Method: 52251

Analysis of Plasticizer Contaminants in Beverages and Milk using an Automated System Based on Turbulent-flow Chromatography Coupled to LC-MS/MS

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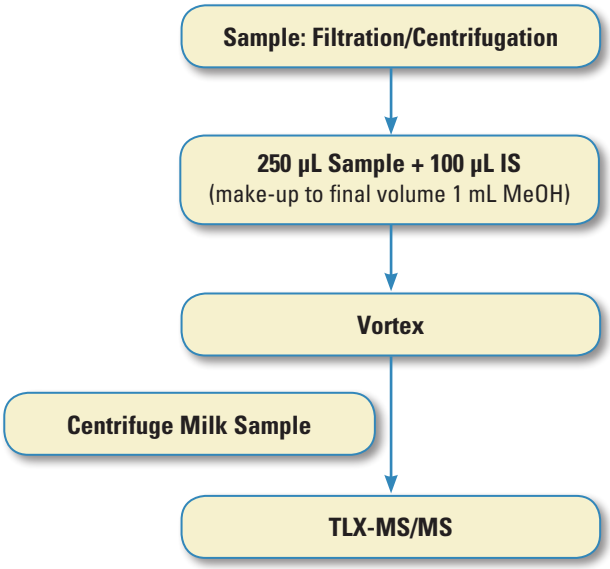
Key Words

- Transcend TurboFlow (TLX)
- TSQ
- Adipate
- Beverages and Milk
- Phthalates
- Plasticizer

1. Schematic of Method



2. Introduction

Phthalates are endocrine active chemicals used in a variety of consumer products. In some markets, restricted levels of certain phthalates are permitted for use in food contact materials, but they are not permitted as direct food additives.

Phthalates have been used to deliberately adulterate beverages and sports drinks in Taiwan, and phthalates and other plasticizers are widely found as ubiquitous contaminants particularly in fatty foodstuffs. Contamination arises from numerous sources such as the environment and food packaging. Cross-contamination with phthalates can easily arise during trace analysis in the laboratory and there are significant advantages in minimizing sample handling through online automated analysis. A method using online Thermo Scientific TurboFlow chromatography is presented.

3. Scope

This method can be applied to the determination of 10 phthalates and one adipate plasticizer at concentrations ranging from 0.05 to 100 mg/L (DMP, DEP, DPP, BBzP, DiBP, DcHP, DHP, DEHP, DEHA, DiNP, DiDP). The method has been validated for 11 plasticizers in beverages and milk.

4. Principle

This method employs an automated sample preparation technique involving sample concentration, cleanup and analytical separation in a single run using online sample prep with a Thermo Scientific Transcend TLX system powered by TurboFlow™ technology. TurboFlow technology serves as a novel sample preparation technique due to its special flow profile and both size exclusion and reversed phase column chemistry. TurboFlow technology enables very effective separation of matrix and target compounds resulting in relatively clean sample extracts. Identification of plasticizers is based on ion-ratios using selected reaction monitoring (SRM) of characteristic transition ions, and quantification of one of the selected SRM ions using labeled internal standards and external calibration.

5. Reagent List

		Part Number
5.1	Acetone, HPLC grade	A9494
5.2	Acetonitrile Optima, for LC-MS	A9554
5.3	Water Optima, for LC-MS	W64
5.4	Methanol Optima, for LC-MS	A4564
5.5	Formic acid	A11750
5.6	Isopropanol, HPLC grade	A4514

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6. Calibration Standards

6.1 Standards

6.1.1	Dimethyl phthalate (DMP)	Sigma Aldrich
6.1.2	Diethyl phthalate (DEP)	Sigma Aldrich
6.1.3	Diisobutyl phthalate (DIBP)	Sigma Aldrich
6.1.4	Dioctyl phthalate (DOP)	Sigma Aldrich
6.1.5	Butyl benzyl phthalate (BBzP)	Sigma Aldrich
6.1.6	Dihexyl phthalate (DHP)	Dr. Ehrenstorfer
6.1.7	Di-2-ethylhexyl adipate (DEHA)	Sigma Aldrich
6.1.8	Di(2-ethylhexyl)phthalate (DEHP)	Sigma Aldrich
6.1.9	Dicyclohexyl phthalate (DCHP)	Sigma Aldrich
6.1.10	Diisononyl phthalate (DiNP)	Sigma Aldrich
6.1.11	Diisodecylphthalate (DiDP)	Sigma Aldrich

