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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Quantitative Determination of Nine Type of Organophosphate Flame Retardant (OPFR) Metabolites in Human Urine

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User Benefits

- The method has been validated and prepared to analyze OPFR metabolites using LC-MS/MS.
- The sample preparation includes steps such as enzymatic hydrolysis and simple SPE method.
- TBA ion-pairing reagent is used as a mobile phase to increase retention and provide sufficient volatilization for ESI.

■ Introduction

Flame retardants activated by an ignition source are substances used to reduce flammability and retard combustion. They include a diverse group of chemicals added to manufactured materials such as plastics, textiles, surface finishes and coatings. Toxicological data suggest that certain organophosphate flame retardants (OPFRs) may be reproductive toxins and may also have carcinogenic and neurotoxic properties. Many OPFR studies have been reported on environmental samples such as water and air, and recently studies have been conducted to dust samples in indoor environments such as houses and offices in addition to food matrices and consumer products.[1]. An exposure assessment conducted by the Consumer Product Safety Commission referred to TDCPP (Tris(1,3-dichloroisopropyl) phosphate) as a suspected carcinogen[2]. However, due to the lack of epidemiological research on human exposure of OPFRs, biological monitoring studies on OPFRs that potentially affect human health are needed. Accordingly, this application news introduces quantitative analysis method for detecting 9 OPFR metabolites in human urine, and some target compounds are shown in Fig. 1.

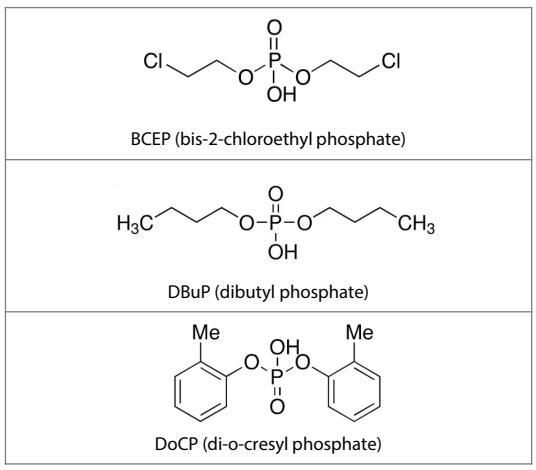

Fig. 1 Examples of Structures of OPFR metabolites

■ Analytical Conditions

In this analysis, a Shimadzu liquid chromatography mass spectrometer LCMS-8060NX was used for optimization of 9 OPFR metabolites. The instrumental conditions and MRM conditions are as shown in the Table 1 and Table 2, respectively. TBA ion-pairing reagent is used as a mobile phase to increase retention and provide sufficient volatilization for ESI[1].

The chromatogram of the standard solution (10 ng/mL) by pretreatment of synthetic urine is shown in Fig. 2.

Table 1 Instrumental Conditions

Liquid chromatograph	Nexera™ X3
Column	: ACE Excel 2 C18-AR (3.0 x 150 mm, 2 µm)
Flow rate	: 0.4 mL/min
Mobile phase (A)	: 1 mM Acetic acid in water/MeOH = 80/20 (v/v)
Mobile phase (B)	: 1 mM Tributylamine, 1mM Acetic acid in MeOH/water = 95/5 (v/v)
Gradient	: 20 % B (0.0 - 1.0 min) - 40 % B (2.0 min) - 80 % B (8.0 min) - 100 % B (8.5 - 10.5 min) - 20 % B (10.51 -14.0 min)
Oven temp.	: 45 ˚C
Injection volume	: 20 µL
Mass spectrometer	LCMS-8060NX
Interface	: ESI
Data acquisition	: MRM mode, Negative mode
Interface temp.	: 300 ˚C
DL Temp.	: 150 ˚C
Heat block Temp.	: 400 ˚C
Nebulizing Gas Flow	: 3.0 L/min
Drying Gas Flow	: 10.0 L/min
Heating Gas Flow	: 10.0 L/min

Table 2 Multiple Reaction Monitoring (MRM) Conditions

Compound name	Type	Quantifier ion (m/z)	Qualifier ion (m/z)
BCEP	Target	221 >35	223 >35
BCPP		249 >35	251 >35
BDCPP		317 >35	319 >35
DBuP		209 >79	209 >153
DBzP		277 >79	277 >63
DoCP		277 >107	277 >169
DpCP		277 >107	277 >169
DPhP		249 >93	249 >155
TBBA		437 >393	437 >79
BCEP-d8	ISTD	229 >35	231 >35
BCPP-d12		261 >35	263 >35
BDCPP-d10		329 >35	327 >35
DBuP-d18		227 >79	227 >163
DBzP-d10		287 >79	287 >63
DoCP-d14		291 >114	291 >175
DpCP-d14		291 >114	291 >112
DPhP-d10		259 >98	259 >159
TBBA-13C6		443 >399	443 >81

