula:

$$\text{The content of each phthalate in sample (ppm)} = \frac{C \times V}{\mathrm{M}}$$

where

C: the concentration of each phthalate in the sample solution calculated by the standard curve (μg/mL)

V: the make up volume of sample (mL)

M: the weight of sample (g)

LC/MS/MS operating conditions:

Mobile phase: methanol: 5 mM ammonium acetate solution (9:1, v/v)

Inject volume: 10 μL

Flow rate: 0.35 mL/min

Capillary voltage: 3.2 kV

Ion source temperature: 120℃

Desolvation temperature: 500℃

Cone gas flow rate: 100 L/hr

Desolvation flow rate: 800 L/hr

Detection mode: multiple reaction monitoring (MRM). Transition ion, cone voltage and collision energy are shown as follows:

Analyte	Detection ion (*m/z*) precursor ion > product ion	Cone voltage (V)	Collision energy (eV)
BBP	313 > 149*	17	11
	313 > 205	17	7
	313 > 239	17	5
DBP	279 > 149*	20	14
	279 > 205	20	7
DEHP	391 > 149*	19	20
	391 > 167	19	14
	391 > 279	19	9
DNOP	391 > 149*	18	12
	391 > 261	18	10
	391 > 121	18	40
DINP	419 > 149*	15	26
	419 > 275	15	12
	419 > 293	15	13
DIDP	447 > 149*	18	25
	447 > 289	18	9
	447 > 307	18	11

*Quantification ion for each phthalate.

Note:

1. All the parameters can be adjusted according to equipment if above conditions are not applicable.
2. The relative intensity is defined as the ratio of the peak area of two ion pairs (≦100%). The tolerance range is as follows:

Relative intensity (%)	Tolerance range (%)
> 50	± 20
> 20 ~ 50	± 25
> 10 ~ 20	± 30
≦ 10	± 50

Remarks: Further validation shall be done when interference compounds appeared in samples.