6.2 Internal Standards

6.2.1	Bis(2-ethylhexyl) phthalate-3,4,5,6-d_4 (d_4-DEHP)	Dr. Ehrenstorfer
6.2.2	Diisobutyl phthalate-3,4,5,6-d_4 (d_4-DIBP)	Dr. Ehrenstorfer
6.2.3	Dihexyl phthalate 3,4,5,6-d_4 (d_4-DHP)	Sigma Aldrich

7. Standards Preparation

Rinse all glassware with methanol before pipetting plasticizer standards. Prepare fresh working standard mixtures weekly.

7.1 Stock Solution

Prepare individual standard stock solution of plasticizer standards in methanol at a concentration of 1 mg/mL in amber flasks. Store the standard stock solutions at 4 °C.

7.2 Working Mixture

Prepare two working mixtures. Prepare plasticizer mixture 1 (including DMP, DEP, DPP, BBzP, DiBP, DcHP and DHP) at 40 µg/mL in methanol. Prepare plasticizer mixture 2 (including DEHP, DEHA, DiNP, DiDP) at 200 µg/mL. Store the standard mixtures at 4 °C.

7.3 Stock Standard Solution of Internal Standard

Prepare individual internal standard stock solutions in methanol at a concentration of 1 mg/mL. Store the standard stock solutions at 4 °C.

7.4 Working Standard Solution of Internal Standard

Prepare a standard working mixture of internal standards in methanol at a final concentration of 5 µg/mL. Store the standard stock solutions at 4 °C.

8. Apparatus

		Part Number
8.1	Transcend™ TLX-1 system	
8.2	Thermo Scientific TSQ Quantum Access Max triple stage quadrupole mass spectrometer	
8.3	Precision balance	02225102
8.4	Sartorius analytical balance	14557812
8.5	Thermo Scientific Barnstead EASYpure II water	0905043
8.6	Ultrasonic bath Elmasonic S40H	15335101
8.7	Vortex shaker	14505141
8.8	Vortex standard cap	3205026
8.9	Centrifuge, Thermo Scientific Hereaus, Thermo Scientific Multifuge X3	503260260

9. Consumables

		Part Number
9.1	Thermo Scientific Hypersil GOLD, 150 × 2.1 mm 3 µm	25003152130
9.2	C18 XL 0.5 × 50 mm	CH953280
9.3	LC vials	3205111
9.4	LC vial caps	3151266
9.5	Pipette Finnpipette 10–100 µL	14386318
9.6	Pipette Finnpipette 100–1000 µL	14386320
9.7	Pipette Finnpipette 500–5000 µL	14386321
9.8	Pipette holder	14245160
9.9	Pipette Pasteur soda lime glass 150 mm	136786A
9.10	Pipette suction device	3120891
9.11	Pipette tips 0.5–250 µL, 500/box	21377144
9.12	Pipette tips 1–5 mL, 75/box	2137750
9.13	Pipette tips 100–1000 µL, 200/box	2137746
9.14	Glass tube	14957E

10. Glassware

		Part Number
10.1	Fisherbrand, Beaker, 25 mL	FB100250
10.2	Fisherbrand, Pasteur pipette	1367830
10.3	Fisherbrand, Volumetric flask, 10 mL	FB40010
10.4	Fisherbrand, Volumetric flask, 100 mL	10205C
10.5	Fisherbrand, Volumetric flask, 1000 mL	10205F

11. Procedure

11.1 Sample Preparation and Spiking

Samples of orange juice, bitter lemon and milk were spiked as described below.