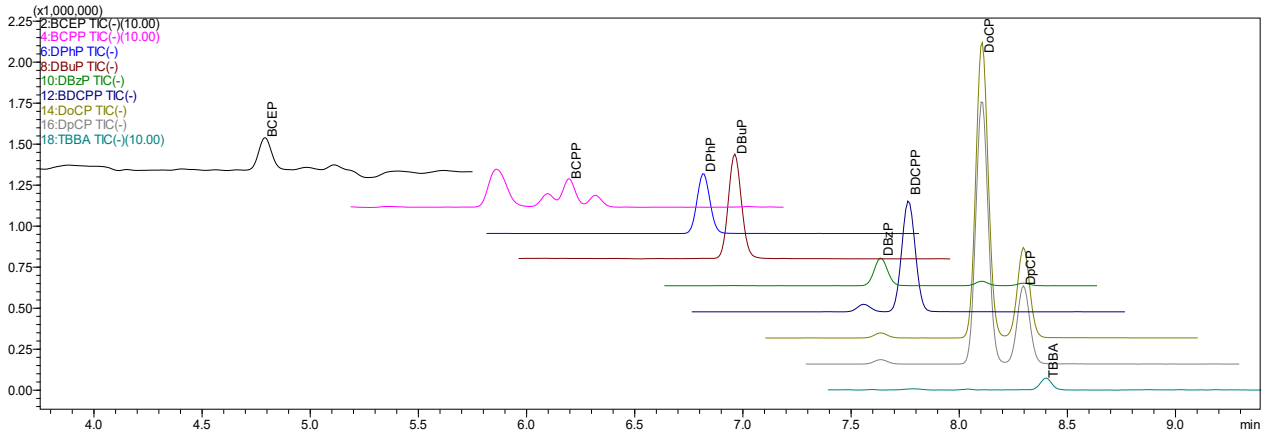

Fig. 2 MS Chromatogram of 9 OPFR Metabolites (concentration in urine sample : 10 ng/mL)

■ Sample Pretreatment

The detail of pretreatment process is shown in Fig. 3. The urine samples were prepared through SPE purification and concentration after enzymatic hydrolysis.

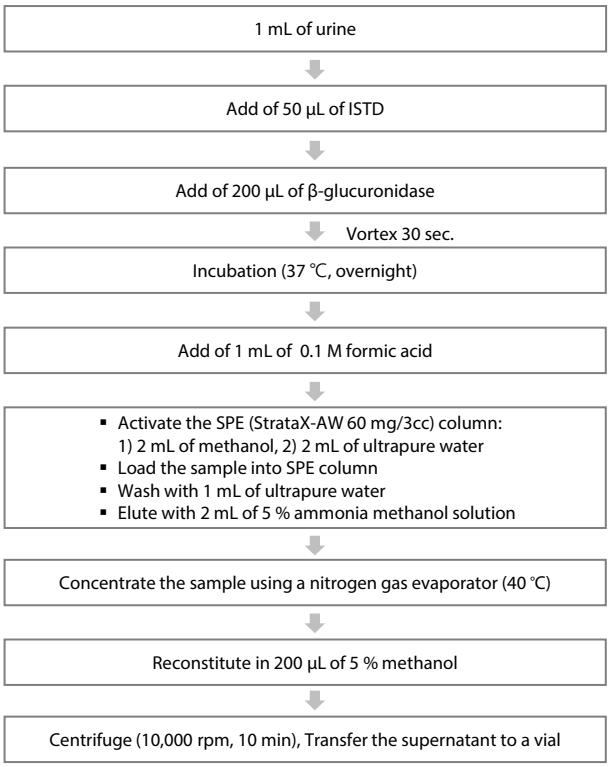

Fig. 3 Sample Preparation Protocol

■ Results and Discussion

Calibration curve of OPFR metabolites

The standard solutions for the calibration curves were prepared by adding mixed standard solution to synthetic urine as shown in Fig. 3. Good linearity was obtained in the range of calibration curve from 0.4 ng/mL to 20 ng/mL and the correlation coefficients (r^2) for each compound was 0.99 or more (Table 3).

Recovery rate and Method detection limit

The synthetic urine was pretreated by adding the mixed standard solution at a level of 2.5 ng/mL, which is the middle concentration of the calibration curve. And then the recovery rate was evaluated through five repeated analysis. The results were ranged from 85 % to 110 %, and the relative standard deviation (%RSD) was within 12 %.

The detection limit of the method was calculated by multiplying 3.14 to the standard deviation obtained by seven repeated analysis of the lowest calibrator concentration. The obtained detection limits were between 0.1 ng/mL and 0.4 ng/mL depending on compounds (Table 3).

Table 3 Validation Results for OPFR Metabolites Analytical Method

Name	r^2	Recovery rate (%) ± %RSD, *n*=5	MDL (ng/mL) *n*=7
BCEP	0.998	101 ±8	0.2
BCPP	0.997	110 ±4	0.2
BDCPP	0.995	109 ±12	0.2
DBuP	0.997	99 ±12	0.4
DBzP	0.995	96 ±3	0.1
DoCP	0.996	101 ±8	0.1
DpCP	0.996	95 ±3	0.1
DPhP	0.998	85 ±8	0.1
TBBA	0.999	94 ±10	0.2

■ Conclusion

The simultaneous analysis of 9 OPFR metabolites in human urine was carried out using a Nexera X3 with an LCMS-8060NX system. We demonstrated excellent performances of the system for calibration curve and recovery rate test within 14 minutes. The correlation coefficients (r^2) for the calibration curve were 0.99 or higher for all compounds, and the recovery rate was ranged from 85 % to 110 %. Method detection limits were between 0.1 ng/mL and 0.4 ng/mL depending on the compounds.

<Reference>

1. Syrago-Styliani E. Petropoulou, Myrto Petreas, June-Soo Park, Analytical methodology using ion-pair liquid chromatography–tandem mass spectrometry for the determination of four di-ester metabolites of organophosphate flame retardants in California human urine, Journal of Chromatography A, 1434(2016) 70–80
2. Kate Hoffman, Julie L. Daniels, Heather M. Stapleton, Urinary Metabolites of Organophosphate Flame Retardants and Their Variability in Pregnant Women, Environ Int. 2014 Feb; 63: 169–172.

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