Orange Juice

11.1.1 Squeeze one orange into a beaker (rinsed with methanol). Centrifuge (5 min at 5000 rpm) the juice by using 15 mL glass centrifuge tubes (rinsed with methanol). Spike in an LC vial, 250 µL of juice with 100 µL of IS working mixture and the appropriate amount of plasticizer standard working mixture. Fill up to a final volume of 1 mL with methanol and vortex for 10 sec.

Milk, (1.5% Fat) Pasteurized, Homogenized

11.1.2 Pipette 250 µL of milk directly into an LC vial. Add 100 µL of IS working mixture and the appropriate amount of plasticizer standard mixture. Fill up to final volume of 1 mL with methanol, vortex for 10 sec. Put the vial into a 15 mL glass centrifuge tube and centrifuge (5 min at 5000 rpm) to remove protein. Transfer the supernatant into an LC vial by the help of a glass pasteur pipette.

Bitter Lemon

11.1.3 Pipette 250 µL of bitter lemon (market sample) directly into an LC vial and spike with 100 µL of IS working mixture and the appropriate amount of plasticizer standard working mixture. Fill up to a final volume of 1 mL with methanol and vortex for 10 sec.

12. LC Operating Conditions

LC analysis is performed on a Transcend TLX System. The LC conditions are as follows:

TurboFlow Column: C18 XL, 0.5 × 50 mm
Analytical Column: Hypersil™ GOLD C18, 150 × 2.1 mm, 3 µm
Total Run Time: 22 min
Mobile Phase: A: acetonitrile (0.1% formic acid)
B: methanol (0.1% formic acid)
C: water (0.1% formic acid)

12.1 Injector Settings

Injector: Thermo Scientific Pal injector with 100 µL injection syringe volume
Wash solvents for the Autosampler
Solvent 1: acetonitrile/isopropanol/acetone (40/40/20)
Solvent 2: methanol

- Pre clean with solvent 1 [steps]: 3
- Pre clean with solvent 2 [steps]: 3
- Pre clean with sample [steps]: 0
- Filling speed [µL/s]: 50
- Filling strokes [steps]: 1
- Injection port: LC Vlv1 (TX channel)
- Pre inject delay [ms]: 500
- Post inject delay [ms]: 500
- Post clean with solvent 1[steps]: 5
- Post clean with solvent 2 [steps]: 5
- Valve clean with solvent 1[steps]: 5
- Valve clean with solvent 2 [steps]: 5

Injection Volume: 10 µL
Tray Temperature: 10 °C

13. Mass Spectrometric Conditions

MS analysis is carried out using a TSQ Access MAX™ triple quadrupole mass spectrometer controlled by Thermo Scientific Aria software. Data acquisition and processing is performed using Thermo Scientific Xcalibur 2.1 software.

13.1 Mass Spectrometer Conditions

Ionization: Electrospray (ESI)
Polarity: Positive ion mode
Spray Voltage [V]: 3500
Ion Sweep Gas Pressure [arb]: 0
Vaporizer Temperature [°C]: 350
Sheath Gas Pressure [arb]: 50
Aux Gas Pressure [arb]: 15
Capillary Temperature [°C]: 270
Collision Gas Pressure [mTorr]: 0
Cycle Time [s]: 0.8
Scan Mode: timed selected reaction monitoring (tSRM)

The mass spectrometer is programmed with 28 timed segments which is set up for the 11 target plasticizers and 3 labeled internal standards. For each compound one quantifier and one qualifier ion is monitored, including the respective labeled internal standard. The program of segments for tSRM events is shown in Table 2.

	Loading Pump									Eluting Pump				
Step	Step [min]	sec	Flow [mL/min]	Grad	A	B	C	Tee	Loop	Flow [mL/min]	Grad	A	B	C
1	0	30	2.0	Step	–	–	100	–	Out	0.7	Step	40	30	30
2	0.5	60	0.4	Step	40	30	30	T	In	0.3	Step	40	30	30
3	1.5	330	1.0	Step	–	100	–	–	In	0.7	Ramp	1	98	1
4	7.0	480	0.7	Step	–	75	25	–	In	0.7	Step	1	98	1
5	15.0	240	0.5	Step	–	–	100	–	Out	0.7	Ramp	40	30	30
6	19.0	180	0.5	Step	–	–	100	–	Out	0.7	Step	40	30	30

Table 1: TLX-LC gradient program

Analyte	Parent	Production 1 (CE)	Production 2 (CE)	Start Time [min]	Stop Time [min]	Tube Lens	Ion Ratio	Retention Time [min]
DMP	194.84	163.09 (11)	77.26 (33)	0.5	2.0	49	0.23	1.40
DEP	223.08	149.08 (18)	121.13 (28)	0.0	2.0	52	0.11	1.56
DPP	251.10	149.07 (16)	121.13 (32)	0.5	2.5	57	0.10	2.37
DiBP	279.13	149.08 (19)	121.10 (31)	1.0	4.0	57	0.07	3.33
d_4-DiBP	283.13	153.09 (20)	69.30 (46)	1.0	4.0	56	0.07	3.30
BBzP	313.09	91.20 (28)	149.05 (12)	1.0	4.0	60	0.72	3.23
DcHP	331.17	149.08 (25)	167.07 (12)	2.5	4.5	59	0.46	4.70
DHP	335.17	149.05 (16)	121.11 (38)	3.0	6.0	60	0.08	5.75
d_4-DHP	339.19	153.05 (17)	125.10 (39)	3.0	6.0	68	0.08	5.73
DEHA	371.25	129.11 (14)	111.13 (22)	4.0	8.0	72	0.35	7.08
DEHP	391.18	149.06 (25)	121.05 (45)	4.5	7.5	72	0.09	7.03
d_4-DEHP	395.26	153.07 (24)	171.07 (13)	4.5	8.0	68	0.32	7.02
DiNP	419.29	149.03 (26)	85.28 (14)	4.5	7.5	76	0.67	7.48
DiDP	447.31	149.01 (30)	85.23 (17)	5.0	8.5	78	0.77	7.90

Table 2: Parameters for tSRM analysis, ion ratios and retention times of plasticizers in methanol (CE = collision energy)

14. Calculation of Results

14.1 Identification

Identification of plasticizers is indicated by the presence of ions measured in selected reaction ion monitoring mode (SRM) corresponding to the retention times of appropriate standards. For peak identification the ion ratios are checked. The ion ratios for sample extracts should fall within the tolerance of the corresponding standard (657/2002 EC[1]).

15. Interpretation of Results

15.1 Quantification

By comparing the peak areas of the samples with those of external calibration the quantification of the standards in the spiked samples is carried out.

It employs internal standardization using peak area ratios for standards in methanol. D_4-DiBP is used as the internal standard for DMP, DEP, DPP, DiBP and BBzP; d_4-DHP is used as the internal standard for DHP; and d_4-DEHP is used as the internal standard for DEHP, DEHA, DiNP and DiDP. Plot the calibration curves as the relative peak areas (analyte versus the corresponding internal standard) as a function of concentrations. The plasticizer concentration (c_p) in the samples is determined from the equation:

$$C_p = \left(\frac{A_p}{A_{IS}}\right) - b/a$$

C_p – plasticizer concentration in mg/L

A_p – peak area of the plasticizer

A_{IS} – peak area of internal standard

b – the y-intercept

a – the slope of calibration curve

16. Method Performance

Single laboratory method performance characteristics were established by spiking experiments with three samples (homemade orange juice, bitter lemon and milk, see 10.1) with a mixture of plasticizer standards.

Method accuracy was assessed at three different spiking levels of plasticizers (low, mid and high level). Other validation parameters included specificity, linearity range and robustness.

16.1 Specificity

Using tSRM the specificity was confirmed based on the presence of transition ions at the correct retention time corresponding in timed segments to the plasticizer standards in methanol (Table 2). Specificity determination was based on ion ratio confirmation according to allowed variations in 657/2002 EC (Table 2). The deviation for retention time was in the range of ±2.5%.

16.2 Linearity and Calibration Curve

The linearity of calibration curves is assessed over the range from 0.05 to 5 mg/kg (DMP, DEP, DPP, BBzP, DiBP, DcHP and DHP) and 2–100 mg/L (DEHP, DEHA, DiNP, DiDP). In all cases, the correlation coefficients of linear functions are >0.985. The calibration curves are created from eight calibration standards which are injected in each batch in duplicate starting from zero value up to the highest calibration concentration. Between each calibration level methanol is injected as blank.

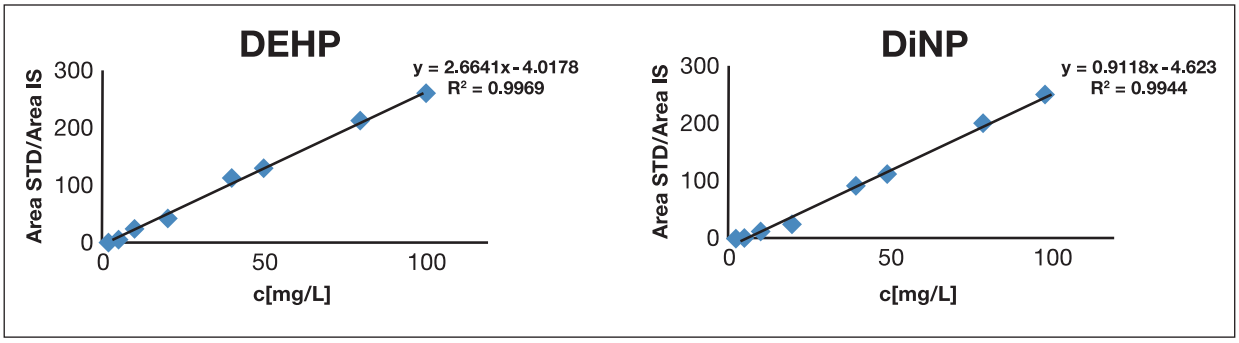

Figure 1: Calibration curve of DEHP and DiNP with d_4-DEHP as internal standard

16.3 Accuracy

Method Accuracy and Precision (Table 3) is evaluated by recovery studies spiking bitter lemon, orange juice and milk at three concentration levels (at low, mid and high levels) of plasticizers. Six replicates are prepared for each experiment, in accordance with EU guidelines. The samples are spiked at 0.2, 2 and 4 mg/L (DMP, DEP, DPP, BBzP, DiBP, DcHP and DHP) and 8, 40 and 80 mg/L (DEHP, DEHA, DiNP, DiDP) levels.

Found concentrations (mg/L), recovery, standard deviation (STDV) and relative standard deviation (%RSD) are calculated.

Analyte	Orange Juice Rec % (%RSD)	Bitter Lemon Rec % (%RSD)	Milk Rec % (%RSD)
DMP	94.5 (5)	102.4 (6)	111.0 (4)
DEP	97.6 (4)	111.0 (5)	108.7 (3)
DPP	106.7 (4)	112.4 (4)	118.0 (3)
BBzP	103.3 (3)	90.0 (4)	98.9 (2)
DiBP	115.8 (4)	90.0 (8)	92.2 (3)
DcHP	77.8 (4)	81.0 (5)	69.0 (3)
DHP	96.3 (4)	96.1 (5)	93.8 (3)
DEHP	97.2 (15)	85.7 (8)	78.2 (9)
DEHA	64.8 (14)	70.4 (7)	76.1 (7)
DiNP	84.5 (12)	75.9 (8)	71.9 (3)
DiDP	72.6 (10)	71.2 (1)	*

Table 3: Average recovery and RSD of plasticizer standards in orange juice, bitter lemon and milk

** DiDP is not applicable for milk matrix*

17. Conclusion

A method for the determination of plasticizers in beverages and milk has been developed to enable fast and cost-effective automated determination of selected plasticizers. Online TurboFlow sample preparation coupled to the analytical HPLC separation equipped with a triple quadrupole detector enables very selective and effective determination of plasticizers. Elimination of time-consuming steps like liquid liquid extraction (LLE) or solid phase (SPE), followed by evaporation and reconstitution enables high sample throughput. Reducing sample treatment steps reduces the probability of cross contamination as well.

18. Reference

1. Commission Decision 657/2002 of August 2002 on implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results. Official journal of the European communities, L221, 8-36, 2002.

19. Annex

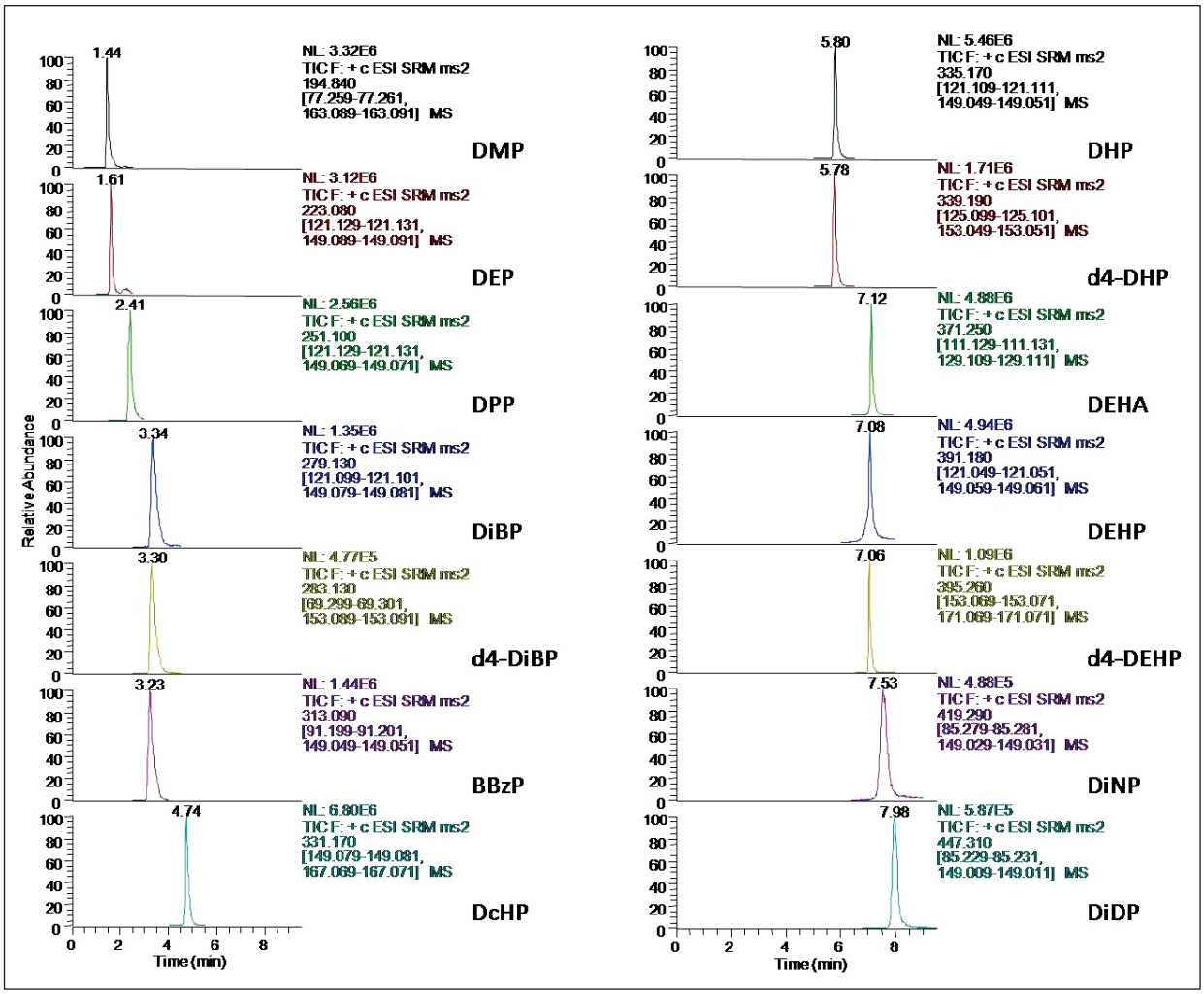

Figure 2: Chromatogram: Separation of plasticizer standards in methanol (c = 1.5 mg/L) at TLX-LC gradient program (Table 1)

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